Efficient oil extraction and use, and production of feed grade protein concentrate and seed meal from *Jatropha curcas* seeds for inclusion in monogastric, fish and ruminant feeds

Project Code: 0330799A

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Structure of the report

The report is presented in two parts: Part A and Part B. Part A starts with Executive Summary and is then followed by four main sections. First section deals with Introduction, problems needed to be addresses and project objectives; second section presents Work plan, collaborating institutions and cooperation amongst them, progress made as 'Targets Envisaged' against 'Targets Achieved', and grants obtained for manpower and objectives achieved; third section describes in details the Work accomplished and results obtained by Biology (detoxification of kerneal meal and protein isolate and their utilization), Engineering (optimization of oil extraction and use of oil and shells as energy sources) and Economics (economic and financial analysis) groups of the University of Hohenheim, Stuttgart; and fourth section has a List of Publications that emanated from the Project. Part B deals with the work accomplished by the German Private Companies. It has three sections: 5, 6 and 7, that contain work conducted and results obtained, respectively by Phyto-Energy Consulting and Engineering, Shareneback, Dr. Otto GmbH, Wittenberge and JatroSolutions GmbH, Stuttgart. Part B is submitted separately since it is not to be made public on the request of the private companies.

Notes:

1. *Jatropha curcas* and *Jatropha* have been used interchangeably in this document. All these expressions mean the same.
2. References for sections are given at the end of each section, and not in a collated form at the end of the report.
3. The formatting and structure of different sections and especially of references might slightly differ since these have been taken from our different publications, and also different sections have been prodcued by different partners of the Project.

Acknowledgements

We thank the German Federal Ministry of Education and Research (BMBF) and Ministry of Science and Technology (MOST) of China for providing the financial support to conduct this work. This work could not have been completed without outstanding cooperation and collaboration of all the German and Chinese groups including the German private industries, which is gratefully acknowledged. Adminstrative and partial financial support by the University of University, Stuttgart is also appreciated. Lastly but not the least, excellent assistance and guidance from Dr. M. Weber and Ms. Birgit Liebs, who were the Project Officer and Finance Oficers of this project enabled smooth implementation of the project, which is also thankfully acknowledged.

Project Website: http://Jatropha.uni-hohenheim.de

(Most of the work presented in this report has been published. Full papers can be downloaded from the Project Website)

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Executive Summary

Jatropha curcas L. is an oil-bearing shrub belonging to the family of *Euphorbiaceae*, widely distributed in many Latin American, Asian, and African countries. *Jatropha* has considerable potential for the tropical and sub-tropical regions including China, as it is a versatile oil plant with many economical and ecological attributes. Many oil importing countries have plans to undertake massive cultivation of *Jatropha* to produce renewable fuel oil locally and reclaim wasteland for food production. When the project started the *Jatropha* seed cake and kernel meal obtained as by-products can best be utilized as a fertilizer since it is toxic. In addition, the efficiency of oil extraction from *Jatropha* seeds and its use in cooking stoves or stationary engines as an energy source was far from satisfactory. The overall objective of the project was to enhance economic viability and sustainability of *Jatropha*-based small and large scale oil production systems by introducing innovative industrial and livestock production systems. Specifically, we aimed to: i) utilize the by-products of the oil production units, *Jatropha* seed meal and kernel meal as livestock feed, and ii) enhance the efficiency of production and utilization of oil and shells as energy sources in small scale operations.

Three technologies: detoxification of *Jatropha* kernel meal, detoxification of *Jatropha* protein isolate, and preparation and detoxification of *Jatropha* protein isolate (one-step method) have been developed and EU and PCT patents have been filed. These will be made public in August 2010 by the Patent Office. The guidelines have been developed for using the detoxified *Jatropha* kernel meal (DJKM) and detoxified *Jatropha* protein isolate (DJPI) in the feed industry. These *Jatropha* products can be used to provide a source of nutrients for fish and shrimp. Fish meal protein can be replaced by 50% and 75% by DJKM and DJPI respectively in fish and shrimp diets. For turkey, initial results based on amino acid digestibility are very promising; however, further studies are required to determine the protein replacement level of the traditional protein sources in diets. In pig diets, 50% of protein from the traditional protein sources can be replaced by DJKM.

A technology has also been developed to isolate kernel meal from screw pressed *Jatropha* cake containing shells. This technology is based on a physical principle of sieving and separates undigested shells from high quality protein. This technology will enable the use of this high quality protein as a feedstock for various applications including detoxification to obtained detoxified kernel meal.

A process has also been developed to clean *Jatropha* oil for use in cooking stoves and stationary engines. It has been established that the exhausts from the stove and the stationary engine is

free of phorbol esters, the main toxins in *Jatropha* oil. A de-shelling machine for obtaining kernels from *Jatropha* seeds has been developed. In addition, potential of shells for use as energy source has been evaluated (it has high energy content) and a prototype of furnace has been developed.

The financial assessment and value chain analysis undertaken in this study were much constrained by the fact that plantations have not reached maturity and yields were low, and that seed processing and detoxification units are presently not operating. As a consequence, the computations had to be based on certain assumptions and the results of this preliminary investigation need to be interpreted with caution. Nevertheless, the report showed that there are many factors currently constraining the development of the *Jatropha* based biofuel sector in China. The detoxification of *Jatropha* kernel meal and protein isolate is a promising strategy to improve the profitability of the *Jatropha* processing units but without additional efforts to raise *Jatropha* seed production efficiency and reduce input costs, this bio-refinery activity might not be a profitable venture in the near future in China.

With the development of the technologies through this project, small, medium and large scale industries would benefit through production of efficient de-shelling, seed pressing and oil extracting units for production of oil for direct use as an energy source or after its transesterification as biodiesel, and through construction of detoxification plants and their accessories. On the other hand, both small and large scale oil producing industries would generate higher profit through production of value-added products, detoxified kernel meal and protein isolate for use in livestock feed, and seed shells for use as a source of energy.

Other associated indirect positive effects would be the aavailability of CO_2 neutral fuel in remote rural areas, wasteland reclamation for food production, furthering development of an alternative high value agricultural crop, employment generation, reduced atmospheric pollution, and carbon sequestration for emission trading.

This project has also opened avenues for use of phorbol esters isolated from *Jatropha* oil (before its use as biodiesel) for use as bio-pesticide, -insecticides and –molluscicide.

1. **Introduction, problems needed to be addresses and project objectives**

Jatropha curcas is a multipurpose shrub or small tree belonging to the family of Euphorbiaceae with many attributes and multiple uses. In many countries, it has been used to prevent or control erosion, reclaim land, and for live fencing. Recently, it is also being planted as a commercial crop, but currently it grows mainly in the wild. Its seeds contain about 25–35% oil that can be used as fuel directly or as a substitute to diesel in the transesterified form. Many oil importing countries have plans to undertake massive cultivation of *Jatropha* to produce renewable fuel oil locally and reclaim wasteland for food production. *Jatropha* plantations have been initiated at a large scale in a number of countries, to name a few, China, India, Madagascar, Myanmar, Indonesia, Egypt, Zimbabwe, Tanzania. The surging prices of crude oil in the international markets have resulted in several private and corporate players showing interest in the field.

The overall objective of the project was to enhance economic viability and sustainability of *Jatropha*-based small and large scale oil production systems by introducing innovative industrial and livestock production systems. Specifically, we aimed to: i) utilize the by-product of the oil production units, *Jatropha* seed meal as livestock feed, and ii) enhance the efficiency of production and utilization of oil in small scale operations. When the project started the *Jatropha* seed cake obtained as a by-product can best be utilized as a fertilizer since it is toxic. The efficiency of oil extraction from *Jatropha* seeds and its use in cooking stoves as an energy source was far from satisfactory. Therefore, the specific objectives of the project were:

1. Preparation of kernel meal and protein concentrate free of antinutrients and toxic factors, and their utilization in the diets of various livestock species
2. Development of efficient de-shelling technology.
3. Optimization of screw-pressing technology for extraction of oil and production of seed meal.
4. Development of technology for cleaning of *Jatropha* crude oil in order to reach the developed standard for use in plant oil stoves.
5. Development of small scale combustion systems (furnace) for using shells as energy source.
6. Economic evaluation of the detoxification process, and its accounting in the overall economic assessment of *Jatropha*-based small and large scale oil production systems.

2. Work plan, collaborating institutions and cooperation amongst them, and progress made

2.1 Work plan

An overview of the project activities is presented in Figure 1a,b,c. Figure 2 gives Project Framework Matrix, showing various components of the project and integration of activities of German and Chinese groups. These figures are self explanatory. The work packages of the German groups for the main phase (July 2007 to June 2009) and the extension phase (July 2009 to June 2010) are listed below.

Main Phase

- Preparation of detoxified kernel meal and protein isolate, establishment of their non-toxic nature using fish and their utilization in fish diets (responsible group: Germany 1)
- Development of technology for separating shells and kernel from screw-pressed shell-containing seed meal; and design and development of a pilot plant for production of detoxified feed-grade protein concentrate and seed meal (responsible groups: Germany 4 and 1)
- Development of efficient de-shelling technology; optimization of screw-pressing technology for extraction of oil and production of seed meal; development of technology for cleaning of *Jatropha* crude oil for use in plant oil stoves, and development of small scale combustion systems for using shells as energy source (responsible groups: Germany 2, 3 and 1)
- Intellectual property management and technology transfer to farmers (responsible group: Germany 5)
- Financial and economic evaluation (responsible group: Germany 6)
- Histopathological evaluation of organs (responsible group: Germany 7)
- Provide support to the filing and defence of patent, and knowledge management (responsible group: Germany 5)

Extension phase

- Use of detoxified kernel meal in shrimp and turkey diets (responsible group: Germany 1 and 8)
- Development of a method for determination of shells in screw pressed cake using NIRS (responsible group: Germany 1)
- Detoxification of protein concentrate using adsorbents (responsible group: Germany 1)

- Evaluation of various byproducts accrued through the production of *Jatropha* biodiesel as livestock diet (responsible group: Germany 1)
- Separation of shells from screw pressed cake using sieving (responsible group: Germany 1)
- Screw pressed based extraction of *Jatropha* oil from kernels with different proportions shells (responsible group: Germany 2)
- Purification of *Jatropha* oil through continued processes and development of a standard to use *Jatropha* oil in plant oil stoves (responsible group: Germany 2)
- Development of a *Jatropha* kernel de-shelling unit (responsible group: Germany 2)
- Quantification of phorbol esters in the stove and stationary engine exhausts (responsible groups: Germany 1, 2 and 5)
- Provide continued support to the filing and defence of patent, and knowledge management (responsible group: Germany 5)

German institutions (8) were responsible for: i) detoxifying the kernel meal and protein isolate and upscale the detoxification process, and their use in fish, shrimp and turkey diets ii) developing improved technologies for de-shelling, oil extraction, and use of oil and shells as energy source, and iii) conducting financial and economic analysis; and Chinese groups (8) were responsible for evaluating the use of the detoxified kernel meal as a substitute for soybean meal in large animal diets.

2.2 Collaborating institutions and cooperation amongst them

Following institutions took part in the project. Figure 2 shows the project management and coordination structure. An excellent collaboration and cooperation was realised throughout the project. It was possible due to: transparency of the decision making process and involvement of all the teams in the decison making; sincerety and committments of all the teams; and excellent communication between groups. The collaboration with Chinese groups which was managed through the coordinator was very effective. The private companies from Germany were responsive to the needs of the project and worked hand-in-hand with the public institutions and *vice-a versa*. Each and every group contributed tot he achievement of the project objectives.

Germany

Universität Hohenheim, Stuttgart

1. Institute for Animal Production in the Tropics and Subtropics (480b), *Prof. Dr. Klaus Becker*.
2. Institute of Agriculture Engineering (440e), *Prof. Dr. Joachim Müller*
6. Institute for Agricultural Economics and Social Sciences in the Tropics and Subtropics (490), *Prof. Dr. Thomas Berger*
7. Institut für Umwelt- und Tierhygiene sowie Tiermedizin mit Tierklinik (460), *Prof. W. Amselgrüber*
8. Institute for Animal Nutrition (450), *Prof. Markus Rodehutskot.*

3. *Phyto-Energy Consulting and Engineering,* Am Hang 2, D-21379 Scharnebeck.
4. *Dr. Otto GmbH, Zur Karthane 8, 19322 Wittenberge.*
5. *JatroSolutions GmbH*, Wollgrasweg 49, 70599 Stuttgart.

China

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2. Institute of Feed Science, College of Animal Sciences, *Prof. Dr. Yi-zhen Wang*
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7. College of Forestry and Horticulture, *Dr. Meng Ye*
8. College of Information and Engineering Technology, *Dr. Liao Nianhe*

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6. Chinese Academy of Agricultural Sciences (CAAS), Zhongguanchun Nandajie 12, 100081 Beijing, Institute of Feed Science, *Prof. Dr. Qi-yu Diao*

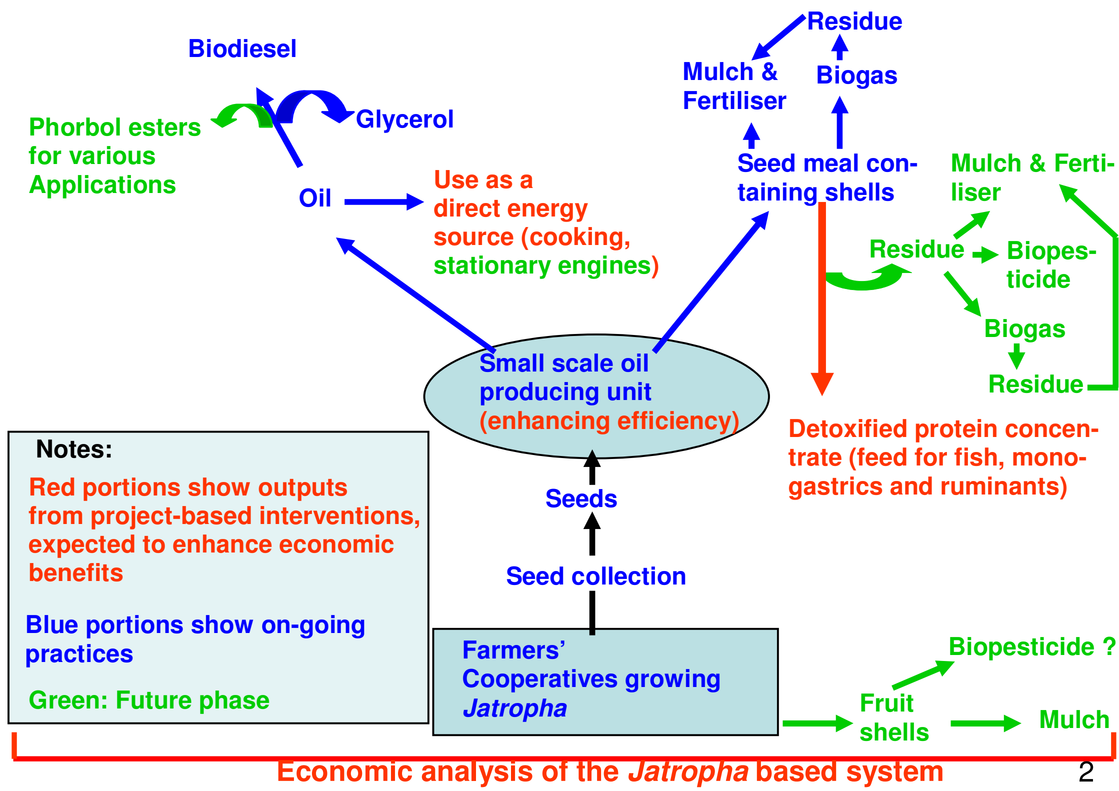
Figure 1a

Figure 1. An overview of the project activities

A diagrammatic presentation of:

- **ongoing practices (in blue font),**
- **intervention-led changes through the proposed project (in red font), and**
- **potential future activities (in green font)**

1


Small-scale oil producing unit
Figure 1b
Biodiesel
Glycerol
Phorbol esters for various Applications
Oil
Use as a direct energy source (cooking, stationary engines)
Residue
Mulch & Fertiliser
Biogas
Seed meal con-taining shells
Mulch & Ferti-liser
Residue
Biopes-ticide
Biogas
Residue
Small scale oil producing unit (enhancing efficiency)
Detoxified protein concen-trate (feed for fish, mono-gastrics and ruminants)
Notes:
Red portions show outputs from project-based interventions, expected to enhance economic benefits
Blue portions show on-going practices
Green: Future phase
Seeds
Seed collection
Farmers' Cooperatives growing Jatropha
Biopesticide ?
Fruit shells
Mulch
Economic analysis of the Jatropha based system
2

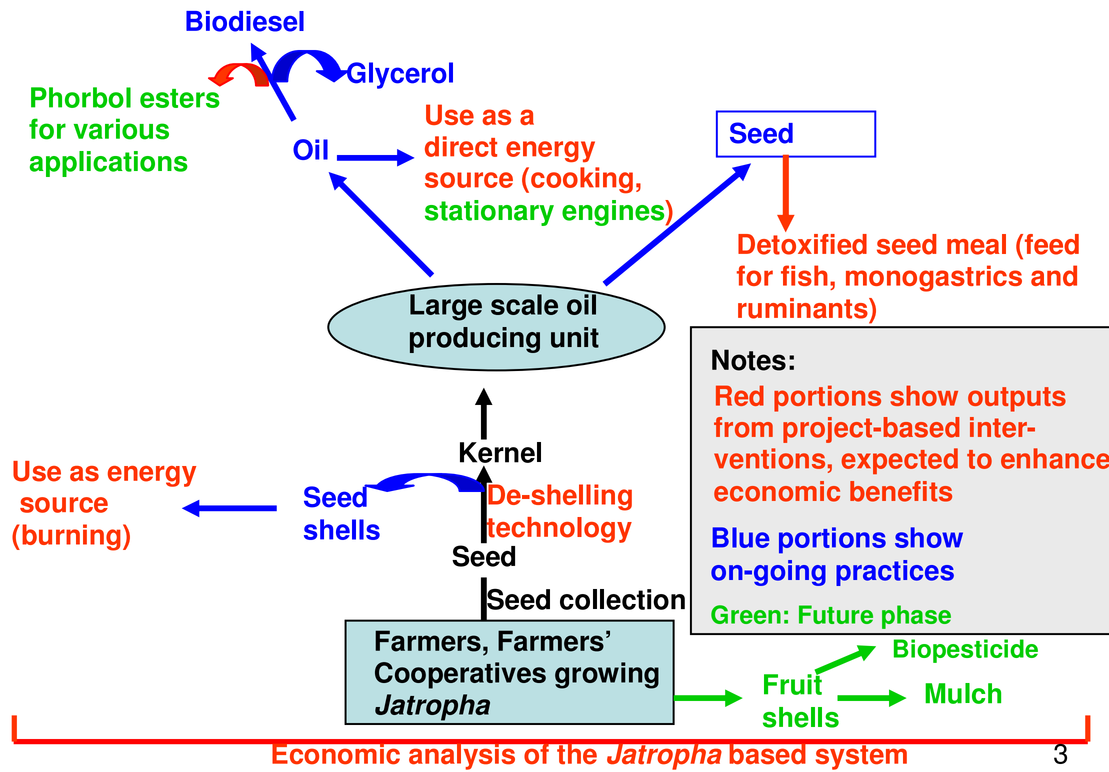
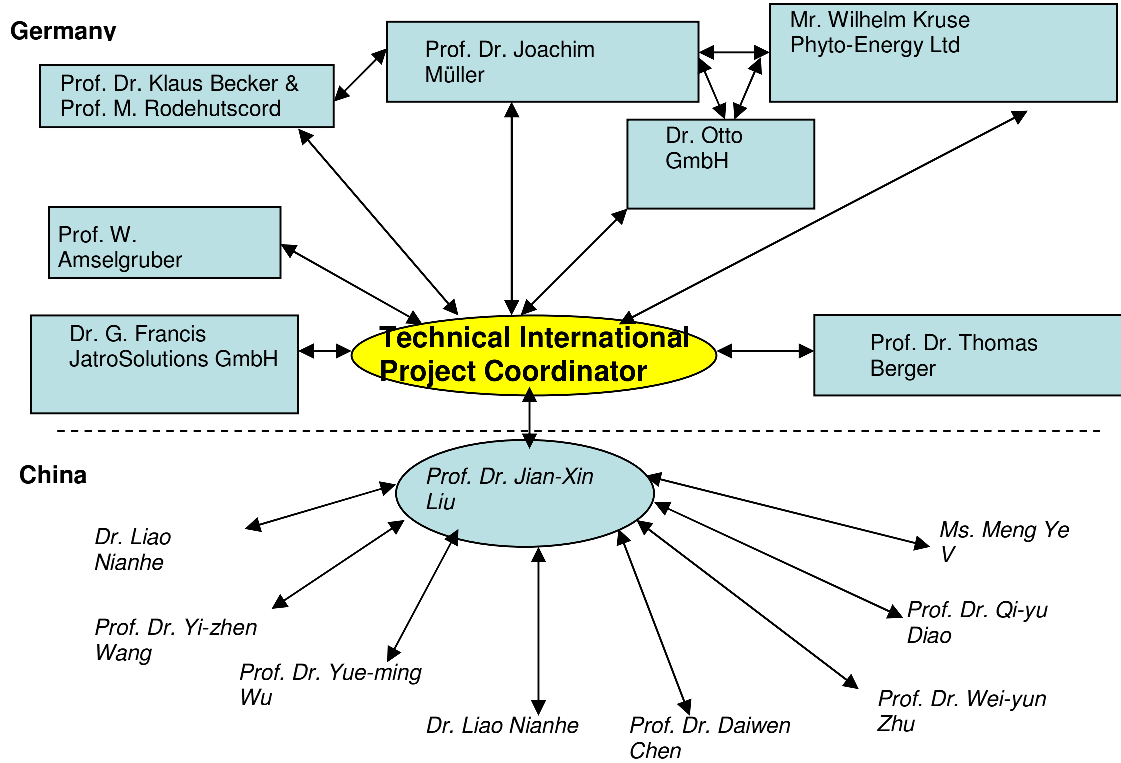

Large-scale oil producing unit
Figure 1c
Biodiesel
Glycerol
Phorbol esters for various applications
Oil
Use as a direct energy source (cooking, stationary engines)
Seed
Detoxified seed meal (feed for fish, monogastrics and ruminants)
Large scale oil producing unit
Notes:
Red portions show outputs from project-based inter-ventions, expected to enhance economic benefits
Blue portions show on-going practices
Green: Future phase
Kernel
Use as energy source (burning)
Seed shells
De-shelling technology
Seed
Seed collection
Farmers, Farmers' Cooperatives growing Jatropha
Biopesticide
Fruit shells
Mulch
Economic analysis of the Jatropha based system
3

Figure 2. Project Management and Coordination



2.3 Progress made

Information on the 'Targets Envisaged' against 'Targets Achieved', in a tabular form, is given below. All activities have been completed satisfactorily, and as per the work plan.

Targets and status of their completion
University of Hohenheim, Stuttgart

Sr. No.	Targets	Completed (Yes/No)	Remarks
1	Preparation of kernel meal (obtained from large scale oil producing units) free of toxin and antinutritional factors and its use in fish and livestock diets (responsible institutes: Inst 480b and Inst 460) Herstellung von Samenmehl (gewonnen von großen ölproduzierenden Anlagen) frei von Toxinen und Antinutritiven und dessen Nutzung in Fisch und Nutztierfutter (verantwortliche Institute: Inst. 480b und Inst. 460)	Yes	See Sections 3.1.1 (3.1.1.1 to 3.1.1.8) of Report. The process developed has been filed for EU and IPT patents. Detoxified kernel meal has been found successfully included in the diets of fish, shrimp, turkey, poultry and pigs Siehe Abschnitt 3.1.1 (3.1.1.1 to 3.1.1.8) des Berichts. Der Herstellungsprozess wurde als EU und IPT Patent eingereicht. Entgiftetes Samenmehl konnte erfolgreich in Fisch-, Schrimp-, Puten-, Geflügel- und Schweinefutter eingesetzt werden.
2	Preparation of protein concentrate, from screw pressed cake (obtained from small scale oil producing units), free of toxin and antinutritional factors and its use in fish and livestock diets (responsible institutes: Inst 480b and Inst 460) Herstellung von Proteinkonzentrat aus Samenkuchen gewonnen mit Schneckenpressen (gewonnen aus kleineren ölproduzierenden Anlagen) frei von Toxinen und Antinutritiven und dessen Nutzung in Fisch und Nutztierfutter (verantwortliche Institute: Inst. 480b und Inst. 460)	Yes	See Section 3.1.2 (3.1.2.1 to 3.1.2.3) of Report. The process developed has been filed for EU and IPT patents. Detoxified protein isolate has been found successfully included in the diets of fish and turkey Siehe Abschnitt 3.1.2 (3.1.2.1 to 3.1.2.3) des Berichts. Der Herstellungsprozess wurde als EU und IPT Patent eingereicht. Entgiftetes

			Proteinisolat konnte erfolgreich im Futter von Fischen und Puten eingesetzt werden.
3	Identification of de-shelling technology (Inst 440e and Phyto-Energy) Bestimmung einer Schältechnologie	Yes	See Section 5 of Report (not made public). The technology was not found to be entirely satisfactory. Hence it was decided to develop a de-shelling machine at University of Hohenheim (see Section 3.2 (Objective 1) of report and target for the extension phase: July 2009 to February 2010). Siehe Abschnitt 5. des Berichts. Die Technologie war nicht zufrieden stellend. Deshalb wurde entschieden, eine Schälmaschine an der Universität Hohenheim zu entwickeln. (Siehe Abschnitt 3.2 (Objective 1) des Berichts und Ziel für die Verlängerungsphase Juli 2009 bis Februar 2010).
4	Optimization of screw press technology (Inst 440e and Inst 480b) Verbesserung der Schneckenpressentechnologie (Inst. 440e und Inst. 480b)	Yes	See Section 3.2 (Objectives 1 and 2) of Report. Siehe Abschnitt 3.2 (Objectives 1 and 2) des Berichts
5	Development of a procedure for cleaning of *Jatropha* crude oil for use in plant oil stoves (Inst 440e) Entwicklung eines Verfahrens, um *Jatropha* Rohöl in Pflanzenölkochern zu verwenden.	Yes	See section 3.2 (Objectives 2.1 and 3) of Report Siehe Abschnitt 3.2 (Objectives 2.1 and 3) des Berichts
6	Development of a small scale combustion systems for using shells as fuel available (inst 440e) Entwicklung eines kleinen Verbrennungssystem zur Nutzung der Schalen als Brennmittel (Inst. 440e)	Yes	See Section 3.2 (Objective 4) of Report Siehe Abschnitt 3.2 (Objectives 4) des Berichts
7	Economic and financial analyses of *Jatropha*	Partially	See Section 3.3 of Report

	cultivation and production in China (Inst 490d) Ökonomische und finanzielle Analyse von *Jatropha*kultivierung und Produktion in China (Inst. 490d)	completed Teilweise fertig	Siehe Abschnitt 3.3 des Berichts

Targets for Dr. Otto GmbH and status of their completion

Sr. No.	Targets Ziele	Completed (Yes/No)	Remarks
1	Upscaling of laboratory scale detoxification process for kernel meal Erweiterung des Entgiftungsprozess für Samenmehl vom Labormaßstab.	Yes	A pilot plant has been constructed with a capacity of producing 6.5 kg of detoxified material per 24 h. See Section 6 of Report (not made public) Eine Pilotanlage wurde erstellt mit einer Herstellungskapazität von 6.5 kg entgiftetem material in 24 h. Siehe Abschnitt 6 des Berichts (nicht öffentlich)
2	Upscaling of laboratory scale detoxification process for protein isolate Erweiterung des Entgiftungsprozess für Proteinisolat vom Labormaßstab	Yes	A pilot plant has been constructed with a capacity of producing 6.5 kg of detoxified material per 24 h. See Section 6 of Report (not made public) Eine Pilotanlage wurde erstellt mit einer Herstellungskapazität von 6.5 kg entgiftetem material in 24 h. Siehe Abschnitt 6 des Berichts (nicht öffentlich)
3	Development of a process for mechanical separation of shells from screw pressed cake Entwicklung eines Verfahrens, um die Schalen vom Presskuchen zu trennen	Yes	The process adopted by Dr. Otto GmbH was not successful. In the extension phase (July 2009-February 2010, this task was revisited by Inst 480b and was successfully achieved. See Section 3.1.4 of Report. Das angewandte Verfahren des Dr. Otto GmbH war nicht erfolgreich. Während der Verlängerungsphase (Juli 09 – Februar 2010) wurde das

			Problem vom Inst. 480b nochmals, diese Mal mit Erfolg, behandelt. Siehe Abschnitt 3.1.4 des Berichts.

Targets for JatroSolutions GmbH and status of their completion

Sr. No.	Targets	Completed (Yes/No)	Remarks
1	Überwachung und Beurteilung der Verwendbarkeit der im Projekt entwickelten Technologien in Anbetracht auf die Nachfrage am Markt.	Yes	Done on regular basis# Wurde regelmäßig durchgeführt
2	Sorgt für eine reibungslosen Wissensaustausch zwischen den Partnern.	Yes	Done on regular basis# Wurde regelmäßig durchgeführt
3	Folgende neu entwickelte Verfahren könnten zum Patent angemeldet werden: i. Entgiftung des *Jatropha* Samenmehls ii. Optimierung des Ertrags und der Entgiftung von *Jatropha* Proteinkonzentrat iii. Anlage zur kommerziellen Entgiftung von *Jatropha* Samenmehl und Proteinkonzentrat iv. Wirtschaftlicher Prozess zur Produktion von Öl mit Brennstoffqualität zur direkten Nutzung und v. Technologie zur Verbrennung von Samenschalen in kleineren Verbrennungsmotoren	Yes	Patent filed for processes i) to iii). It was decided that the processes listed under iv) and v) should be published and not patented# Patent für die Verfahren i) bis iii) wurde eingereicht. Es wurde entschieden, die Verfahren unter iv) und v) zu publizieren und nicht zu patentieren
4	Durchführung von Training Workshops einschließlich China (in Kooperation mit dem Projektkoordinator)	Yes	Two persons from Chinese counterparts were trained on the detoxification process and on quantification of toxins# Zwei Mitarbeiter der chinesischen Partner wurden für das Entgiftungsverfahren und die Bestimmung von

			Toxinen geschult.
5	Erstellung von Richtlinien zur Futterherstellung und Entwurf von wissenschaftlichen Publikationen (in Kooperation mit dem Projektkoordinator)	Yes	Publications see Section 4 Siehe Abschnitt 4 des Berichts. Preparation of guidelines for feed production# Erstellung von Richtlinien zur Futterherstellung

See Section 7 of Report (not made public); Siehe Abschnitt 7 des Berichts (nicht öffentlich).

Targets for the University of Hohenheim partners for the period July 2009 to June 2010 and status of their completion

Arbeitsplan, Juli 2009 bis June 2010

Aktivität	Completed (Yes/No)	Remarks
1 Fütterungsversuche mit Shrimps (Verantwortlich: Inst 480b) (Use of detoxified kernel meal in shrimp diet)	Yes	See Section 3.1.1 (3.1.1.6) of Report Siehe Abschnitt 3.1.1 (3.1.1.6) des Berichts.
2. Aminosäureverdaulichkeit von entgiftetem *Jatropha*-Samenmehl in Geflügel (Hühnern) (Verantwortlich: Inst 480b and Inst 450) und Dr. Otto GmbH (Amino acid digestibility of detoxified kernel meal in poultry)	Yes	See Section 3.1.1 (3.1.1.8) of Report. The poultry experiments were done by Chinese groups, and hence the Hohenheim group decided to evaluate the amino acid digestibility in Turkey. Siehe Abschnitt 3.1.1 (3.1.1.8) des Berichts. Die Geflügelexperimente wurden den chinesischen Partnern durchgeführt. Deshalb entschied die Hohenheimer Gruppe, die Aminosäure Verdaulichkeit in Puten zu evaluieren.
3. Entwicklung einer Methode zur Bestimmung des Schalenanteils im *Jatropha* Samenkuchen mit Hilfe der NIRS Technik (Verantwortlich: Inst 480b) (Development of a method for determination of shells in screw pressed cake using NIRS)	Partially completed due to late receipt of NIRS machine	See Section 3.1.5 of Report. Further work on this is required. Siehe Abschnitt 3.1.5 des Berichts (mehr Arbeit getan werden sollte).
4. Proteinkonzentratentgiftung mittels Adsorbern (Verantwortlich: Inst 480b) (Detoxification of protein concentrate using adsorbents)	Yes	See Section 3.1.2.5 of Report Siehe Abschnitt 3.1.2.5 des Berichts.
5. Evaluierung von verschiedenen, bei der Produktion von Biodiesel durch Veresterung anfallenden Beiprodukten als Tiernahrung (Verantwortlich: Inst 480b)	Yes	See Section…3.1.1 (3.1.1.8) of Report Siehe Abschnitt 3.1.1 (3.1.1.8) des Berichts.

(Evaluation of various byproducts accrued through the production of *Jatropha* biodiesel as livestock diet)		
6. Trennung der Schalen vom Samenkuchen durch Sieben (Verantwortlich: Inst 480b) (Separation of shells from screw pressed cake using sieving)	Yes	See Section 3.1.3 of Report Siehe Abschnitt 3.1.3 des Berichts.
7. Schraubpressen basierte Ölextraktion von Kernen mit verschiedenen Schalenanteilen (Verantwortlich: Inst 440e and Inst 480b) (Screw pressed based extraction of *Jatropha* oil from kernels with different proportions shells)	Yes	See Section 3.2 (Objectives 2) of Report Siehe Abschnitt 3.2 (Objective 2) des Berichts.
8. Reinigung von *Jatropha*öl mittels eines kontinuierlichen Prozesses und Erstellung eines Standards für *Jatropha*öl zur Nutzung in Pflanzenölkochern. (Verantwortlich: Inst 440e) Purification of *Jatropha* oil through continued processes and development of a standard to use *Jatropha* oil in plant oil stoves. (Responsible: Inst. 440e)	Yes	See Section 3.2 (Objective 3) of Report Siehe Abschnitt 3.2 (Objective 3) des Berichts.
9. Entwicklung einer *Jatropha*samen-Schälmaschine. (Verantwortlich: Inst 440e von UH) Development of a *Jatropha* kernel de-shelling unit (responsible Inst. 440e)	Yes	See Section 3.2 (Objective 1) of Report Siehe Abschnitt 3.2 (Objective 1) des Berichts.
10. Feinabstimmungen beim Entgiftungsprozess von Samenmehl und Patent Bearbeitung. (Verantwortlich: Dr. Otto GmbH, Inst 480b und JatroSolutions GmbH) Fine tuning of detoxification process for kernel meal and patent processing (responsible: Dr. Otto GmbH, Inst. 480b and JatroSolutions GmbH)	Partially completed (constraint: lack fo time)	See Section 6 of Report (not made public) Siehe Abschnitt 6 des Berichts.
11. Bewertung des Vorhandenseins von Phorbolestern im Abgas eines Pflanzenölkochers. (Verantwortlich: Inst 440e und Inst 480b)	Yes	See Section 3.2 (Objective 3.1) of Report. In addition to stove exhaust, phorbol esters were

(Quantification of phorbol esters in the stove exhaust)		also determined in the exhaust of a stationary engine (See Section 7 of Report; not made public) Siehe Abschnitt 3.2 (Objective 3.1) und Abschnitt 7 des Berichts.
12. Optimierung des Upscale-Prozesses für die Produktion und Entgiftung von Proteinkonzentrat. (Verantwortlich: Dr. Otto GmbH und Inst 480b) Optimization of the upscaled process for the production of detoxified protein concentrate)	Yes	See Section 6 of Report (not made public). Siehe Abschnitt 6 des Berichts (nicht öffentlich).
13. Beschreibung der Hemmnisse für die Ausdehnung der *Jatropha*-Technologie (Verantwortlich: Inst 490d) Description of constraints for expansion of the *Jatropha* technology (responsible: Inst. 490d)	Yes	See Section 3.3 of Report Siehe Abschnitt 3.3 des Berichts.
14. Kontinuierliche Unterstützung in Patent Angelegenheiten, Wissenstransfer und Management der deutschen und chinesischen Projektpartnern. (Verantwortlich: Jatrosolutions GmbH) (Provide continued support to the filing and defense of patent, and knowledge management)	Yes	See Section 7 of Report (not made public) Siehe Abschnitt 7 des Berichts (nicht öffentlich)..

2.4. Grants obtained for manpower and objectives achieved

1. Salaray for Project Coordinator for the entire period was obtained through the project. For such a large project in which many public and private institutions participated, it was necessary to have a Project Coordinator. The Project Coordinator submitted the project reports in time, coordinated writing of publications, patent filing and activities with Chinese teams, and managed completion of all project activities on time. He was also responsible for a major component of technology development: detoxification of kernel meal and protein isolate and their utilization as livestock feed.
2. One scientist position was used for economic and financial analysis. The scientist employed on this position managed data collection work in China in collaboration with the Chinese groups and was also responsible for data analysis. The study was completed satisfactorly.
3. Four PhD students were employed under this project. Two worked on detoxification of kernel meal and protein isolate and their utilization as animal feed and on the utilization of other value added products such as phorbol esters and byproducts of biofuel industry. The other two worked on development of efficient de-shelling technology; optimization of screw-pressing technology for extraction of oil and production of seed meal; development of technology for cleaning of *Jatropha* crude oil in order to reach the developed standard for use in plant oil stoves; and development of small scale combustion systems (furnace) for using shells as energy source. These tasks have also been completed.
4. Three technicians were also recruited through the project. Two technicians contributed to the analysis of samples obtained from the work on detoxification of kernel meal and protein isolate and optimization of oil extraction and its use as energy sources; and the thirds one on histopathological analysis of biological samples ontained from the studies. Their contribution towards completion of objectives was vital.

The activities could not have been completed without having the above human resources.

2.5 Processes and technologies developed through the project and their impact

EU and PCT patent applications for three technologies: detoxification of *Jatropha* kernel meal, detoxification of *Jatropha* protein isolate, and preparation and detoxification of *Jatropha* protein

isolate (one-step method) have been favourable evaluated by the patent offices. These will be made public in August 2010. A number of feed manufacturing and engineering companies have contacted us for buying the rights.

The guidelines developed for using the detoxified kernel meal and protein isolate will find place in the feed industry.

A technology has also been developed to isolate kernel meal from screw pressed *Jatropha* cake containing shells. This technology is based on a physical principle of sieving and separates undigested shells from high quality protein.

A processes has also been established to clean *Jatropha* oil for using in cooking stoves and stationary engines. A de-shelling machine for obtaining kernels from *Jatropha* seeds has been developed. In addition, potential of shells for use as energy source evaluated and a prototype of furnace has been developed.

Through these technologies, small, medium and large scale industries would benefit through production of efficient seed de-shelling, seed pressing and oil extracting units for production of oil for use as biodiesel, and through construction of detoxification plants and their accessories. On the other hand, both small and large scale oil producing industries would generate higher profit through production of value-added products, detoxified kernel meal and protein isolate for use in livestock feed, and seed shells for use as a source of energy.

Other associated positive effects are the availability of CO_2 neutral fuel in remote rural areas, wasteland reclamation for food production, furthering development of an alternative high value agricultural crop, employment generation, reduced atmospheric pollution, and carbon sequestration for emission trading.

This project has also opened avenues for use of phorbol esters isolated from *Jatropha* oil (before its use as biodiesel) for use as bio-pesticide, -insecticides and –molluscicide.

(*The findings of this project have global implications since Jatropha plantations have been initiated at a large scale in a number of countries, to name a few, India, Madagascar, Myanmar, Indonesia, Egypt, Zimbabwe, Tanzania; and this would put Germany in a position of comparative advantage.)*

3 Work accomplished and Results obtained

3.1 University of Hohenheim, Stuttgart (Biology Group: Inst 480b, Inst 460 and Inst 450; Germany 1, 7 and 8 respectively)

Contents

3.1.1 Preparation of kernel meal free of toxin and antinutritional factors and its use in fish and livestock diets

3.1.1.1 Detoxified *Jatropha curcas* kernel meal as a dietary protein source: Growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings

3.1.1.2 Utilization of a byproduct from *Jatropha* biodiesel industry as a fish meal replacer in common carp *Cyprinus carpio* L. diet

3.1.1.3 Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio* L.) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal

3.1.1.4 Dietary inclusion of detoxified *Jatropha curcas* kernel meal: Effects on growth performance and metabolic efficiency in common carp, *Cyprinus carpio* L.

3.1.1.5 Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus mykiss*) juveniles fed detoxified *Jatropha curcas* kernel meal

3.1.1.6 Substitution of fish meal by *Jatropha curcas* kernel meal: Effects on growth performance and body composition of white leg shrimp (*Penaeus vannamei*)

3.1.1.7 Effects of replacing soybean meal by detoxified *Jatropha curcas* kernel meal in the diet of growing pigs on their growth, serum biochemical parameters and visceral organs

3.1.1.8 Amino acid digestibility of detoxified *Jatropha curcas* L. kernel meal and protein isolate in turkeys

3.1.2 Preparation of protein isolate free of toxin and antinutritional factors and its use in fish and livestock diets

3.1.2.1 Evaluations of the nutritional value of *Jatropha curcas* protein isolate in common carp (*Cyprinus carpio* L.)

3.1.2.2. Comparative nutritional evaluation of *Jatropha curcas* protein isolate and soy protein isolate in common carp (*cyprinus carpio* l.) fingerlings (in this study protein isolate prepared by 'One-Step Method' was used)

3.1.2.3 Detoxification of protein isolate using adsorbents

3.1.3 Challenges and opportunities for using byproducts from the production of biodiesel from *Jatropha* oil as livestock feed

3.1.4 Separation of shells from screw pressed cake using sieving

3.1.5 Development of a method for determination of shells in screw pressed cake using NIRS

3.1.6 Additional related side work that emanated from the project (only title and summary of the work are presented here

3.1.6.1 *Jatropha platyphylla*, a new non-toxic *Jatropha* species: Physical properties and chemical constituents including toxic and antinutritional factors of seeds

3.1.6.2 Dietary inclusion of *Jatropha platyphilla* kernel meal in the diet of Nile tilapia *(Oreochromis niloticus L.):* growth, metabolic, nutritional and haematological

3.1.6.3 Are *Jatropha curcas* phorbol esters degraded by rumenmicrobes?

3.1.6.4 Nutritional, Biochemical, and Pharmaceutical Potential of Proteins and Peptides from *Jatropha*: Review

3.1.6.5 Optimization of conditions for the extraction of phorbol esters

from *Jatropha* oil

3.1.6.6 Quality of Biodiesel Prepared from Phorbol Ester Extracted

3.1.6.7 Toxicity of *Jatropha curcas* phorbol esters in mice

3.1.6.8 Fate of *Jatropha Curcas* phorbol esters in soil

3.1.1 Preparation of kernel meal free of toxin and antinutritional factors and its use in fish and livestock diets (main responsible institute: Germany 1)

A method for detoxification of *Jatropha* kernel meal has been developed and filed for EC and PCT patent (PCT/EP2010/051779). It will be open to public in August 2010. The method has also been upscaled to a pilot plant stage.

3.1.1.1 Detoxified *Jatropha curcas* kernel meal as a dietary protein source: Growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings

Introduction

Ongoing intensification of aquaculture in tropical countries has made it essential to develop suitable diets for carp using alternative protein sources. Traditionally, fish meal (FM) has been the main source of dietary protein for fish. In recent years, its increasing cost, decreasing availability in the market and poor quality have stimulated several studies on its partial or complete substitution with alternative protein sources (Kaushik *et al.* 1995; Fournier *et al.* 2004). Since, FM is a limited primary source and plants are widely available and reasonably priced, the use of plant protein sources in aqua feeds should be considered (SOFIA 2007). Our previous studies demonstrate that plant protein (*Moringa oleifera* leaf meal and *Sesbania aculeate* seed meal) could partially replace FM in the diet of tilapia, *Oreochromis niloticus* and common carp, *Cyprinus carpio* (Hossain *et al.* 2001; Richter *et al.* 2003; Dongmeza *et al.* 2006).

Soybean meal (SBM) is one of the most nutritious of all plant protein sources (Lovell 1988). Soybeans are the leading oilseed crop produced globally, and its production for 2004–2005 was around 200 mmt (Gatlin *et al.* 2007). Because of its high protein content, high digestibility, relatively well-balanced amino acid profile, reasonable price, and steady supply, SBM is widely used as a cost-effective feed ingredient for many aquaculture animals (Storebakken *et al.* 2000). It is currently the most commonly used plant protein source in fish feeds (El-Sayed 1999). However, SBM competes with human food and hence there is a need to identify other protein rich plant resources that could be used in fish diets.

Jatropha curcas (physic nut) is a hardy plant, thrives on degraded land and requires limited amounts of nutrients and water. Its seeds have been extensively investigated as a source

of oil. The seed kernel contains about 60% oil that can be converted into biodiesel upon transesterification and used as a substitute for diesel fuel (Makkar *et al.* 2007b). The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 58 to 62 % crude protein (Makkar *et al.* 2008; Makkar & Becker 2009). The levels of essential amino acids (except lysine) are higher in *Jatropha* kernel meal than in the FAO reference protein for a growing child of 3–5 years (Makkar & Becker 1999). However the presence of high levels of antinutrients like trypsin inhibitor, lectin and phytate (Makkar *et al.* 2008) and the major toxic components phorbol esters (PE_S) (Makkar & Becker 1997) restrict their use in fish feed.

Heat labile antinutrients, like protease inhibitors, and lectins are easy to inactivate by moist heating. A method for detoxification of *Jatropha* kernel meal has been developed in our laboratory. It is based on removal of PEs and inactivation of trypsin inhibitors and lectin by heat treatment. The aim of this experiment was to evaluate the nutritional value of detoxified *Jatropha* kernel meal (DJKM) in common carp, and compare it with that of SBM and FM.

Material and methods

Preparation of the Jatropha kernel meal

Jatropha seeds were obtained from India and deshelled manually to obtain kernels. Defatting of *Jatropha* kernals was done using petroleum benzene (b.p. 40–60 ^{0}C) in a Soxhlet apparatus. Organic solvents were used to detoxify defatted *Jatropha* kernel meal. Two duration of PE removal were investigated: shorter (30 min) and longer (60 min) and the detoxified meals so obtained were designated as J_a and J_b respectively (patent application has been filed for the process of detoxification). After removal of PEs, the meal was autoclaved (121 ^{0}C) to remove heat labile antinutrients like trypsin inhibitor and lectin.

Diet formulation

FM (Seelöwe fishmeal) was procured from Vereinigte Fishmeh werke Cuxhaven GmbH & Co KG, Cuxhaven, Germany; and wheat meal was purchased from a local market. SBM (dehulled,

defatted and roasted) was obtained from Institute of Animal Nutrition (450), University of Hohenheim, Germany. Soya protein isolate (SUPRO® 500E IP) was purchased from Solae Europe S.A., 2, Chemin du Pavillon, CH-1218 Le Grand-Saconnex, Geneva, Switzerland. Source of sunflower oil was Thomy, Nestle Deutchland AG, 41415 Neuss; Reg. Trademark of Societe des, Produits Nestle S.A.. Vitamin premix and mineral premix were procured from Altromin Spezialfutter GmbH & Co. KG, Lage, Germany. Source of lysine is Merck KGaA, 64271 Darmstadt, Germany.

Prior to feed formulation, the proximate composition of defatted *Jatropha* meal, wheat meal, SBM, soya protein isolates and FM was determined. A total of seven isonitrogenous and isoenergetic diets were formulated. Experimental diets containing crude protein 38%, crude lipid 8%, vitamin premix 2%, mineral premix 2% and titanium oxide (TiO_2) 1% were prepared. Lysine monohydrochloride (lysine 80% in this salt) was supplemented at the rate of 1% of DJKM inclusion in the diet. Each experimental feed, except the control, contained 500 FTU phytase (NATUPHOS 5000G, BASF, Ludwigshafen) per kg. The inclusion levels of the DJKM and SBM were as follows:

Control diet was prepared with FM and wheat meal, without any DJKM and SBM. The plant protein fed groups were: S_{50}: 50% of FM protein replaced by SBM; S_{75}: 75% of FM protein replaced by SBM; J_{a50}: 50% of FM protein replaced by DJ_aKM; J_{a75}: 75% of FM protein replaced by DJ_aKM; J_{b50}: 50% of FM protein replaced by DJ_bKM; J_{b75}: 75% of FM protein replaced by DJ_bKM. The final mixture of each diet was made into 2 mm diameter moist pellets (using a Bosch, Type UM60ST 2-M, Robert Bosch Hausgerät GmbH, 70839 Gerlingen, Germany) and then freeze-dried (Table 1).

Table 1 Composition of the experimental diets (g kg^{-1} feed)

Ingredients	Experimental diets						
	Control	J_{a50}	J_{a75}	J_{b50}	J_{b75}	S_{50}	S_{75}
Fish meal	507.5	253.7	126.3	253.7	126.3	253.7	126.3
Soyabean meal	-	-	-	-	-	342.1	513
[1]Wheat meal	402.5	381.5	372	390	384.1	271	206
Jatropha meal	-	249.5	372	242.5	361.9	-	-
Soya concentrate	-	3.5	7	2	5	22	32
Sunflower oil	40	61.8	72.7	61.8	72.7	61.2	72.7
[2]Vitamin premix	20	20	20	20	20	20	20
[3]Mineral premix	20	20	20	20	20	20	20
TiO_2	10	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000	1000
Phytase (FTU/kg)	-	500	500	500	500	500	500
Lysine monohydrochloride (g)	-	2.5	3.7	2.4	3.6	-	-

Control: FM and wheat meal, without any DJKM and SBM
J_{a50}: 50% of FM protein replaced by DJaKM
J_{a75}: 75% of FM protein replaced by DJaKM
J_{b50}: 50% of FM protein replaced by DJbKM
J_{b75}: 75% of FM protein replaced by DJbKM
S_{50}: 50% of FM protein replaced by SBM
S_{75}: 75% of FM protein replaced by SBM
[1]Whole wheat meal.
[2]Vitamin premix (g or IU kg-1 premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
[3]Mineral premix (g kg-1): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67; $CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

Experimental system and animals

Common carp (*Cyprinus carpio* L.) fingerlings (about 2.0 – 3.0 g) from the Institute of Fisheries Ecology and Aquaculture of the Federal Research Center for Fisheries at Ahensburg, Germany were transferred to the University of Hohenheim, Stuttgart, Germany and kept in two 500 l capacity tanks for acclimatisation. They were fed the Hohenheim standard fish diet containing

approximately 38% protein, 8% lipid, 10% ash and with a gross energy content of 18.5 kJ g^{-1} dry matter. After an acclimatisation period of 20 days, 252 fish were randomly distributed into seven groups with four replicates; each replicate contained nine fish (av. wt. 3.2 ± 0.07 g) in an aquarium (45 l capacity). All the aquaria were supplied with water from a recirculatory system. The system was subjected to a photoperiod of 12 h light : 12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 26.2 – 27.1°C, pH 7.0 – 7.5, dissolved oxygen 6.9 – 7.4 mg l^{-1}, total NH_3 0.1– 0.2 mg l^{-1}, nitrite 0.07 – 0.1 mg l^{-1} and nitrate 1–3 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation above 80%. One day before start of the experiment, the fish were starved and during the experimental period fish were fed at 16 g feed per kg metabolic body mass ($kg^{0.8}$) per day (equal to five times their maintenance energy requirement). In a preliminary study, feed at seven times maintenance energy requirement was offered to fish. This resulted in substantial presence of uneaten feed in the aquaria. However, no feed was left in the aquaria when feeds at five times maintenance energy requirement were offered. Since the aim of the study was to evaluate the performance of fish fed a diets containing detoxified *Jatropha* kernel meal, high level of feed consumption was preferred, in order to elicit adverse effects if any due to the presence of the detoxified kernel meal.

Total feed per day was split into five equal portions and each portion was given at 8:00, 10:30, 13:00, 15:30 and 18:00 h. The feeds were dispensed using an automatic feeder. Fish were weighed individually at the beginning of the experiment (av. wt. 3.2 ± 0.07 g) and at weekly intervals during the experimental period to adjust the feeding level for the subsequent week. The fish were not fed on the weighing day. During last 2 weeks of the experiment, fish were fed with a diet containing a marker (TiO_2) for digestibility measurement (Mamun *et al.* 2007 and Dongmeza *et al.* 2009). The faeces collection was qualitative, as the experimental diets contained an inert marker (TiO_2). During last two weeks of the experiment, faeces were collected daily. After each feeding the aquaria were controlled for remaining feed; generally, there were no feed residues left. Every day prior to the faeces collection, aquaria were siphoned out to clean any residues. Faeces subsequently excreted by the fish were collected in separate beakers for each aquarium by siphoning with a short small pipe (Mamun *et al.* (2007). The collected mixture of water and faeces was centrifuged at 4000 × g for 10 min, the supernatant discarded and the faeces

were then stored at -20°C until analysis. For the analysis, faeces from all the experimental periods from the same fish were pooled.

At start of the experiment, 18 fish of the same population were also killed and preserved at −20 °C for analysis of the initial body composition.

The experiment was terminated after 8 weeks and the fish were killed. Two fish per replicate were carefully dissected to obtain the intestine and stored in liquid nitrogen for determination of digestive enzymes activities. Two fish per replicate were stored at −20 ^{o}C for chemical composition analysis. Prior to the determination of the proximate composition, the fish were autoclaved at 121°C for 20 min, thoroughly homogenised using an Ultra-Turrax T25, frozen overnight and freeze-dried.

Extraction and estimation of phorbol esters (PEs) by high-performance liquid chromatography, and determination of antinutrients

PEs were determined according to Makkar *et al.* (2007a), which was based on the method of Makkar *et al.* (1997). Briefly, 0.5 g of the *Jatropha* meal and dried whole body fish sample were extracted four times with methanol. A suitable aliquot was loaded on a high-performance liquid chromatography (HPLC) reverse-phase C_{18} LiChrospher 100, 5 µm (250 x 4 mm I.D. from Merck (Darmstadt, Germany) column. The column was protected with a head column containing the same material. The separation was performed at room temperature (23 ^{o}C) and the flow rate was 1.3 ml/min using a gradient elution (Makkar et al., 2007a). The four-PEs peaks were detected at 280 nm and appeared between 25.5 and 30.5 min. The results were expressed as equivalent to a standard, phorbol-12-myristate 13-acetate. Detection limit of PEs is 3µg/g meal.

Trypsin inhibitor activity was determined essentially according to Smith *et al.* (1980) except that the enzyme was added last as suggested by Liu & Markakis (1989). Analysis of the lectin content was conducted by haemagglutination assay (Makkar et al., 1997). The haemagglutination activity was expressed as the minimum amount of material (mg mL^{-1} assay medium) that produced agglutination. The minimum amount was the amount of material mL^{-1} assay medium in the highest dilution that was positive for agglutination; the lower this value, the higher the lectin activity. The phytic acid content of samples was determined by a

spectrophotometric procedure (Vaintraub & Lapteva 1988). Results were expressed as g phytic acid per 100 g material using phytic acid sodium salt (Sigma, St Louis, MO, USA) as standard. Non-starch polysaccharides were estimated according to Englyst *et al.* (1994).

Amino acid analysis

The amino acid compositions of FM, DJKM, SBM, soya protein concentrate and wheat meal were determined using an automated amino acid analyser after hydrolysing the samples with 6 M HCl at 110 ^{0}C for 24 h (Bassler & Buchholz 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The tryptophan contents of the above-mentioned samples were quantified spectrophotometrically by the method of Pinter-Szakacs & Molnar-Perl (1990).

Biochemical analysis

The proximate composition of diet ingredients, diets and whole body of fish was determined using the standard methods of the Association of Official Analytical Chemists (AOAC 1990). Samples of animal origin (fish bodies and FM) were analysed for dry matter (DM), ash, crude protein (CP) and lipid (ether soluble lipid). Gross energy (GE) of diet ingredients, diets and fish bodies was determined with bomb calorimeter (IKA C7000) using benzoic acid as a standard.

Growth parameters

Growth performance and diet nutrient utilisation were assessed in terms of body mass gain (BMG), specific growth rate (SGR), metabolic growth rate (MGR), feed gain ratio (FGR), protein efficiency ratio (PER), protein productive value (PPV), lipid production value (LPV) and energy production value (EPV). These were calculated as follows:

BMG (%) = [(Final body mass - initial body mass) / Initial body mass] X 100; SGR = [(ln final body mass in g) - ln initial body mass in g) / number of trial days] X 100; MGR = (Body mass gain in g) / [{(initial body mass in g / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)^$^{0.8}$}/2] / number of trial days (Dabrowski et al., 1986); FGR = dry feed fed (g)/body mass gain (g); PER = fresh

body mass gain (g)/crude protein fed (g); PPV (%) = [(final fish body protein in g - initial fish body protein in g) / total protein consumed in g] X 100; LPV (%) = [(final fish body lipid in g - initial fish body lipid in g) / total crude lipid consumed in g] X 100; EPV (%) = [(final fish body energy - initial fish body energy)/(gross energy intake)] X 100.

Digestibility measurement and Fractional feed efficiency

Titanium dioxide in the feed and faeces was determined according to the method described by Richter *et al.* (2003). The percentage of apparent dry matter digestibility of diets was calculated according to Maynard *et al.* (1981).

Apparent dry matter digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces)}] X 100

Apparent digestibility coefficients (ADCs) of nutrients and energy of the experimental diets were calculated according to Maynard & Loosli (1969).

The nutrient and energy digestibility were obtained using the following formula:

Apparent nutrient digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces) X (% Nutrient or energy in faeces) / (% Nutrient or energy in feed)}] X 100

ADC of the test ingredients ADC were calculated based on the digestibility of the reference diet and the test diets using a equation used by Bureau *et al.* (1999):

$ADC_I = ADC_T + ((1\text{-}s)\ D_R/sD_I)\ (ADC_T - ADC_R)$

where: ADC_I = Apparent digestibility coefficient of test ingredient; ADC_T = Apparent digestibility coefficient of test diet; ADC_R = Apparent digestibility coefficient of the reference diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = % nutrient (or kJ/g gross energy) of the test ingredient; s = Proportion of test ingredient in test diet mash.

Fractional feed efficiency of nutrients and gross energy = (Nutrient and energy retained in the whole body/Digestible nutrient and gross energy) x 100

Digestible nutrients and energy = Total intake of nutrients and gross energy through feed X digestibility coefficient.

Relative intestinal length (RIL), hepatosomatic index (HSI), and intestinal somatic index (ISI)

RIL was measured and is expressed in relation to each animal weight expressed in mm g^{-1}.

RIL, HSI, and ISI are calculated as indicated below:

RIL = Intestine length (mm)/ body mass (g)

HSI = Liver mass (g) X 100 / body mass (g)

ISI = Intestine mass (g) X 100 / body mass (g)

Digestive enzymes assay

The reducing sugars produced due to the action of glucoamylase and α- amylase on carbohydrate was estimated using dinitro-salicylic-acid (DNS) method (Rick & Stegbauer 1974). Amylase activity was expressed as mmole of maltose released from starch per min at 37 ^{0}C. Protease activity was determined by the casein digestion method of Drapeau (1974). One unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to $\Delta 0.001A_{280}$ per minute at 37 ^{0}C and pH 7.8. Lipase activity was assayed by the method of Cherry & Crandell (1932). One unit of enzyme was the hydrolysis of 1.0 microequivalent of fatty acid from a triglyceride in 24 h at pH 7.7 at 37^{0}C.

Statistical analysis

All data were subjected to a one-way analysis of variance ANOVA and the significance of the differences between means was tested using Duncan's multiple range test ($P<0.05$). The software used was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). Values are expressed as means ± standard deviation.

Results

Phorbol esters and antinutrients content in defatted Jatropha kernel meal and whole body fish

PE content in untreated defatted *Jatropha* kernel meal was 1.8 mg/g. However, PEs in detoxified *Jatropha* kernel meal (J_a and J_b) and dried whole body fish were undetectable. Trypsin inhibitor

was not detected in autoclaved DJKM whereas phyate level in J_a and J_b were 8.9% and 9.1% respectively.

Fish behaviour and feed intake

Based on the visual observation during feeding time, palatability or acceptability of feed was good and the behaviour of fish was normal. There was no mortality during the entire experimental period.

It was particularly noted that the fish in the groups J_{a75} fed very slowly on their feed. Sometimes they ingested the feed pellets and spit them out after a few seconds and it was repeated 2 to 3 times before finally ingesting and swallowing the pellets, whereas fish in all other dietary groups fed actively on the experimental diets throughout the experiment.

Amino acid profile of experimental diets, proximate composition of experimental diets and whole body of fish

Proximate composition, antinutrients and amino acid compositions of feed ingredients are shown in Table 2. Amino acid compositions of experimental diets are shown in Table 3. All experimental diets have almost similar amino acid composition. The proximate composition of the different experimental diets (% dry matter) and whole body tissue (wet basis %) are presented in Tables 4 and 5 respectively. Diets contained about 38% crude protein and 18.5 kJ/g gross energy and were isonitrogenous and isoenergetic. Dry matter, crude lipid and ash were in the range of 94.4–96.1%, 8.3–8.8% and 10.3–11.1% respectively. There was no significant difference ($P > 0.05$) in moisture content, crude protein and ash of the whole body among the groups. Highest crude lipid deposition was observed in J_{b75} group, which is not statistically different ($P > 0.05$) to all plant protein fed groups, except J_{a75} group, wherein lowest lipid deposition was observed.

Table 2 Proximate composition, antinutrients content and amino acid composition of feed ingredients

	Fish meal	*Jatropha* meal (J_a)	*Jatropha* meal (J_b)	Soyabean meal	Soya protein concentrate	Wheat meal
Proximate composition (g kg $^{-1}$)						
Dry matter	940	941	945	955	940	941
Crude protein	635	646	665	471	900	143
Crude lipid	88	13.2	11.4	11.7	10	16.3
Crude ash	142	125	137	21.4	4.0	1.4
Crude fibre		89	91	38	10	25
Gross energy (KJ/g)	21.1	18.5	18.3	18.2	-	18.7
Antinutrients						
Trypsin inhibitor (mg trypsin inhibited per g sample)	ND	ND	ND	ND	ND	-
Lectin[a]	ND	ND	ND	ND	ND	
Phytate (% dry matter)	-	9.5	9.3	2.41	-	-
Amino acids composition (g kg $^{-1}$)						
Asparginine	60.5	66.1	68.7	66.6	122.8	7.2
Threonine	23	20.3	22	17.8	31.1	3.7
Serine	25.5	27.3	30.6	24.4	46	6.3
Glutamine	79.4	99.4	112.1	93.8	174.9	44.9
Glycine	59.8	27.6	31.5	21.3	37.2	5.6
Alanine	43.3	27	29.4	21.4	40.9	4.6
Cystine	4.3	2.2	2.3	6.5	9.8	2.9
Valine	29.3	28.5	31.6	21.2	37.4	5.1
Methionine	16	10.2	10.6	6.2	12.1	2

Iso leucine	22.8	24.3	26.7	19.6	36.5	4.2
Leucine	41.6	44.4	46.7	35.7	68.1	9.1
Tyrosine	14.8	15.5	18.8	15.8	31	3.3
Phenylalanine	21.8	27	30.4	24.3	43.2	6.5
Histidine	17.7	19.6	21.7	14.4	24.4	3.4
Lysine	40.9	19.7	23.3	29.1	52.1	3.3
Arginine	35.3	67.4	69.7	36	67.9	5.4
Proline	36.9	30.5	32.2	28.2	50.2	14.5
Tryptophan	4.9	7	7.1	6.4	10.4	1.4
Non-starch polysaccharides (NSP) (g kg $^{-1}$)						
Rhamnose	-	-	3	0	-	-
Fucose	-	-	1	0	-	-
arabinose	-	-	31	24	-	-
Xylose	-	-	20	11	-	-
Mannose	-	-	5	6	-	-
Galactose	-	-	14	42	-	-
Glucose	-	-	57	32	-	-
Glucuronic acid	-	-	0	0	-	-
Galacturonic acid	-	-	30	24	-	-
Total-NSP	-	-	160	140	-	-

J_a and J_b: Detoxified *Jatropha* kernel meal obtained from shorter (30 min) and longer (60 min) duration of detoxification process respectively;

ND: Not detected

[a:]Minimum amount of material (mg mL^{-1} assay medium) that produced agglutination.

Table 3 Amino acid composition of the experimental diets (g kg^{-1} feed)

Amino acids	Control	J_{a50}	J_{a75}	J_{b50}	J_{b75}	S_{50}	S_{75}
Essential							
Arginine	20.09	28.07	32.02	28.10	32.10	24.23	26.21
Histidine	10.35	10.76	10.96	11.13	11.52	10.87	11.10
Iso leucine	13.26	13.58	13.74	13.97	14.34	14.43	14.97
Leucine	24.77	25.34	25.63	25.56	25.99	26.73	27.62
Lysine	22.09	19.23	17.79	19.82	18.73	22.37	22.44
Phenylalanine	13.68	14.90	15.52	15.52	16.47	16.56	17.94
Methionine	8.93	7.41	6.64	7.43	6.69	6.99	6.00
Threonine	13.16	12.42	12.05	12.68	12.44	13.61	13.79
Tryptophan	3.05	3.56	3.82	3.53	3.78	4.04	4.52
Valine	16.92	16.62	16.46	17.16	17.28	16.89	16.82
Non essential							
Alanine	23.83	19.62	17.51	19.99	18.08	20.45	18.70
Asparginine	33.60	35.02	35.77	35.06	35.88	42.79	47.22
Cystine	3.35	2.78	2.51	2.80	2.54	4.32	4.79
Glycine	32.60	24.32	20.16	25.07	21.29	24.79	20.82
Glutamine	58.37	62.69	64.93	65.19	68.72	68.25	72.99
Proline	24.56	22.68	21.75	22.93	22.13	24.04	23.72
Serine	15.48	15.85	16.04	16.44	16.94	17.54	18.51
Tyrosine	8.84	8.99	9.08	9.66	10.10	10.74	11.65

Amino acid compositions of the experimental diets were calculated from amino acid profile of individual feed ingredients

See footnotes to Table 1.

Table 4 Proximate compositions of experimental diets (g kg^{-1} dry matter basis)

Treatment*	Dry matter	Crude protein	Crude lipid	Gross energy (kJ/g)	Ash
Control	948	385	87	184	105
J_{a50}	948	384	86	186	108
J_{a75}	954	385	86	185	111
J_{b50}	961	381	88	182	102
J_{b75}	949	382	87	184	100
S_{50}	944	383	83	187	110
S_{75}	949	382	85	194	103

* See footnotes to Table 1.

Table 5 Chemical composition of whole body of common carp (*Cyprinus carpio* L.) fingerlings of different experimental groups at the start and at the end of the experiment (gkg^{-1} wet basis ± SD)

Treatment*	Moisture	Crude protein	Crude lipid	Ash	Gross energy (kJ/g)
Initial fish	796 ± 0.6	133 ± 0.5	30 ± 0.1	44 ± 0.3	48 ± 0.3
Control	766 ± 6.4	146 ± 5.2	49^{b} ± 2.3	22 ± 1.1	58^{a} ± 1.6
J_{a50}	778 ± 18.8	151 ± 5.1	57^{ab} ± 17.8	23 ± 2.0	52^{ab} ± 8.3
J_{a75}	786 ± 7.0	150 ± 2.7	50^{b} ± 6.1	22 ± 0.7	49^{b} ± 2.6
J_{b50}	769 ± 10.5	157 ± 2.4	66^{ab} ± 10.7	22 ± 0.6	59^{a} ± 02.2
J_{b75}	768 ± 05.6	153 ± 3.1	69^{a} ± 7.8	22 ± 1.9	55^{ab} ± 2.3
S_{50}	782 ± 11.5	14.5 ± 10.3	51^{ab} ± 11.0	24 ± 1.3	49^{b} ± 5.1
S_{75}	786 ± 21.0	144 ± 14.9	57^{ab} ± 4.8	22 ± 2.8	50^{b} ± 4.3
SEM	2.8	1.4	2.1	0.3	1.1

Values are mean (n = 4) ± standard deviation.

* See footnotes to Table 1

Mean values in the same column with different superscript differ significantly (P < 0.05).

Growth performance and feed utilization

Weekly body mass developments of fish are given in Figure 1. The growth performance of the fish at the end of the experimental period and the nutrient utilization are presented in Tables 6 and 7. Weekly body mass gain indicates that second week onwards there was differential growth among the group, and lower body mass development was observed in J_{a50}, J_{a75} and S_{75} groups compared to other groups. This trend was maintained till the end of the experiment. Highest BMG, SGR, MGR and EPV were observed for the J_{b50} groups, which were statistically similar to that for control group and significantly ($P < 0.05$) higher than for all other groups. Lowest FGR was observed in control group, which is statistically similar to J_{b50} group. Highest LPV was observed in J_{b50} group, however; it was not significantly different from that of all other groups except J_{a75}. The LPV was lowest in J_{a75} group.

Lowest MGR, PER and PPV were observed in J_{a75} group whereas this group had lowest growth rate as well. In general, we found a decreasing trend of various growth and assimilation parameters (BWG, MGR, FGR, PER, PPV, and EPV) in the fish fed diets containing increasing levels of plant protein in the diet.

Table 6 Initial weight (IW), final weight (FW) and body mass gain (BMG) of common carp (*Cyprinus carpio* L.) fed with experimental diets for eight weeks

Treatment*	IW (g)	FW (g)	BMG (g)
Control	3.2 ± 0.1	32.0^{a} ± 1.96	28.9^{a} ± 1.95
J_{a50}	3.2 ± 0.1	24.9^{d} ± 3.31	21.7d ± 3.24
J_{a75}	3.3 ± 0.0	20.9^{e} ± 2.04	17.7^{e} ± 2.03
J_{b50}	3.2 ± 0.1	33.3^{a} ± 0.64	30.1^{a} ± 0.63
J_{b75}	3.2 ± 0.1	28.3^{bc} ± 1.21	25.1^{bc} ± 1.25
S_{50}	3.3 ± 0.1	30.6^{ab} ± 0.72	27.3^{ab} ± 0.68
S_{75}	3.2 ± 0.1	27.7^{c} ± 0.57	24.5^{c} ± 0.64
SEM	0.01	0.88	0.88

Values are mean (n = 4) ± standard deviation.
* See footnotes to Table 1.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).

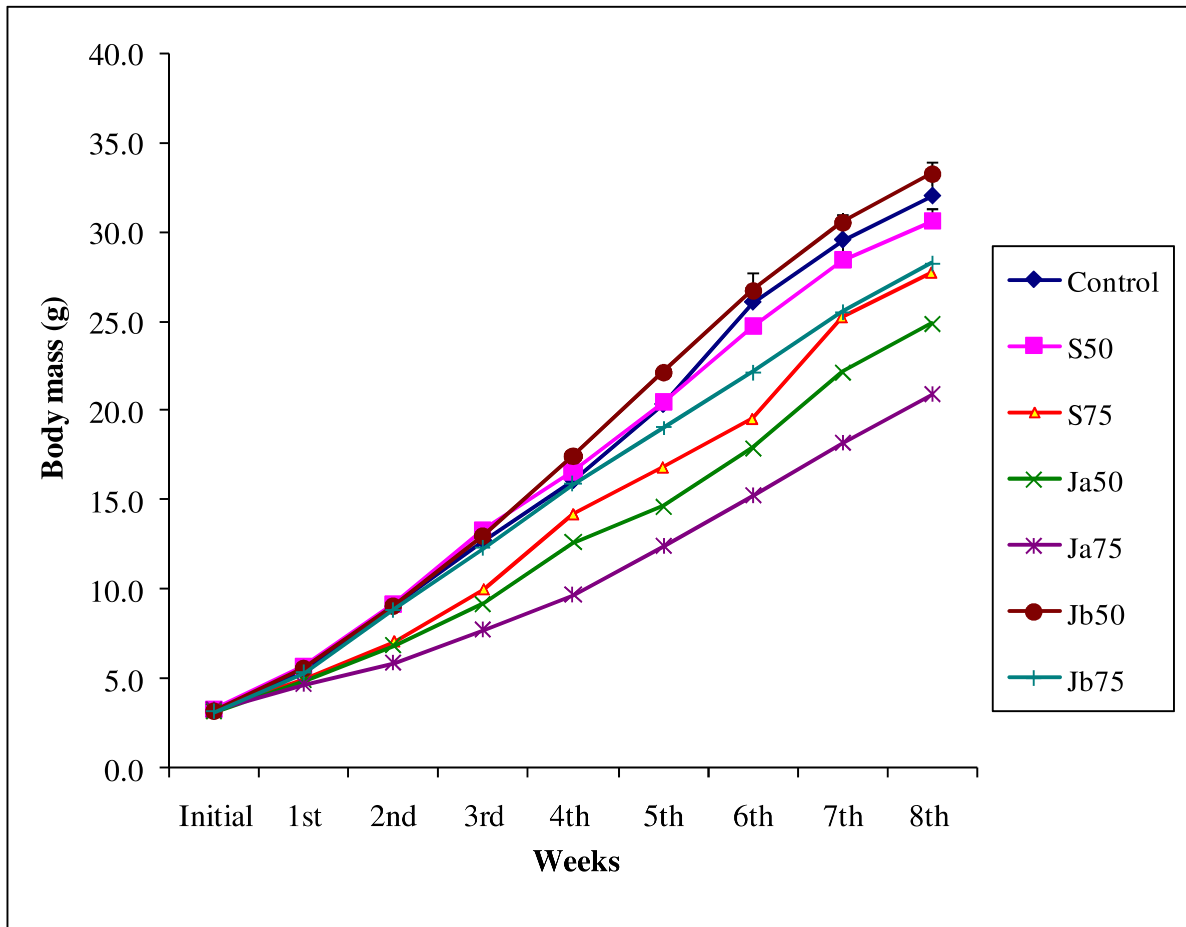


Figure 1: Body mass gain of common carp (*Cyprinus carpio* L.) fed with experimental diets for eight weeks

Table 7 Growth performance and nutrient utilisation of common carp (*Cyprinus carpio* L.) fed with experimental diets for eight weeks

Treatment*	BMG	SGR	FGR	MGR	PER	PPV	LPV	EPV
Control	$917.0^{a} \pm 59.63$	$4.1^{ab} \pm 0.11$	$1.00^{b} \pm 0.05$	$21.7^{a} \pm 0.5$	$2.6^{a} \pm 0.12$	$38.8^{ab} \pm 2.41$	$59.0^{ab} \pm 1.80$	$30.1^{a} \pm 1.91$
J_{a50}	$684.7^{c} \pm 90.12$	$3.7^{d} \pm 0.21$	$1.06^{ab} \pm 0.03$	$19.3^{b} \pm 1.18$	$2.4^{ab} \pm 0.08$	$37.7^{ab} \pm 1.97$	$66.8^{ab} \pm 22.66$	$24.1^{b} \pm 4.76$
J_{a75}	$542.8^{d} \pm 61.53$	$3.3^{e} \pm 0.18$	$1.15^{ab} \pm 0.03$	$17.6^{c} \pm 0.93$	$2.3^{b} \pm 0.05$	$34.7^{b} \pm 0.95$	$54.4^{b} \pm 7.54$	$20.8^{b} \pm 1.33$
J_{b50}	$946.9^{a} \pm 26.34$	$4.2^{a} \pm 0.05$	$1.01^{b} \pm 0.02$	$22.0^{a} \pm 0.18$	$2.6^{a} \pm 0.04$	$41.3^{a} \pm 0.88$	$78.2^{a} \pm 13.04$	$29.5^{a} \pm 0.93$
J_{b75}	$774.0^{bc} \pm 58.23$	$3.9^{cd} \pm 0.12$	$1.21^{a} \pm 0.24$	$20.1^{b} \pm 0.99$	$2.2^{b} \pm 0.37$	$34.4^{b} \pm 5.56$	$72.3^{ab} \pm 13.07$	$23.3^{b} \pm 4.01$
S_{50}	$826.8^{b} \pm 25.95$	$4.0^{bc} \pm 0.05$	$1.06^{ab} \pm 0.02$	$21.1^{ab} \pm 0.20$	$2.5^{ab} \pm 0.06$	$38.0^{ab} \pm 1.87$	$68.4^{ab} \pm 10.39$	$24.2^{b} \pm 2.64$
S_{75}	$758.1^{bc} \pm 39.13$	$3.8^{cd} \pm 0.08$	$1.02^{b} \pm 0.02$	$20.3^{b} \pm 0.32$	$2.6^{a} \pm 0.05$	$37.4^{ab} \pm 4.06$	$69.7^{ab} \pm 5.09$	$23.2^{b} \pm 2.00$
SEM	27.24	0.06	0.02	0.31	0.04	0.71	2.71	0.82

Values are mean (n = 4) ± standard deviation.

* See footnotes to Table 1

Mean values in the same column with different superscript differ significantly (P < 0.05).

BMG (%) - Body mass gain, SGR (%) – Specific growth rate and FGR – Feed gain ratio; MGR (gkg0.8 day-1) - Metabolic growth rate, PER - Protein efficiency ratio, PPV (%)- Protein productive value, LPV (%)-Lipid production value and EPV (%)-Energy production value

Digestibility measurements of diets and feed ingredients

Digestibility of the dry matter, nutrients and energy of different experimental diets and feed ingredients (SBM and DJKM) are given in Tables 8 and 9 respectively. The dry matter and lipid digestibility were statistically ($P < 0.05$) highest in control group and lowest in J_{a50} group. Protein and energy digestibilties were statistically similar ($P > 0.05$) for, control and J_{b50} groups and these values were highest for these groups. This was also reflected in PER and EPV parameters for these two groups.

Highest dry matter digestibility was observed for J_{b50} meal which was statistically similar to that S_{50} and significantly higher than J_{a50} meal. Protein and energy digestibilities for J_{b50} meal was statistically higher than SBM and J_a meal. It was generally observed that as plant protein inclusion increased in the diet, dry matter, protein and energy digestibility of feed ingredients (SBM, J_a and J_b) decreased gradually.

Table 8 Effects of experimental diets on apparent digestibility coefficient of the dry matter, nutrient and energy digestibility (%) in common carp (*Cyprinus carpio* L.) fingerlings

Treatment*	Dry matter digestibility	Protein digestibility	Lipid digestibilty	Energy digestibilty
Control	$84.4^{a} \pm 0.40$	$92.3^{a} \pm 0.45$	$97.2^{a} \pm 0.68$	$87.7^{a} \pm 1.33$
J_{a50}	$73.1^{f} \pm 0.73$	$87.7^{d} \pm 0.75$	$89.7^{d} \pm 0.74$	$77.9^{c} \pm 3.14$
J_{a75}	$74.8^{e} \pm 0.93$	$87.3^{d} \pm 0.94$	$89.2^{d} \pm 1.31$	$78.0^{c} \pm 2.84$
J_{b50}	$82.5^{b} \pm 0.25$	$92.2^{a} \pm 0.39$	$95.0^{b} \pm 0.91$	$87.6^{a} \pm 1.11$
J_{b75}	$80.6^{c} \pm 0.12$	$90.6^{b} \pm 0.07$	$92.1^{c} \pm 0.90$	$83.1^{b} \pm 0.95$
S_{50}	$81.3^{c} \pm 0.45$	$91.3^{b} \pm 0.31$	$94.0^{b} \pm 0.82$	$83.6^{b} \pm 0.51$
S_{75}	$77.9^{d} \pm 0.29$	$88.6^{c} \pm 0.71$	$92.5^{c} \pm 1.11$	$82.8^{b} \pm 1.47$
SEM	0.74	0.39	0.53	0.78

Values are mean ($n = 4$) ± standard deviation.

* See footnotes to Table 2

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Table 9 Dry matter, protein and energy digestibility of feed ingredients

Ingredients	Replacement of fish meal protein (%)	Dry matter digestibility	Protein digestibility	Energy digestibilty
Jatropha	50%	$60.6^{c} \pm 2.33$	$82.3^{d} \pm 1.89$	$61.6^{c} \pm 7.61$
(J_{a})	75%	$43.1^{d} \pm 2.68$	$79.4^{e} \pm 2.10$	$48.6^{d} \pm 12.51$
Jatropha	50%	$77.1^{a} \pm 0.96$	$92.1^{a} \pm 1.12$	$87.4^{a} \pm 4.69$
(J_{b})	75%	$74.7^{ab} \pm 0.31$	$88.9^{b} \pm 0.15$	$74.8^{b} \pm 2.64$
Soyabean	50%	$75.9^{a} \pm 1.22$	$89.8^{b} \pm 0.81$	$75.0^{b} \pm 1.62$
	75%	$72.3^{b} \pm 0.54$	$85.8^{c} \pm 1.27$	$77.6^{b} \pm 3.04$
SEM		2.85	0.96	2.55

Values are mean (n = 4) ± standard deviation.

Mean values in the same column with different superscript differ significantly (P < 0.05).

Fractional feed efficiency

Fractional feed efficiency of all experimental diets is shown in Table 10. Highest crude protein efficiency was observed in J_{b50} group, which was statistically similar to that of control, S_{50}, S_{75}, J_{a50} and J_{a75} groups whereas lowest crude protein efficiency was found in J_{b75} group. Crude lipid efficiency did not differ significantly among the groups. Crude energy efficiency was not statistically different (P>0.05) for control and J_{b50} and significantly higher (P<0.05) compared to that for other groups.

Relative intestinal length (RIL), hepatosomatic index (HSI), and intestinalsomatic index (ISI)

RIL, HSI and ISI of fish fed different diets are presented in Table 11. There was no significant difference in ISI among different groups. HSI value of plant protein fed groups was statistically higher (P>0.05) than for control group. RIL value of plant protein fed groups except J_{a50} and J_{a75} were statistically higher (P>0.05) than for control group.

Table 10 Fractional feed efficiency of experimental diets, based on digestible nutrients

Treatments*	Crude protein efficiency	Crude lipid efficiency	Crude energy efficiency
Control	$0.42^{ab} \pm 0.03$	0.47 ± 0.15	$0.34^{a} \pm 0.02$
J_{a50}	$0.43^{ab} \pm 0.02$	0.57 ± 0.20	$0.31^{abc} \pm 0.06$
J_{a75}	$0.39^{ab} \pm 0.01$	0.47 ± 0.59	$0.27^{c} \pm 0.02$
J_{b50}	$0.45^{a} \pm 0.01$	0.65 ± 0.11	$0.34^{ab} \pm 0.01$
J_{b75}	$0.38^{b} \pm 0.06$	0.61 ± 0.11	$0.28^{bc} \pm 0.05$
S_{50}	$0.42^{ab} \pm 0.02$	0.55 ± 0.85	$0.29^{bc} \pm 0.03$
S_{75}	$0.42^{ab} \pm 0.05$	0.57 ± 0.46	$0.28^{bc} \pm 0.03$
SEM	0.08	0.23	0.09

Values are mean (n = 4) ± standard deviation.

* See footnotes to Table 2

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Table 11 Effects of experimental diets on the relative intestinal length (RIL), hepatosomatic index (HSI) and intestinalsomatic index (ISI) of common carp (*Cyprinus carpio* L.) fingerlings

Treatment*	RIL	HSI	ISI
Control	$2.55^{bc} \pm 0.13$	$2.12^{b} \pm 0.03$	2.99 ± 0.26
J_{a50}	$2.33^{c} \pm 0.10$	$2.20^{b} \pm 0.05$	3.07 ± 0.17
J_{a75}	$2.20^{c} \pm 0.08$	$2.23^{ab} \pm 0.10$	3.12 ± 0.34
J_{b50}	$3.30^{a} \pm 0.18$	$2.24^{b} \pm 0.04$	3.08 ± 0.03
J_{b75}	$2.93^{ab} \pm 0.10$	$2.35^{a} \pm 0.07$	3.09 ± 0.36
S_{50}	$3.10^{a} \pm 0.16$	$2.26^{ab} \pm 0.06$	3.06 ± 0.15
S_{75}	$2.75^{b} \pm 0.13$	$2.36^{a} \pm 0.12$	3.23 ± 0.56
SEM	0.08	0.02	0.11

Values are mean (n = 4) ± standard deviation.

* See footnotes to Table 2

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Digestive enzymes activity

Amylase, protease and lipase enzyme activities in intestine of fish are shown in Table 12. Amylase, protease and lipase activities for control group were higher ($P<0.05$) than for plant protein fed groups.

Table 12 Digestive enzymes activities (U/g protein) and of different experimental groups

Treatment*	Amylase	Protease	Lipase
Control	$14.23^{a} \pm 1.71$	$31.12^{a} \pm 5.04$	$4.85^{a} \pm 0.74$
J_{a50}	$6.94^{c} \pm 0.70$	$13.65^{d} \pm 1.57$	$2.45^{d} \pm 0.54$
J_{a75}	$5.85^{c} \pm 0.97$	$10.51^{e} \pm 0.90$	$2.16^{d} \pm 0.37$
J_{b50}	$11.64^{b} \pm 1.80$	$24.79^{b} \pm 1.92$	$4.43^{ab} \pm 0.38$
J_{b75}	$11.02^{b} \pm 1.59$	$20.12^{c} \pm 2.37$	$4.21^{bc} \pm 0.62$
S_{50}	$10.51^{b} \pm 1.48$	$22.65^{b} \pm 1.89$	$4.16^{ab} \pm 0.61$
S_{75}	$10.47^{b} \pm 0.83$	$19.13^{c} \pm 1.32$	$3.95^{c} \pm 0.56$
SEM	0.32	0.73	0.12

Values are mean (n = 4) ± standard deviation.

* see footnotes to Table 2

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Discussion

The results of the present investigation indicate that J_b meal at low inclusion levels (J_{b50}, 50% of FM protein replaced by DJKM) is a good dietary protein source for carp feed. The performance of SBM fed groups was not as good as of J_b fed groups**,** however it was better than that of J_a fed groups. SBM was found to be a moderately good protein source for carp at the 50% inclusion level, whereas, it was not promising at a high inclusion level (75% of FM protein replacement). J_a proved to be unsuitable even at low inclusion (50%) in carp diet.

Several factors such as acceptability of diets, presence of toxic and antinutritional factors and digestibility of protein and energy in the diets could contribute to the observed variation in the growth responses of carp. Although PEs was

not detected by HPLC in J_a meal, the poor growth response, feed intake and feed utilization of carp fed diets containing J_a meal indicate that J_a meal was not detoxified properly. It is possible that PE might be present in strongly bounded form in the J_a meal, and was not extracted by our protocol for HPLC determination. PE could have been released from J_a meal in the fish intestine during digestion process hindering the growth performance and nutrient utilization. Experimental evidence for the toxic action of PE is well documented in common carp and rat. Feeding trials on common carp and rats with *Jatropha* meal containing PE has been reported to cause marked reduction in feed intake, diarrhoea and depression of growth (Becker & Makkar 1998; Rakshit *et al.* 2008).

Growth performance and nutrient utilization

Growth performance and nutrient utilization of carp fed J_{b50} diet (50% FM protein replacement by DJKM) were better than of those fed SBM based diets and similar to those fed FM based diets. Slightly lower performance of fish fed the diet where 75% of FM protein was replaced by DJKM suggested that the capacity of DJKM to fully sustain growth was slightly lower compared to the diet based on FM only (control diet). The growth data were consistent with the feed conversion data, nitrogen retention and protein efficiency.

The lower growth response of 75% plant protein fed group could be due to lower protein availability from the SBM and DJKM (plant protein structure is much more compact than FM, so digestive enzymes act slowly on plant proteins), poor availability of crystalline lysine added to DJKM groups to equalize lysine content, and/or the presence of antinutrients such as phytate and non-starch polysaccharides (NSP), which are present in high amounts in the kernel meal, which affects adversely the feed utilization.

Our result of growth performance and feed utilization in carp are supported by Hasan *et al.* (1997) in diet for carp wherein groundnut meal substituted 25% of FM protein without evidencing a negative effect on the feed intake and growth performance. Carp are not able to utilize high level (more than 50% of FM protein replacement) of

plant derived protein in the diets because of low palatability, and high fibre and antinutrients content. Similar results were obtained by Viola *et al.* (1982) for carp and by Jakson *et al.* (1982) for tilapia.

Biochemical composition of whole body of fish

Moisture and lipid contents of whole body of fish tend to show greater variation than other carcass components (crude protein, ash and gross energy) and they appear to be inversely related (Atack *et al.* 1979; Focken & Becker, 1993; Hasan *et al.* 1997). Crude lipid content and gross energy content of carp fed different diets did not show large variations, although some of the values were statistically different (Table 4). Significantly higher crude lipid deposition was observed in all plant protein fed groups (except J_{a75} group), whereas lowest lipid deposition was observed in J_{a75} and control groups. Higher value of HSI in plant protein fed groups suggests higher lipid deposition in liver. HSI values of above 2, as observed here, are common in common carp (Yılmaz & Genc 2006). The increase in whole body fat content with the use of dietary plant proteins based diets could be due to the higher content of total carbohydrate in plant protein based diets. The carbohydrates could get converted to lipid in the body by lipogenesis. Hasan *et al.* (1997) in their study on common carp fed with plant protein source such as mustard, sesame, linseed, copra and groundnut oil cakes, substituting for FM protein, also observed significantly higher deposition of crude lipid in whole body.

Whole body moisture and crude protein content was similar for all the groups. Whereas, Hasan *et al.* (1997) observed that carp fed with plant protein (mustard, sesame and linseed oil cake) exhibits lower moisture and higher crude protein content when compared to FM fed groups. However, in the same trial Hasan *et al.* (1997) found that whole body ash content of carp did not differ significantly among all the groups and same trend was followed in our study. In the present study the plant protein based diets were supplemented with phytase and that could have released minerals bound to phytic acid.

Digestibility measurement of experimental diets

SBM and DJKM in combination with FM protein showed excellent dry matter, crude protein, lipid and energy digestibility in the present study. Generally, oil seed meal proteins have digestibilities of 80-95% for fish (Jauncey & Ross 1982) and carp are also reported to be able to digest the plant proteins well, which is generally slightly better than monogastric mammals (National Research Council 1983). The protein digestibility coefficient is a key factor in the evaluation of the quality of the diet for fish and the potential of the diet for the synthesis of new tissue. Dry matter, protein, lipid and energy digestibility of experimental diets were 78-85%, 89-92%, 92-97% and 83-88% respectively, which indicate excellent utilization of feed ingredients. Higher apparent digestibility for protein from DJKM than from SBM implies that a greater amount of protein would have been available to the fish from the DJKM diets. Hasan *et al.* (1994) reported that apparent protein digestibility values ranged between 68.3-72.9% for carp fed plant protein (leucaena leaf meal) based diets. In the present study, crude protein digestibility of DJKM diets were high (above 90%) in common carp suggested DJKM to be an excellent protein source for carp diet. Energy digestibility of plant protein based diets (SBM and DJKM based diets) was considerably lower than protein digestibility due to their high carbohydrate content (Gomes *et al.* 1993; Gouveia *et al.* 1993). The lower lipid digestibility of the fish fed the SBM diet may be associated with the increase in the NSP content, which reduces fat absorption by disturbing micelle formation in the gastro intestinal tract (Krogdahl *et al.* 2003; Gatlin *et al.* 2007; Øverland *et al.* 2009).

Digestibility measurement of feed ingredients

The apparent protein digestibility (92.1%) of J_b meal at 50% replacement of FM was almost as good as FM. The reduction in the digestibility of protein in the fish fed the SBM compared to FM is in agreement with earlier findings (Storebakken *et al.* 2000; Aslaksen *et al.* 2007). However, the apparent digestibility of dry matter was generally poor, especially for plant feed ingredients where values ranged from 39.3% - 76.5%. The low-energy digestibility of these plant derived ingredients could be attributed to

their high-carbohydrate content and poor digestibility by omnivorous fish (Lupatsch *et al.* 1997), although these values exhibit higher (48.3-87.4%) than reported by many researchers for fish. Interestingly it was found that J_b meal at 50% replacement of FM protein in the carp diet has similar apparent energy digestibility as for FM. The results demonstrate that the carp were efficient in digesting protein and energy from the J_b meal, while fish fed the SBM in general exhibited lower digestibilities.

Fractional feed efficiency indicates retained nutrients and energy in whole body to the total digestible nutrients and gross energy. Highest crude protein and gross energy efficiency were observed in J_{b50} group, which indicate that carp have utilized J_b meal and retained nutrients in the body maximally.

Digestive enzyme activities and relative intestinal length (RIL)

Heat labile antinutrients (trypsin inhibitors and lectins) were not detected in the autoclaved DJKM and roasted SBM, whereas heat stable enzyme inhibitors (phytate) were present in those feed ingredients. Antinutritional factors such as phytic acid inhibit activities of some digestive enzymes such as pepsin, trypsin and alpha-amylase (Alarcon *et al.* 1999; Robaina *et al.* 1995), or form complexes with minerals (Teskeredzic *et al.* 1995; Sugiura *et al.* 1999) and proteins (Moyano *et al.* 1999), thereby modifying digestion processes and this could impair intestinal absorption. Carp showed a significant decrease in protease, amylase and lipase activities in intestine on inclusion of plant proteins in the diet. Lower protease activities, the level of which decreased with increase in SBM and DJKM in common carp diets, corresponded to decrease in protein availability from SBM and DJKM. Similar results were observed by Santigosa *et al.* (2008), Sandholm *et al.* (1976) and Krogdahl *et al.* (1994). They found that protein digesting enzyme (trypsin) activity decreases as plant protein inclusion increases in trout diet and they concluded that trypsin is highly sensitive to plant antinutrients. Escaffre *et al.* (1997) observed that increasing levels of dietary soya protein concentrate induced a significant decline in trypsin activity in common carp. The decrease in protease activity at higher inclusion level of DJKM might be caused by the presence of phytate. The lower activity of digestive enzymes in DJKM fed groups was correlated with lower nutrient digestibility of nutrients.

It is known that carnivorous and omnivorous fish require longer time to digest plant protein based diets (Buddington *et al.* 1997). Direct relationship between the amount of dietary plant protein and RIL has been reported earlier in fish (Kramer & Bryant 1995). In carp, plant protein based diets (except J_{a50} and J_{a75} groups) exhibited higher RIL than the control group. In addition, in the present study J_{b75} and S_{75} groups exhibited lower RIL than J_{b50} and S_{50} and higher than control group. In another study, we observed that RIL value increases as the plant protein inclusion increases from 50% and 62.5% FM protein replacement in the common carp diets (Unpublished). This indicates that RIL value increased up to 62.5% replacement of FM protein by plant protein. Lower RIL at higher than 62.5% FM protein replacement by plant protein in common carp indicates that 75% level of replacement of FM is above the threshold level that could elicit higher RIL. Lower RIL of J_{a50} and J_{a75} groups than that of control could be attributed to the lower growth and lower nutrient utilization possible due to the presence of residual PEs.

From a physiological view point, a longer RIL would facilitate an increase in digestibility and retention time by enhancing contact time of the digestive enzymes and the feed components, resulting in increase in their digestion and absorption. Omnivorous fish like common carp species showed compensation mechanisms, such as an increase in RIL and as a result increase in digestive activity, to achieve a digestive balance and growth rates similar to those observed for FM fed group.

Conclusion

The DJKM can replace 50% FM protein in carp diets, without sacrificing the growth and nutrient utilization of fish. The DJKM can be used as one of the promising FM replacers in the diet of common carp. PE, the main toxic principle for *Jatropha* toxicity was not detected in fish muscle tissues, suggesting the fish is safe for human consumption.

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3.1.1.2 Utilization of a byproduct from *Jatropha* biodiesel industry as a fish meal replacer in common carp *Cyprinus carpio* L. diet

Introduction

In recent decades, global aquaculture has been the fastest growing food production sector. The most common aquaculture products are freshwater, omnivorous fish, most of which come from the Cyprinid family. Common carp is one of the widely cultured species, the production of which substantially contributes to the total aquaculture production. For the past several years, one of the main steps in improving fish feeds has been the search for protein source alternatives to fish meal (FM) and determining their nutritional suitability in diets (Mazurkiewicz 2009). In the next decade demand for protein ingredients is expected to exceed annual world supply of FM, which will change the economic and nutritional paradigms of aquafeed industry. The challenge facing the aquaculture industry is to identify economically viable and environmental friendly alternatives to FM, and the challenge for the livestock industry is to find alternatives to conventional feed ingredients such as soybean meal (SBM), groundnut cake, rapeseed oil meal, maize and wheat. Studies on FM replacement in carp are sparse compared to other carnivore fishes. The use of oil seeds as a source of protein in formulating the diet of common carp was evaluated by several authors (Hansan, Macintosh & Jauncey 1997; Hossain & Jauncey 1989; Kumar, Makkar & Becker 2008; Mazurkiewicz 2009).

The SBM can be substituted for FM in carp feed up to 50-60% (Kumar *et al.* 2008). The use of such a high percentage of these components is possible due to good nutritional (total protein content, amino acid composition) and palatable properties (Hasan *et al.* 1997). Presently most of the commercial feeds depend mostly on SBM as a FM replacer. However the over dependence will cause hike in price of SBM and therefore exploration of other inexpensive plant protein sources, particularly those that do not compete with human food is required.

Jatropha curcas (L.) (physic nut) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. It is a hardy plant, thrives on degraded land and requires limited amounts of nutrients and water. Its seeds have been extensively investigated as a source of oil. The seed kernel contains about 60% oil that

can be converted into biodiesel of high quality upon transesterification and used as a substitute for diesel fuel. The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 58 to 62 % crude protein. The levels of essential amino acids (except lysine) are higher in *Jatropha* kernel meal than SBM (Makkar & Becker 2009). However the presences of high concentrations of antinutrients like trypsin inhibitor, lectin and phytate and the major toxic components phorbol esters (PE_S) (Makkar & Becker 1997) restrict their use in fish feed. Heat labile antinutrients, like protease inhibitors, and lectins are easy to inactivate by moist heating. A method for detoxification of *Jatropha* kernel meal has been developed in our laboratory. It is based on extraction and destruction of PEs using organic solvents and inactivation of trypsin inhibitors and lectin by heat treatment.

The International *Jatropha* Organization has projected that in 2017 there will be around 32.72 million hectares of land cultivated worldwide with *Jatropha* producing 160 million tons of seeds and 95% of its total production will be concentrated in Asia (mostly in China and India). *Jatropha* plant can yield up to 4 tons seed per year from one hectare of plantation, which can produce approximately one ton of kernel meal rich in protein. This implies that there is possibility of producing enough *Jatropha* kernel meal to meet growing aquaculture industry demand. Our previous short term (8-week) study (Kumar *et al.* 2008) has shown that detoxified *Jatropha* kernel meal (DJKM) is a good protein source and better than SBM for common carp diet. The present paper is the second in a series, focusing on long term effects of DJKM on growth performance, and on biochemical, hematological and histological changes in common carp *Cyprinus carpio* L. The purpose of this long term study was to capture adverse effect, if any, of feeding a diet containing DJKM and to ascertain the complete detoxification of *Jatropha* kernel meal.

Material and methods

Preparation of the Jatropha meal

Jatropha seeds were purchased from India and deshelled and defatted in Germany. Organic solvents were used to detoxify defatted *Jatropha* kernel meal (patent

application has been filed for the process of detoxification). After removal of PEs, the meal was autoclaved (121 °C for 15 min) to inactivate heat labile antinutrients, trypsin inhibitor and lectin.

Diet formulation, experimental system and animals

Fish meal was purchased from Kurt Becker GmbH, Bremen, Germany. Prior to feed formulation, the proximate composition of DJKM, wheat meal, wheat gluten and FM were determined. Three isonitrogenous and isoenergetic diets were formulated. Experimental diets containing crude protein 38%, crude lipid 10%, vitamin premix 2%, mineral premix 2% and TiO_2 1%. Titanium oxide (TiO_2) was added for digestibility measurement. Lysine was supplemented at the rate of 1.5% of DJKM inclusion in the diet. Each experimental feed, except the control, contained 500 FTU phytase (NATUPHOS 5000G, BASF, Ludwigshafen) per kg. The inclusion concentrations of the DJKM were as follows: Control diet (C_{ontrol}) was prepared with FM and wheat meal, without DJKM; J_{50}: 50% FM protein replaced by DJKM; and $J_{62.5}$: 62.5% FM protein replaced by DJKM. The final mixture of each diet was made into 2 mm diameter moist pellets and then freeze-dried (Table 1).

Table 1 Composition of the experimental diets (g kg^{-1} feed) for common carp (*Cyprinus carpio* L.)

Ingredients	**Experimental diets**		
	C_{ontrol}	**J_{50}**	**$J_{62.5}$**
Fish meal	503	251	189
[1]Wheat meal	417	399	398
Jatropha meal	-	247	310
Wheat gluten	-	9	8
Sunflower oil	40	54	56
[2]Vitamin premix	20	20	20
[3]Mineral premix	20	20	20
Total	1000	1000	1000

TiO_2	10	10	10
Phytase (FTU/kg)	-	500	500
Lysine (g)	-	4.5	6.0

[1]Whole wheat meal.
[2]Vitamin premix (g or IU kg-1 premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
[3]Mineral premix (g kg-1): $CaCO_3$, 336; KH_2PO_4, 50_2; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67;$CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; 3 $NaSeO_3$, 0.02.

Common carp (*Cyprinus carpio* L.) fingerlings from the Institute of Fisheries Ecology and Aquaculture of the Federal Research Center for Fisheries at Ahensburg, Germany were transferred to the University of Hohenheim, Stuttgart, Germany and kept in two 500 l capacity tanks for acclimatisation. After acclimatisation, 36 fish were randomly distributed into three groups with four replicates; each replicate contained three fish (av. wt. 22 ± 0.12 g) in an aquarium (45 l capacity). All the aquaria were supplied with water from a recirculatory system. The system was subjected to a photoperiod of 12 h light : 12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 26.2 – 27.1°C, pH 7.0 – 7.5, dissolved oxygen 6.9 – 7.4 mg l^{-1}, total NH_3 0.1– 0.2 mg l^{-1}, nitrite 0.07 – 0.1 mg l^{-1} and nitrate 1–3 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation above 80%. One day before start of the experiment, the fish were starved and during the experimental period fish were fed at 16 g feed per kg metabolic body mass ($kg^{0.8}$) per day (equal to five times their maintenance requirement) and split into 5 equal rations per day (at 8:00, 10:30, 13:00, 15:30 and 18:00 h). The feeds were dispensed using an automatic feeder. Fish were weighed individually at the beginning of the experiment and at weekly intervals during the experimental period to adjust the feeding level for the subsequent week. The fish were not fed on the weighing day. During last two weeks of the experiment, fish were fed with a diet containing a marker (TiO_2) for digestibility measurement (Mamun, Focken & Becker 2007). During last 2 weeks of the experiment, faeces were collected daily according to Mamun *et al.* (2007). The collected faeces was centrifuged at 4000 × g for 10 min, the supernatant discarded and the faeces were then stored at -20°C until analysis. At start of the experiment, six

fish of the same population were also killed and preserved at −20 °C for analysis of the initial body composition.

The experiment was terminated after 16 weeks and the fish were killed. At the end of experiment, fish were anaesthetized by tricaine methanesulfonate (MS222) at 250 ppm in water. Blood was drawn near caudal peduncle from one fish from each group and transferred into a heparinized tube for hematological study. Blood was drawn from another fish from each group and divided into two equal part, one part was centrifuged at 1500×*g* for 5 min at room temperature (24 ^{O}C) to obtain plasma, which was then stored at −20 ^{O}C for determination of cholesterol and triglycerides. Another part of blood was kept outside at room temperature for few minutes to collect serum. Serum was stored at −20 ^{O}C for lysozyme determination. One fish per group was carefully dissected to isolate intestine and stored in liquid nitrogen for digestive enzymes assay. Muscle was isolated from same fish and stored at −20 ^{O}C for determination of cholesterol and muscle lipid peroxides. One fish per group were stored at −20 ^{O}C for chemical composition analysis. Prior to determination of the proximate composition, the fish were autoclaved at 121 ^{O}C for 20 min, thoroughly homogenised using an Ultra-Turrax T25, frozen overnight and freeze-dried. The University animal welfare committee (University of Hohenheim Germany) approved all experimental procedures involving common carp.

Proximate analysis and determination of phorbol esters, antinutrients and amino acid

The proximate composition of diet ingredients, diets and whole body of fish was determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Phorbol esters (PEs) were determined according to Makkar, Francis and Becker (2007), which was based on the method of Makkar, Becker, Sporer and Wink (1997). The results were expressed as equivalent to a standard, phorbol-12-myristate 13-acetate. Detection limit of PEs by HPLC is 3 µg/g meal. Trypsin inhibitor activity was determined essentially according to Smith, VanMegen, Twaalfhoven and Hitchcook (1980) except that the enzyme was added last as suggested by Liu and Markakis (1989). Analysis of the lectin content was conducted by haemagglutination assay (Makkar *et al.* 1997). Phytate content of samples was determined by a

spectrophotometric procedure (Vaintraub & Lapteva 1988). Non-starch polysaccharides (NSP) were determined according to Englyst, Quigley and Hudson (1994). Amino acid composition of FM, DJKM, wheat gluten and wheat meal was determined using an automated amino acid analyser after hydrolysing the samples with 6 N HCl at 110 ^{0}C for 24 h (Bassler & Buchholz 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. Tryptophan content of the above-mentioned samples was determined spectrophotometrically by the method of Pinter-Szakacs & Molnar-Perl (1990).

Growth and nutrient utilization parameters

Growth performance and diet nutrient utilization were assessed in terms of body mass gain (BMG) = [(Final body mass - initial body mass) / Initial body mass] X 100; specific growth rate (SGR) = [(ln final body mass in g) - ln initial body mass in g) / number of trial days] X 100; metabolic growth rate (MGR) = (Body mass gain in g) / [{(initial body mass in g / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)^$^{0.8}$}/2] / number of trial days; feed gain ratio (FGR) = dry feed fed (g)/body mass gain (g); protein efficiency ratio (PER) = body mass gain (g)/crude protein fed (g); protein productive value (PPV) = [(final fish body protein in g - initial fish body protein in g) / total protein consumed in g] X 100; lipid productive value (LPV) = [(final fish body lipid in g - initial fish body lipid in g) / total crude lipid consumed in g] X 100 and energy productive value (EPV) = [(final fish body energy - initial fish body energy)/(gross energy intake)] X 100.

Digestibility measurement and efficiency of digestible nutrients and gross energy

The percentage of apparent dry matter digestibility of diets was calculated according to Maynard et al. (1981). Apparent dry matter digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces)}] X 100

Apparent digestibility coefficients (ADCs) of nutrients and energy of the experimental diets were calculated according to Maynard & Loosli (1969).

The nutrient and energy digestibility were obtained using the following formula:

Apparent nutrient digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces) X (% Nutrient or energy in faeces) / (% Nutrient or energy in feed)}] X 100

Efficiency of digestible nutrients and gross energy = (Nutrient and energy retained in the whole body/Digestible nutrient and digestible energy) x 100

Digestible nutrients and energy = Total offered of nutrients and gross energy through feed x digestibility coefficient.

Intestinal index (II), hepatosomatic index (HSI), intestinal somatic index (ISI) and digestive enzymes assay

Intestinal index was measured and is expressed in relation to each animal weight and expressed in mm g^{-1}.

II, HSI, and ISI are calculated as indicated below:

II = Intestine length (mm)/ body mass (g), HSI = Liver mass (g) X 100 / body mass (g), ISI = Intestinal mass (g) X 100 / body mass (g).

The reducing sugars produced due to the action of glucoamylase and α- amylase on carbohydrate was estimated using dinitro-salicylic-acid (DNS) method (Rick & Stegbauer 1974). Amylase activity was expressed as mmol of maltose released from starch per min at 37 ^{O}C. Protease activity was determined by the casein digestion method of Drapeau (1974), and one unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to $\Delta 0.001 A_{280}$ per minute at 37 ^{O}C and pH 7.8. Lipase activity was assayed by the method of Cherry & Crandell (1932), and one unit of enzyme was the amount of enzyme that hydrolyses 1.0 microequivalent of fatty acid from a triglyceride in 24 h at pH 7.7 at 37 ^{O}C.

Determination of lipid peroxides, cholesterol and triglyceride

Lipid peroxides in fish muscle were determined using the procedure of Utley et al. (1967). The determinations of the plasma cholesterol and triglycerides were using enzymatic colorimetric kits: Hitado Diagnostic system, Nobiflow cholesterin (kit lot number 60041889) and Nobiflow triglyceride-GPO (kit lot number 60040710) respectively. Muscle cholesterol content was determined using a kit (lot no.

10139050035) (Boehringer Mannheim, Germany). The color intensity was determined photometrically and was directly proportional to the concentration of cholesterol and triglycerides.

Haematological parameters

RBC and WBC were counted by Neubauer's counting chamber of haemocytometer. Care was taken to avoid trapping of air bubbles. The RBC lying inside the five small squares were counted under high power (40X) of light microscope.

Number of RBC/mm^3 = (N x dilution)/area counted x depth of fluid. The Hb content of blood was analyzed by Reflotron Hemoglobin test (REF 10744964, Roche diagnostic GmbH, Manheim Germany). Hct was determined on the basis of sedimentation of blood. Heparinised blood (50 µl) was taken in the hematocrit capillary (Na-heparinised) and centrifuged in the Hematocrit 210 Hettich Centrifuge (Tuttlingen Germany) to obtain hematocrit value. From analyses of Hct, Hb and RBC, the following parameters were calculated: mean cell volume, MCV (fL) = (Haematocrit [in L/L] x 1000) ÷ (RBC count [in millions/µL]); mean corpuscular haemoglobin, MCH (pg) = (Hemoglobin [g/dL] x 10 ÷ (RBC count [in millions/µL]) and mean cell haemoglobin concentration, MCHC [in g/dL] = Haemoglobin [in g/dL] ÷ Haematocrit [in L/L]. Lysozyme activity of serum was measured by EnzChek Lysozyme Assay Kit (E-22013) Leiden, The Netherlands. VetScan Chemistry Analyzer (Scil Animal care company GmbH, Technischer Service, Germany) was used for determinations of alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total calcium (Ca^{++}), creatinine, globulin, glucose, phosphorus, potassium (K^+), sodium (Na^+), total bilirubin (TBIL), total protein, and urea nitrogen (BUN) in heparinized blood.

Statistical analysis

All data were subjected to a one-way analysis of variance ANOVA and the significance of the differences between means was tested using Duncan's multiple range test ($P<0.05$). The software used was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). Values are expressed as means ± standard deviation.

Results and discussion

The crude protein contents of DJKM and FM are similar. Therefore, replacing FM by DJKM can achieve any desired level of FM protein replacement by DJKM protein in the diet on weight-to-weight basis. In this respect DJKM is unique, unlike other plant protein sources such as soybean meal, canola meal and lupin (used generally in fish diets) that have lower crude protein content. In the present study, lysine was added in the DJKM based diets and there was no other change except that the FM was replaced by DJKM; and hence the effects observed could be attributed to the lysine supplemented DJKM.

Phorbol esters and antinutrients content in defatted Jatropha kernel meal

Phorbol esters content in untreated defatted *Jatropha* kernel meal was 1.8 mg/g. However, PEs in DJKM was undetectable (< 3 µg/g). Trypsin inhibitor and lectins were also not detected in DJKM; whereas phyate and NSP levels in DJKM were 9.1% and 16% respectively (Table 2).

Proximate and amino acid composition of feed ingredient and experimental diets

Proximate composition and amino acid profiles of feed ingredients and diets are shown in Tables 2 and 3. Diets contained about 38% crude protein was isonitrogenous. Crude lipid and ash were in the range of 9.9–10.6% and 10.5–12.3% respectively. All experimental diets had almost similar amino acid composition. Essential amino acids were in all diets confirmed to the requirement of the common carp (NRC 1993).

Table 2 Proximate composition and amino acid composition of feed ingredients

	Fish meal	***Jatropha* meal**	**Wheat gluten**	**Wheat meal**
Proximate composition (g kg $^{-1}$)				
Dry matter	940	945	937	941
Crude protein	635	665	856	143
Crude lipid	88	11.4	13.4	16.3
Crude ash	142	82	8.7	14
Gross energy (KJ/g)	21.1	18.3	21.1	18.7
Essential amino acids composition (g kg $^{-1}$)				
Arginine	35.3	69.7	41.1	5.4
Histidine	17.7	21.7	19.7	3.4
Iso leucine	22.8	26.7	42.8	4.2
Leucine	41.6	46.7	68.4	9.1
Lysine	40.9	23.3	16.9	3.3
Phenylalanine	21.8	30.4	30.7	6.5
Methionine	16	10.6	16.7	2
Threonine	23	22	23.2	3.7
Tryptophan	4.9	7.1	13.5	1.4
Valine	29.3	31.6	39.7	5.1
Non-essential amino acids composition (g kg $^{-1}$)				
Alanine	43.3	29.4	20.1	4.6
Asparginine	60.5	68.7	33.5	7.2
Cystine	4.3	2.3	17.1	2.9
Glycine	59.8	31.5	32.5	5.6
Glutamine	79.4	112.1	161.8	44.9
Proline	36.9	32.2	60.3	14.5
Serine	25.5	30.6	43.4	6.3
Tyrosine	14.8	18.8	28.2	3.3

1 **Table 3** Proximate and amino acid composition of experimental diets (dry matter basis)
2 of common carp (*Cyprinus carpio* L.)

	C_{ontrol}	J_{50}	$J_{62.5}$
Proximate (g kg^{-1})			
Dry matter	947	948	948
Crude protein	389	389	381
Crude lipid	106	104	99
Crude ash	123	106	105
Gross energy (KJ/g)	19.9	19.1	18.9
Essential amino acids (g kg^{-1})			
Arginine	20.0	28.60	30.76
Histidine	10.3	11.34	11.58
Iso leucine	13.2	14.38	14.60
Leucine	24.7	26.22	26.51
Lysine	21.9	21.49	22.40
Phenylalanine	13.7	15.85	16.38
Methionine	8.9	7.58	7.24
Threonine	13.1	12.89	12.83
Tryptophan	3.0	3.66	3.79
Valine	16.9	17.55	17.68
Non-essential amino acids (g kg^{-1})			
Alanine	23.7	20.15	19.29
Asparginine	33.4	35.33	35.87
Cystine	3.4	2.96	2.82
Glycine	32.4	25.32	23.56
Glutamine	58.6	66.99	68.92
Proline	24.6	23.54	23.21
Serine	15.4	16.86	17.16
Tyrosine	8.8	9.93	10.16

3

4 *Fish behaviour, feed intake and growth*

5

6 Based on the visual observation during feeding time, palatability or acceptability of feed
7 was good and the behaviour of fish was normal. No left feed was observed in the
8 aquaria.

9 Growth performance and nutrient utilization parameters did not differ
10 significantly ($P > 0.05$) among the three groups (Table 4). Similar to our earlier short
11 term study on carp (Kumar *et al.* 2008), we obtained encouraging results on
12 replacement of FM with DJKM in the present study as well. The growth rate in the
13 present study was relatively lower than our previous short term experiment (Kumar *et*
14 *al.* 2008). The initial size of fish and duration of the experiment has significant
15 influence on growth rate. The present study was carried out for longer term (16 weeks)
16 and the initial fish size was bigger (22 g versus 4 g). In the short term study, growth
17 performance and nutrient utilization of carp fed DJKM based diet (50% FM protein
18 replaced by DJKM) were similar to that of FM based diets. In addition, slight but
19 significantly lower ($P > 0.05$) performance of fish fed the diet in which 75% of FM
20 protein was replaced by DJKM suggested that the capacity of DJKM to fully sustain
21 growth at this level of incorporation was slightly lower compared to the diet based on
22 FM only (control diet).

23 **Table 4** Growth performance and nutrient utilization in common carp (*Cyprinus carpio* L.) juveniles fed with experimental diets for 16
24 weeks

Treatment	IBM (g)	FBM (g)	BMG (%)	SGR (%)	MGR (g $kg^{0.8}$ day^{-1})	FGR	PER	PPV (%)	LPV (%)	EPV (%)
C_{ontrol}	21.5 ± 0.74	148 ± 29.4	588 ± 120	1.7 ± 1.71	8.5 ± 0.76	1.7 ± 0.26	1.6 ± 0.16	26.5 ± 4.26	43.0 ± 14.99	20.1 ± 4.56
J_{50}	21.6 ± 1.04	137 ± 24.6	531 ± 82	1.6 ± 1.59	7.9 ± 0.15	1.8 ± 0.05	1.4 ± 0.02	26.1 ± 0.29	36.5 ± 6.34	17.6 ± 4.04
$J_{62.5}$	21.8 ± 1.03	122 ± 36.5	460 ± 139	1.4 ± 1.36	6.8 ± 1.32	2.1 ± 0.48	1.2 ± 0.26	21.9 ± 3.88	29.4 ± 4.04	15.4 ± 5.69
SEM	0.24	5.95	43.56	0.07	0.36	0.13	0.08	1.22	3.42	1.27

25 IBM- Initial body mass, FBM- Final body mass, BMG - Body mass gain, SGR – Specific growth rate and MGR - Metabolic growth rate;
26 FGR – Feed conversion ratio, PER - Protein efficiency ratio, PPV - Protein productive value, LPV - Lipid productive value and EPV -
27 Energy productive value
28 Values are mean (n = 4) ± standard deviation.
29 For all parameters, mean values in the same column were not significantly different ($P < 0.05$).
30

31 *Chemical composition of whole body of fish*

32

33 Whole body chemical composition is shown in Table 5. Highest whole body lipid was
34 observed in C_{ontrol} group, which was statistically not different ($P > 0.05$) from that in J_{50}
35 group and was lowest in J_{62} group; whereas, crude protein in whole body had reverse
36 trend (Table 4). In our study whole body moisture content does not show any inverse
37 relation with whole body lipid but Hasan *et al.* (1997) found inverse relation of moisture
38 with lipid content. Lower whole body lipid content on replacing more than 50% FM
39 protein by DJKM, which resulted in decreased accretion of lipid in liver appear to lower
40 the hepatosomatic index in $J_{62.5}$ group. Hasan *et al.* (1997) and Mazurkiewicz (2009)
41 observed opposite trend. In their study common carp fed with plant proteins such as
42 mustard, sesame, linseed, copra, groundnut oil cakes and legume-rapeseed mixture
43 substituting 17-75% FM protein, significantly higher ($P < 0.05$) deposition of crude
44 lipid in whole body has been observed. Gross energy and ash content of the whole body
45 did not differ significantly ($P > 0.05$) among the groups.

46 Efficient protein synthesis requires sufficient availability of all essential amino
47 acids. Unbalanced amino acid concentrations in diet resulted in increased protein
48 degradation, and thereby increased protein turnover (Martin, Vilhelmsson, Mèdale,
49 Watt, Kaushik & Houlihan 2003). Cheng, Hardy and Usry (2003) reported that the plant
50 protein (SBM) based diets lower nitrogen retention in fish because these diets have less
51 digestible energy and an amino acid profile that is suboptimal for muscle growth.
52 Interestingly in our study whole body protein content was higher in DJKM fed groups.
53 Similarly Hasan *et al.* (1997) and Mazurkiewicz (2009) also found that the body protein
54 content increased significantly ($P < 0.05$) when plant protein replaced FM in common
55 carp diet. The similar profile of amino acid content in DJKM (Table 3) and FM along
56 with supplementation of lysine in test diets might resulted in the increased protein
57 accretion in test groups J_{50} and $J_{62.5}$ compared to control group. In addition, proper
58 combination of FM along with plant protein efficiently increased protein retention in
59 carp (Jahan, Watanabe, Kiron & Satoh 2003). This indicates that DJKM containing
60 diets contained optimum digestible energy and balanced amino acid profile optimal for
61 carp growth.

62

63 **Table 5** Chemical composition of whole body of common carp (*Cyprinus carpio* L.)
64 juveniles of different experimental groups at the start and at the end of the
65 experiment (% wet basis ± SD)
66

Treatment	Moisture	Crude protein	Crude lipid	Ash	Gross energy (kJ/g)
Initial fish	78.1 ± 0.41	14.5 ± 0.08	3.6 ± 0.21	3.4 ± 0.14	4.4 ± 0.11
C_{ontrol}	75.2 ± 0.65	16.3^{b} ± 0.34	5.8^{a} ± 1.13	2.4 ± 0.24	5.6 ± 0.39
J_{50}	75.8 ± 2.08	17.3^{a} ± 0.26	5.1^{a} ± 1.57	2.5 ± 0.36	5.2 ± 0.82
$J_{62.5}$	76.1 ± 0.87	17.5^{a} ± 0.58	3.7^{b} ± 0.17	3.0 ± 0.21	4.8 ± 0.19
SEM	0.41	0.22	0.42	0.12	0.19

67 Values are mean (n = 4) ± standard deviation.
68 Mean values in the same column with different superscript differ significantly (P < 0.05).
69

70 *Digestibility measurement and efficiency of digestible nutrients and energy*

71

72 Apparent digestibility coefficient of dry matter, protein, lipid and energy were highest
73 in C_{ontrol} group, followed by J_{50} and $J_{62.5}$ groups; all being significantly different (Table
74 6). Growth performance and nutrient utilization parameters were not statistically
75 different (P > 0.05) amongst the groups although a numerically descending trend was
76 noticed as DJKM increased in the carp diets. It is possible that this lack of statistical
77 difference was because of high variability of replicates within the treatment groups. The
78 decrease in digestibility coefficients in J_{50} and $J_{62.5}$ might have contributed to decrease
79 in growth performance and nutrient utilization.
80 Generally, oil seed meal proteins have digestibilities of 80-95% for fish
81 (Jauncey & Ross 1982) and carp are also reported to be able to digest the plant proteins
82 well, generally slightly better than monogastric animals (National Research Council
83 1983). The protein digestibility coefficient is a key factor in the evaluation of the quality
84 of the diet for fish and the potential of the diet for the synthesis of new tissues. DJKM in
85 combination with FM protein showed excellent dry matter, crude protein, lipid and
86 energy digestibility in the present study. Dry matter, protein, lipid and energy
87 digestibility of experimental diets were 70-75%, 79-86%, 80-90% and 73-82%
88 respectively, which indicate excellent utilization of feed ingredients. Hasan *et al.* (1994)

89 reported that apparent protein digestibility values ranged between 68.3-72.9% for carp
90 fed plant protein (leucaena leaf meal) based diets. In the present study, crude protein
91 digestibility of DJKM diets were high (above 79%) in common carp suggested DJKM
92 to be an excellent protein source for carp diet. Energy digestibility of DJKM protein
93 based diets was considerably lower than protein digestibility due to their high
94 carbohydrate content (Gouveia, Oliva Teles, Gomes & Rema 1993). The lower lipid
95 digestibility of the fish fed the DJKM based diets may be associated with the increase in
96 the NSP content, which reduces fat absorption by disturbing micelle formation in the
97 gastro intestinal tract (Øverland, Sørensen, Storebakken, Penn, Krogdahl & Skrede
98 2009).

99 Efficiency values of digestible nutrients and energy indicate retained nutrients
100 and energy in whole body relative to the total digestible nutrients and digestible energy
101 ingested. Efficiency of digestible nutrients and energy of diets did not differ
102 significantly among the three groups, indicating that common carp has utilized DJKM
103 well and retained nutrients in the body maximally and similar to control group. The
104 value for the efficiency of digestible protein, lipid and energy were in the range of 28-
105 31%, 37-41% and 18-25% respectively (Table 6).

106 **Table 6** Effects of experimental diets on the dry matter, nutrient and energy digestibility (%); digestive enzymes activities (U/mg protein);
107 and utilization of digestible protein, lipid and energy in common carp (*Cyprinus carpio* L.) fingerlings

Treatment	**Dry matter digestibility**	**Protein digestibility**	**Lipid digestibility**	**Energy digestibility**	**Amylase**	**Protease**	**Lipase**	**DPE (%)**	**DLE (%)**	**DEE (%)**
C_{ontrol}	74.9[a] ± 0.98	85.9[a] ± 0.98	89.6[a] ± 0.51	82.0[a] ± 1.62	17.4[a] ±1.32	36.5[a] ± 1.31	6.8[a] ± 0.29	30.8 ± 4.95	40.8 ± 7.11	24.5 ± 2.85
J_{50}	71.5[b] ± 0.64	83.2[b] ± 0.80	86.2[b] ± 0.76	77.7[b] ± 1.83	13.6[b] ± 0.83	28.1[b] ± 0.83	5.3[b] ± 0.32	31.4 ± 0.41	49.8 ± 17.3	22.7 ± 4.38
$J_{62.5}$	69.6[b] ± 1.32	78.6[c] ± 0.37	80.1[c] ± 1.65	73.4[c] ± 0.49	11.0[c] ± 0.76	20.7[c] ± 1.24	4.3[c] ± 0.22	27.8 ± 4.92	36.6 ± 4.32	18.3 ± 3.74
SEM	0.83	1.09	1.43	1.35	0.84	1.96	0.31	1.29	3.75	1.42

108 DPE: digestible protein efficiency; DLE: digestible lipid efficiency; DEE: digestible energy efficiency
109 Values are mean (n = 4) ± standard deviation.
110 Mean values in the same column with different superscript differ significantly ($P < 0.05$).

111 *Digestive enzyme activities and intestinal index*

112

113 The results of digestive enzymes revealed that the activities are altered significantly due
114 to replacement of FM by DJKM (Table 6). Digestive enzyme (amylase, protease and
115 lipase) activities were highest in C_{ontrol} group, followed by J_{50} and $J_{62.5}$ groups; all being
116 significantly different. Heat labile antinutrients (trypsin inhibitors and lectins) were not
117 detected in the DJKM, whereas heat stable enzyme inhibitor (phytate) was present in
118 DJKM. Phytate inhibits activities of digestive enzymes such as pepsin, trypsin and
119 alpha-amylase (Alarcon, Moyano & Diaz 1999), or form complexes with minerals
120 (Sugiura, Raboy, Young, Dong & Hardy 1999) and proteins (Moyano, Martinez, Diaz
121 & Alarcon 1999), thereby modifying digestion processes and this could impair intestinal
122 absorption. Carp showed a significant decrease in protease, amylase and lipase activities
123 in intestine on inclusion of plant proteins in the diet. Lower protease activities, the level
124 of which decreased with increase in DJKM in common carp diets, corresponded to
125 decrease in protein availability from DJKM. Similar results were observed by Santigosa
126 *et al.* (2008). They found that protein digesting enzyme (trypsin) activity decreases as
127 plant protein inclusion increases in trout diet and they concluded that trypsin is highly
128 sensitive to plant antinutrients. Escaffre, Zambonino Infante, Cahu, Mambrini, Bergot
129 and Kaushik (1997) observed that increasing levels of dietary soya protein concentrate
130 induced a significant decline in trypsin activity in common carp. The decrease in
131 protease activity at higher inclusion level of DJKM might be caused by the presence of
132 phytate (Kumar *et al.* 2009). The lower activity of digestive enzymes in DJKM fed
133 groups was correlated with lower nutrient digestibility.

134 It is known that carnivorous and omnivorous fish require longer time to digest
135 plant protein based diets (Buddington, Krogdahl & Bakke-McKellep 1997). Direct
136 relationship between the amount of dietary plant protein and intestinal index has been
137 reported earlier in fish (Kramer & Bryant 1995, Kumar *et al.* 2009). In carp, DJKM
138 based diets exhibited higher intestinal index than the control group. Intestinal index
139 value increases as the plant protein inclusion increases in the common carp and trout
140 diets (Santigoga, Sánchez, Médale, Kaushik, Pérez-Sánchez & Gallardo 2008; Kumar *et*
141 *al.* 2009). From a physiological view point, a longer intestinal index would facilitate an
142 increase in retention time and digestibility by enhancing contact time of the digestive

143 enzymes and the feed components, resulting in increase in their digestion and
144 absorption. Omnivorous fish like common carp species showed compensation
145 mechanisms, such as an increase in intestinal index and as a result increase in digestive
146 activity, to achieve a digestive balance and growth rates similar to those observed for
147 FM fed group.
148
149 *Cholesterol and triglycerides, muscle lipid peroxide; and blood glucose level*
150
151 Cholesterol and triglyceride levels in plasma, and muscle cholesterol concentration were
152 highest in C_{ontrol} group, followed by J_{50} and $J_{62.5}$ groups; all being significantly different
153 whereas muscle lipid peroxide value did not differ significantly ($P > 0.05$) among the
154 three groups (Table 7). Detoxified *Jatropha* kernel meal reduced cholesterol level in
155 plasma and muscle as compared to control group. The decrease in plasma cholesterol
156 concentrations in fish fed diets with plant proteins is in accordance with the results of
157 Yamamoto, Suzuki, Furuita, Sugita, Tanaka and Goto (2007) and Kumar *et al.* (2008).
158 In terrestrial animals, plant products are generally considered to have a
159 hypocholesteromic effect, mainly due to the relatively high levels of estrogeno-mimetic
160 isoflavones (Setchell & Cassidy 1999). In humans, different plant constituents have
161 been reported to lower plasma cholesterol concentration (Wester 2000). Although
162 cholesterol metabolism in animals and fish could differ, the fish hypocholesterolemia in
163 response to dietary plant protein supply could be due either to an increased excretion of
164 bile salts, to an inhibition of cholesterol intestinal absorption, or just to the withdrawal
165 of FM rather than to the direct effects of plant protein (Kaushik, Coves, Dutto & Blanc
166 2004). Serum triglycerides act as a short-term indicator of feeding or nutritional status
167 (Bucolo & David 1973). The decrease in whole body fat content in plant protein fed
168 group ($J_{62.5}$) along with the decrease in plasma triglyceride concentrations also suggest
169 lipid mobilisation in these groups. Shimeno, Kumon, Ando, Mima and Ueno (1993)
170 observed similar trend in the yellowtail. Blood glucose level was not affected by dietary
171 treatments. The blood glucose concentrations were similar in all groups (Table 8).
172 Similar trend was shown in fish fed diets containing yellow lupin as a substitute for FM
173 (Glencross, Carter, Duijster, Evans, Dods, McCafferty, Hawkins, Maas & Sipsas 2004).

On the other hand, Kikuchi (1999) observed that dietary inclusion of SBM and corn gluten in fish diet increased blood glucose level.

Blood chemistry

The WBC counts and Hb content did not differ significantly (P > 0.05) among the three groups (Table 8). RBC counts were higher in DJKM fed groups whereas MCV, MCH, MCHC exhibited opposite trend (Table 8). As the DJKM content increased, an increase in the RBC count was observed. Plant ingredients may cause early release of immature erythrocytes (Hemre, Sanden, Bakke-Mckellep, Sagstad & Krogdahl 2005), increasing the RBC count. Consequently, MCV value was changed at the same time. Lower MCV was observed in DJKM protein fed groups than control group (Table 8). Similarly, significant reduction of MCV on increase in the content of plant proteins in salmon diet was observed (Hemre *et al.* 2005). As this observation appeared to coincide with increased spleen size (Hemre *et al.* 2005), it was suggested that some of the plant ingredients might cause early release of immature erythrocytes. The Hb and Hct assays are normally used as general indicators of fish health (NRC 1981). Hematocrit level in all groups was within the normal range and did not differ significantly (P > 0.05) among the groups (Sun, Chen & Chang 1995). In the present study higher Hct content was observed in DJKM fed groups, which is in contrast with the observation of Soltan, Hanafy and Wafa (2008), who observed that FM protein replaced by mixture of plant proteins in Nile tilapia diets that led to lower Hct content. This was attributed to the binding of phytate to minerals (iron) and/or amine group of amino acids causing their low availabilities in the body and increase in erythrocyte fragility.

199 **Table 7** Cholesterol and triglyceride (mg/dl) level in plasma; and muscle cholesterol (mg/100 g) level and muscle lipid peroxide (nMol
200 MDA (malondialdehyde) /100g tissue) level, intestinal index (II) (mm g^{-1}), hepatosomatic index (HSI) and intestinalsomatic index (ISI) of
201 common carp (*Cyprinus carpio* L.) fingerlings

Treatment	**Plasma cholesterol**	**Plasma triglycerides**	**Muscle cholesterol**	**Muscle lipid peroxide**	**Intestinal index**	**HSI**	**ISI**
Control	$152^{a} \pm 5.4$	$102^{a} \pm 4.5$	$141^{a} \pm 22$	1.98 ± 0.89	$2.24^{c} \pm 0.07$	1.4 ± 0.12	1.7 ± 0.29
J_{50}	$131^{b} \pm 6.4$	$88^{b} \pm 5.2$	$112^{b} \pm 14$	2.21 ± 1.13	$2.78^{b} \pm 0.10$	1.3 ± 0.17	1.9 ± 0.33
$J_{62.5}$	$108^{c} \pm 7.2$	$72^{c} \pm 6.9$	$86^{c} \pm 18$	2.56 ± 1.12	$3.17^{a} \pm 0.10$	1.1 ± 0.03	2.1 ± 0.31
SEM	2.41	2.55	6.34	0.84	0.17	0.06	0.11

202 Values are mean (n = 4) ± standard deviation.
203 Mean values in the same column with different superscript differ significantly ($P < 0.05$).

204 The concentration of total protein in blood is used as a basic index for health and
205 nutrititional status in fish (Martinez 1976). Among the blood proteins, albumin and
206 globulin are the major proteins that play a significant role in the immune response.
207 Albumin is used as an indicator of liver impairment (Silverman, Christenson & Grant
208 1986). Blood protein did not differ significantly ($P > 0.05$) among the three groups
209 (Table 8), indicating that there are no nutritional deficiencies and no impaired protein
210 metabolism in the liver.

211 Lysozyme has an important role in nonspecific immune response and it is found
212 in mucus, serum and ova of fish. Innate immunity due to lysozyme is caused by lysis of
213 bacterial cell wall and this stimulates the phagocytosis of bacteria. The suppression of
214 the non-specific immune capacity by high concentrations of dietary soybean proteins
215 has been reported in rainbow trout (Burrells, Williams, Southgate & Crampton 1999).
216 However, other reports wherein SBM was fed to rainbow trout and Atlantic salmon or
217 alginate to Atlantic salmon, increased values of different non-specific immune
218 parameters have been reported, which have been interpreted as immunostimulating
219 effects of plant protein sources (Krogdahl, Bakke-McKellep, Røed & Bæverfjord 2000).
220 In the present study lysozyme activity was statistically not different ($P > 0.05$) amongst
221 the groups (Table 8) but numerically it was higher in DJKM fed groups, indicating
222 immunostimulating effect of DJKM in common carp.

223 **Table 8** Effects of experimental diets on the haematological parameters (RBC (10^6 cells/mm^3), WBC (10^3 cells/mm^3), Hb (g/dl), Hct (%),
224 MCV (fL), MCH (pg), MCHC (g/dl), albumin (g/dl), globulin (g/dl) and total protein (g/dl) in the blood and lysozyme activity (IU/ml) in
225 the serum of common carp (*Cyprinus carpio* L.) fingerlings

Treatment	RBC	WBC	Hb	Hct	MCV	MCH	MCHC	Albumin	Globulin	Total protein	Lysozyme activity
C_{ontrol}	$1.32^c \pm 0.02$	0.78 ± 0.04	5.0 ± 0.00	$32^b \pm 5.10$	$380^a \pm 6.3$	$37.9^a \pm 0.63$	$17.7^a \pm 1.70$	2.25 ± 0.48	0.53 ± 0.10	2.78 ± 0.39	404 ± 98
J_{50}	$1.41^b \pm 0.06$	0.79 ± 0.06	5.2 ± 0.50	$40^{ab} \pm 12.31$	$372^a \pm 28.3$	$37.2^a \pm 2.83$	$13.6^{ab} \pm 4.34$	2.05 ± 0.71	0.78 ± 0.26	2.83 ± 0.50	505 ± 122
$J_{62.5}$	$1.52^a \pm 0.06$	0.77 ± 0.06	5.0 ± 0.00	$45^a \pm 4.57$	$330^b \pm 12.8$	$33.0^b \pm 1.28$	$11.3^b \pm 1.18$	2.13 ± 0.05	0.75 ± 0.10	2.88 ± 0.13	500 ± 54
SEM	0.03	0.01	0.10	2.81	11.41	0.81	1.08	0.18	0.06	0.10	26

226 Values are mean (n = 4) ± standard deviation.

227 Mean values in the same column with different superscript differ significantly ($P < 0.05$)

228 MCV: Mean cell volume (fL); MCH: Mean corpuscular hemoglobin (pg); MCHC**:** Mean corpuscular hemoglobin concentration (g/dl)

229 IU- The amount of enzyme required producing a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25^0C, using
230 a suspension of *Micrococcus lysodeikticus* as the substrate.

231 *Metabolic enzymes and blood ions*

232

233 Alkaline phosphatase and ALT are released into blood during organ damage (Racicot,
234 Gaudet & Leray 1975). Alkaline phosphatase level rises during bile duct obstruction,
235 and in intrahepatic infiltrative diseases of the liver. Alanine transaminase also called
236 serum glutamic pyruvate transaminase is an enzyme present in hepatocytes (liver cells).
237 When liver cell is damaged, it leaks into the blood. Alanine transaminase rises
238 dramatically in acute liver damage (Racicot *et al.* 1975). Thus, detection of blood level
239 of ALP and ALT gives information on the damage of organs and in particular of liver
240 cells. Concentrations of ALP and ALT were similar in all the diets (Table 9), indicating
241 normal organ function on feeding of DJKM. Hemre *et al.* (2005) and Sanden, Krogdahl,
242 Bakke-McKellup, Buddington and Hemre (2006) also reported similar results on
243 feeding SBM containing diets to fish.

244 Blood urea nitrogen concentrations were in the normal ranges (Wedemeyer
245 1996). Blood urea nitrogen concentrations are thought to be associated with liver or gill
246 dysfunction (Stoskopf 1993), as these are the sites of urea production and excretion,
247 respectively. Blood urea nitrogen concentration did not differ significantly ($P > 0.05$)
248 among the three groups (Table 9), suggesting that DJKM fed groups were normal and
249 healthy. Total bilirubin is an indicator of liver dysfunction (Tietz 1986) was similar for
250 all groups. Creatinine used as an indicator of kidney damage or malfunction (Tietz
251 1986). Creatinine is a degraded product of creatine, which is involved in muscle energy
252 metabolism. Blood creatinine is normally quite stable. Its level in the blood becomes
253 elevated if kidney function is impaired. Creatinine was highest in control group but it
254 was within the normal range (Tietz 1986). Creatinine is a metabolite of animal protein
255 and its highest level in control is due to highest content of FM in this group (Table 9).
256 DJKM based diets were supplemented with phytase that leads to increased release of
257 phosphorus, calcium and other ions from feed and making them available for common
258 carp. Similar blood ions were observed in DJKM fed groups and control group (Table
259 9). Our preliminary histological findings demonstrate that common carp did not show
260 any abnormal changes in intestine and liver.

261 **Table 9** Effects of experimental diets on alkaline phosphatase (ALP, U/l), alanine transaminase (ALT, U/l), glucose (mg/dl), total
262 bilirubin (TBIL, mg/dl), blood urea nitrogen (BUN, mg/dl) and creatinine (mg/dl) in blood, blood ions (calcium (mg/dl), phosphorus mg/dl,
263 sodium (mmol) and potassium (mmol) of common carp (*Cyprinus carpio* L.) fingerlings

Treatment	**ALP**	**ALT**	**Glucose**	**TBIL**	**BUN**	**Creatinine**	**Calcium**	**Phosphorus**	**Sodium**	**Potassium**
C_{ontrol}	61 ± 6.0	80 ± 17.2	115 ± 14	0.30 ± 0.00	3.75 ± 0.50	1.25^{a} ± .42	10.15 ± 0.93	8.03 ± 4.15	131 ± 2.63	5.9 ± 2.86
J_{50}	65 ± 29.6	63 ± 16.1	118 ± 31	0.28 ± 0.05	4.75 ± 0.96	0.68^{b} ± 0.15	10.15 ± 0.45	9.03 ± 2.23	132 ± 4.57	3.6 ± 2.29
$J_{62.5}$	85 ± 24.4	65 ± 13.1	127 ± 33	0.28 ± 0.05	3.75 ± 0.96	0.56^{b} ± 0.00	10.38 ± 1.16	11.38 ± 1.44	131 ± 2.38	2.3 ± 1.65
SEM	6.64	4.66	7.29	0.01	0.06	0.25	0.24	0.85	0.89	0.75

264 Values are mean (n = 4) ± standard deviation.

265 Mean values in the same column with different superscript differ significantly ($P < 0.05$).

266 1 U = 16.66 nKat/l; nKat = Amount of glandular kallikrein which cleaves 0.005 mmol of substrate per minute).

Conclusions

The results of the long term feeding study showed that the detoxified *Jatropha* kernel meal fed groups exhibited good growth performance (almost five times increased in fish body mass after 16 weeks). Also biochemical and hematological parameters were within the normal ranges and similar to those in control group (fish meal fed group). The current study demonstrates that the DJKM can be used as one of the promising fish meal replacers in the diets of common carp. It can replace 50% of FM protein without sacrificing fish yield.

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3.1.1.3 Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio* L.) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal

Introduction

Traditionally, fish meal (FM) has been the main source of dietary protein for fish. In recent years, its increasing cost, decreasing availability in the market and poor quality have stimulated several studies on its partial or complete substitution with alternative protein sources (Kaushik et al., 1995; Fournier et al., 2004). Soybean meal (SBM) is currently the most commonly used plant protein source in fish feeds (Yue and Zhou, 2009). The price of SBM, which is the main protein source for cultured animals, has increased sharply (Azaza et al., 2009). Nowadays, maize is using as an energy source in fish to reduce feed cost and also being used to satisfy the rising demand of the fast-growing bio-fuels industry (Azaza et al., 2009).

According to FAO (2008), the price of SBM increased to new record level, an increase of 60% from early May 2007. Based on the current supply and demand forecasts for the coming years, prices can be expected to remain high. This phenomenon has limited the expansion and profitability of aquaculture enterprises in most developing countries (Tacon, 2007). In many countries, SBM and soybean oil used in feed formulation are imported, which increase feed costs. This is the case in Europe and many tropical countries, especially in sub-Saharan Africa where soybean production is fairly limited due mainly to climatic and geographical constraints.

Most modern, nutrient-dense, aquaculture diets use some inclusion of plant protein ingredients. Many such ingredients have been assessed experimentally, but with the notable exception of SBM, few are used commercially (Carter and Hauler, 2000). The high cost of protein sources, their restricted availability and the unpredictability of their markets, increase the need for alternative sources of protein in fish feed.

Jatropha curcas (L.) (physic nut) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. It is a hardy plant, thrives on degraded land and requires limited amounts of nutrients and water. The International *Jatropha* Organization has projected that in 2017 there will be around 32.72 million hectares of land cultivated worldwide with *J. curcas,* producing 160 million tons of seeds and 95% of the total production will be in Asia (Siang, 2009). *Jatropha* seeds have been extensively investigated

as a source of oil. The seed kernel contains about 60% oil that can be converted into biodiesel of high quality upon transesterification and used as a substitute for diesel fuel (Makkar et al., 2007). The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 58 to 62 % crude protein (Makkar et al., 2008). The levels of essential amino acids, except lysine in *J. curcas* kernel meal are higher than in SMB (except lysine). However, the presence of high levels of antinutrients such as trypsin inhibitor, lectin and phytate (Makkar et al., 2008) and the major toxic components phorbol esters (PE_S) (Makkar and Becker, 1997) restrict their use in fish feed. Heat labile antinutrients, protease inhibitors, and lectins are easy to inactivate by moist heating (Makkar and Becker, 2009). A method for detoxification of *Jatropha* kernel meal has been developed in our laboratory. It is based on extraction of PEs using organic solvents and inactivation of trypsin inhibitors and lectin by heat treatment.

Jatropha plant can yield up to four tons seed per year from one hectare of plantation, which can produce approximately one ton of kernel meal rich in protein (Makkar and Becker, 1997). This means that there is a possibility of producing enough *Jatropha* kernel meal to meet growing aquaculture industry demand. Our previous studies have shown that detoxified *Jatropha* kernel meal (DJKM) is a good protein source for carp (*Cyprinus carpio*) (Kumar et al., 2008) and rainbow trout (*Oncorhynchus mykiss)* diets (Kumar et al., 2009). This study reports the physiological, haematological and histological responses of adding DJKM and SBM to common carp diets.

Material and methods

Preparation of the Jatropha meal

Jatropha seeds were obtained from India and deshelled manually to obtain kernels. Defatting of *Jatropha* kernels was done using petroleum benzene (b.p. 40–60 ^{0}C) in a Soxhlet apparatus. Organic solvents were used to detoxify defatted *Jatropha* kernel meal. Two durations of PE removal were investigated: shorter (30 min) and longer (60 min) and the detoxified meals so obtained were designated as J_a and J_b respectively (patent application has been filed for the detoxification process). After removal of PEs, the meal was autoclaved (121 ^{o}C) to remove heat labile antinutrients, trypsin inhibitor and lectin.

Diet formulation

Fish meal (Seelöwe fishmeal) was procured from Vereinigte Fischmehlwerke Cuxhaven GmbH & Co KG, Cuxhaven, Germany; and wheat meal was purchased from a local market. Extracted soybean meal (dehulled, defatted and roasted) was obtained from the Institute of Animal Nutrition (450), University of Hohenheim, Stuttgart, Germany. Soya protein isolate (SUPRO® 500E IP) was purchased from Solae Europe S.A., 2, Chemin du Pavillon, CH-1218 Le Grand-Saconnex, Geneva, Switzerland. Prior to feed formulation, the proximate composition of defatted *Jatropha* kernel meal, wheat meal, SBM, soya proteins isolate and FM was determined. A total of seven isonitrogenous and isoenergetic diets were formulated. Experimental diets containing crude protein 38%, crude lipid 8%, vitamin premix 2%, mineral premix 2% and TiO_2 1% were prepared. Lysine monohydrochloride (lysine 80% in this salt) was supplemented at the rate of 1% of DJKM inclusion in the diet. Each experimental feed, except the control, contained 500 FTU phytase (NATUPHOS 5000G, BASF, Ludwigshafen) per kg. The inclusion levels of the DJKM and SBM were as follows: Control diet was prepared with FM and wheat meal, without any DJKM and SBM. S_{50}: 50% of FM protein replaced by SBM; S_{75}: 75% of FM protein replaced by SBM; J_{a50}: 50% of FM protein replaced by DJ_aKM; J_{a75}: 75% of FM protein replaced by DJ_aKM; J_{b50}: 50% of FM protein replaced by DJ_bKM; J_{b75}: 75% of FM protein replaced by DJ_bKM. The final mixture of each diet was made to 2 mm diameter moist pellets and then freeze-dried (Table 1).

Table 1 Composition of the experimental diets (g kg^{-1} feed)

Ingredients	Experimental diets						
	Control	J_{a50}	J_{a75}	J_{b50}	J_{b75}	S_{50}	S_{75}
Fish meal	507.5	253.7	126.3	253.7	126.3	253.7	126.3
Soyabean meal	-	-	-	-	-	342.1	513
[1]Wheat meal	402.5	381.5	372	390	384.1	271	206
Jatropha meal	-	249.5	372	242.5	361.9	-	-
Soya concentrate	-	3.5	7	2	5	22	32
Sunflower oil	40	61.8	72.7	61.8	72.7	61.2	72.7
[2]Vitamin premix	20	20	20	20	20	20	20
[3]Mineral premix	20	20	20	20	20	20	20
TiO_2	10	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000	1000
Phytase (FTU/kg)	-	500	500	500	500	500	500
Lysine monohydrochloride (g)	-	2.5	3.7	2.4	3.6	-	-

Control: FM and wheat meal, without any DJKM and SBM
J_{a50}: 50% of fish meal protein replaced by detoxified *Jatropha* kernel meal (30 min)
J_{a75}: 75% of fish meal protein replaced by detoxified *Jatropha* kernel meal (30 min)
J_{b50}: 50% of fish meal protein replaced by detoxified *Jatropha* kernel meal (60 min)
J_{b75}: 75% of fish meal protein replaced by detoxified *Jatropha* kernel meal (60 min)
S_{50}: 50% of fish meal protein replaced by soybean meal
S_{75}: 75% of fish meal protein replaced by soybean meal
[1]Whole wheat meal.
[2]Vitamin premix (g or IU kg^{-1} premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
[3]Mineral premix (g kg^{-1}): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4 \cdot 7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4 \cdot H_2O$, 3.12; $ZnSO_4 \cdot 7H_2O$, 4.67; $CuSO_4 \cdot 5H_2O$, 0.62; KI, 0.16; $CoCl_2 \cdot 6H_2O$, 0.08; ammonium molybdate, 0.06; 3 $NaSeO_3$, 0.02.

Experimental system and animals

Common carp (*Cyprinus carpio* L.) fingerlings (about 2.0–3.0 g) from the Institute of Fisheries Ecology and Aquaculture of the Federal Research Center for Fisheries at Ahrensburg, Germany, were transferred to the University of Hohenheim, Stuttgart, Germany and kept in two 500 l capacity tanks for acclimatisation. They were fed a standard fish diet containing approximately 38% protein, 8% lipid, 10% ash and with a gross energy content of 18.5 kJ g^{-1} dry matter. After an acclimatisation period of 20 days, 252 fish were randomly distributed into seven groups with four replicates; each replicate contained nine fish in an

aquarium (45 l capacity). All the aquaria were supplied with water from a recirculatory system. The system was subjected to a photoperiod of 12 h light : 12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 26.2–27.1°C, pH 7.0–7.5, dissolved oxygen 6.9–7.4 mg l^{-1}, total NH_3 0.1–0.2 mg l^{-1}, nitrite 0.07–0.1 mg l^{-1} and nitrate 1–3 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation above 80%. One day before start of the experiment, the fish were starved and during the experimental period fish were fed at 16 g feed per kg metabolic body mass ($kg^{0.8}$) per day (equal to five times their maintenance energy requirement). No feed was left in the aquaria when the feeds at five times maintenance energy requirement were offered. Since the aim of the study was to evaluate the performance of fish fed diets containing DJKM, high level of feed consumption was preferred, in order to elicit adverse effects if any due to the presence of the detoxified kernel meal.

Total feed per day was split into five equal portions and each portion was given at 8:00, 10:30, 13:00, 15:30 and 18:00 h. The feeds were dispensed using an automatic feeder. Fish were weighed individually at the beginning of the experiment (av. wt. 3.2 ± 0.07 g) and at weekly intervals during the experimental period to adjust the feeding level for the subsequent week. The fish were not fed on the weighing day.

The experiment was terminated after 8 weeks and the fish were killed. At the end of experiment, fish were anaesthetized by tricaine methanesulfonate (MS222) at 250 ppm in water. Blood was drawn near caudal peduncle from two fish from each replicate and transferred into a heparinized tube. Blood from one fish was used for haematological study and the other was centrifuged at 1500×*g* for 5 min at room temperature (24 °C) to obtain plasma, which was then stored at –20 °C for determination of cholesterol and triglycerides. Blood was drawn from one fish per replicate for serum collection. Serum was stored at –20 °C for lysozyme determination. Fish, one per replicate were carefully dissected to isolate muscle and stored at –20 °C for determination of cholesterol and muscle lipid peroxides.

The University animal welfare committee (University of Hohenheim Germany) approved all experimental procedures involving common carp.

Extraction and estimation of phorbol esters (PEs) by high-performance liquid chromatography, and determination of antinutrients

PEs were determined according to Makkar et al. (2007), which was based on the method of Makkar et al. (1997). Briefly, 0.5 g of the *Jatropha* kernel meal and dried whole body fish sample were extracted four times with methanol. A suitable aliquot was loaded on a high-performance liquid chromatography (HPLC) reverse-phase C_{18} LiChrospher 100, 5 µm (250 x 4 mm I.D. from Merck (Darmstadt, Germany) column. The column was protected with a head column containing the same material. The separation was performed at room temperature (23 ^{o}C) and the flow rate was 1.3 ml/min using a gradient elution (Makkar et al., 2007). The four-PEs peaks were detected at 280 nm and appeared between 25.5 and 30.5 min. The results were expressed as equivalent to a standard, phorbol-12-myristate 13-acetate. Detection limit of PEs is 3µg/g meal.

Trypsin inhibitor activity was determined essentially according to Smith et al. (1980) except that the enzyme was added last as suggested by Liu and Markakis (1989). Analysis of the lectin content was conducted by haemagglutination assay (Makkar et al., 1997). The haemagglutination activity was expressed as the minimum amount of material (mg mL^{-1} assay medium) that produced agglutination. The minimum amount was the amount of material mL^{-1} assay medium in the highest dilution that was positive for agglutination; the lower this value, the higher the lectin activity. Phytate content of samples was determined by a spectrophotometric procedure (Vaintraub and Lapteva, 1988). Results were expressed as g phytic acid per 100 g material using phytic acid sodium salt (Sigma, St Louis, MO, USA) as standard. Non-starch polysaccharides (NSP) were estimated according to Englyst et al. (1994).

Amino acid analysis

The amino acid compositions of FM, DJKM, SBM, soya protein concentrate and wheat flour were determined using an automated amino acid analyser after hydrolysing the samples with 6 M HCl at 110 ^{0}C for 24 h (Bassler and Buchholz, 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The tryptophan contents of the above-mentioned samples were determined spectrophotometrically by the method of Pinter-Szakacs and Molnar-Perl (1990).

Proximate analysis

The proximate composition of diet ingredients and diets was determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Samples of FM were analysed for dry matter, ash, crude protein and lipid. Gross energy of diet ingredients and diets was determined with bomb calorimeter (IKA C7000) using benzoic acid as a standard.

Spleen Index (SI)

Spleen Index is calculated as indicated below:
Spleen Index = spleen mass (g) / body mass (g)

Growth parameters

Growth performance in terms of body mass gain (BMG), Specific growth rate (SGR) and metabolic growth rate (MGR) were calculated as follows
BMG = final body mass (g) - initial body mass (g)
SGR (% day^{-1}) = [(ln final body mass in g) - ln initial body mass in g) / number of trial days] X 100; MGR (g . $kg^{0.8}$. day^{-1}) = (Body mass gain in g) / [{(initial body mass in g / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)$^{\wedge 0.8}$}/2] / number of trial days (Dabrowski et al., 1986).

Cholesterol and triglyceride estimation

The determinations of the plasma cholesterol and triglycerides were using enzymatic colorimetric kits: Hitado Diagnostic system, Nobiflow cholesterin (kit lot number 60041889) and Nobiflow triglyceride-GPO (kit lot number 60040710) respectively. Muscle cholesterol content was determined using a kit (lot no. 10139050035) (Boehringer Mannheim, Germany). The color intensity was determined photometrically and was directly proportional to the concentration of cholesterol and triglycerides in the plasma sample.

Assay of lipid peroxides

Lipid peroxides in fish muscle were determined using the procedure of Utley et al. (1967).

Total erythrocyte count (RBC) and total leucocyte count (WBC)

Red blood cells and WBC were counted by Neubauer's counting chamber of haemocytometer. Care was taken to avoid trapping of air bubbles. The RBC lying inside the five small squares were counted under high power (40X) of light microscope.
The following formula was used to calculate the number of RBC per mm^3 of the blood sample:
Number of RBC/mm^3 = (N x dilution)/area counted x depth of fluid
N= Number of cells counted

2.12. Hemoglobin (Hb) and hematocrit (Hct) content

The Hb content of blood was analyzed by Reflotron Hemoglobin test (REF 10744964, Roche diagnostic GmbH, Manheim Germany). Hematocrit was determined on the basis of sedimentation of blood. Heparinised blood (50 µl) was taken in the hematocrit capillary (Na-heparinised) and centrifuged in the Hematocrit 210 Hettich Centrifuge (Tuttlingen Germany) to obtain hematocrit value.

From analyses of Hct, Hb and RBC, the following parameters were calculated: mean cell volume, MCV (fL) = (Hematocrit [%] x 1000) ÷ (RBC count [millions/µL]); mean cell hemoglobin, MCH (pg) = (Hemoglobin [g/dL] x 10 ÷ (RBC count [in millions/µL]) and mean cell hemoglobin concentration, MCHC [g/dL] = Hemoglobin [g/dL] ÷ Hematocrit [%].

Lysozyme activity in serum

Lysozyme activity of serum was measured by EnzChek Lysozyme Assay Kit (E-22013) Leiden, The Netherlands. The assay measures lysozyme activity on *Micrococcus lysodeikticus* cell wall, which is labeled to such a degree that the fluorescence is quenched. Lysozyme action relieves this quenching; yielding a dramatic increase in fluorescence that is proportional to lysozyme activity. The fluorescence increase was measured by using spectrofluorometer that detects fluorescein. Lysozyme hydrolyzes β-(1-4)-glucosidic linkages

between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues present in the mucopolysaccharide cell wall of the microorganism.

Blood parameters analysis by Vet Scan

VetScan Chemistry Analyzer (Scil Animal care company GmBh, Technischer service, Germany) was used for determinations of alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total calcium, creatinine, globulin, glucose, phosphorus, potassium, sodium, total bilirubin (TBIL), total protein, and urea nitrogen (BUN) in heparinized blood.

Histopathological studies

Immediately after killing the fish a total of 144 histological samples of different segments of the intestinal tract were fixed in Bouin´s fluid or in methanol/acetic acid for 48 h or 24 h respectively. The intestine was thereby subdivided in fore-, mid- and hindgut. After dehydration and embedding in paraffin serial sections of 5 µm thickness were prepared and processed for conventional histopathological studies. Haematoxylin and eosin (H&E) was used for staining the tissue sections.

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) and the significance of the differences between means was tested using Duncan's multiple range test ($P < 0.05$). The software used was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). Values are expressed as means ± standard deviation.

esults

Phorbol esters and antinutrient contents in defatted Jatropha kernel meal

Phorbol ester content in defatted *Jatropha* kernel meal was 1.6 mg/g. However PEs in the J_a and J_b detoxified *Jatropha* kernel meal was reduced to undetectable level. The sensitivity of the method is 5 µg/g kernel meal.

Trypsin inhibitor and lectins were not detected in SBM DJ_aKM and DJ_bKM. Phytate level in SBM, DJ_aKM and DJ_bKM was 9.5%, 9.3% and 2.5% respectively whereas NSP level in SBM, DJ_aKM and DJ_bKM was 14%, 16% and 16% on dry matter basis respectively.

Proximate composition, antinutrients and amino acid profile

Proximate composition and amino acid contents of feed ingredients are shown in Table 2. All experimental diets had almost similar amino acid composition. All the diets containing essential amino acid are essentially as per the requirement of the common carp (NRC, 1993). The proximate composition of the different experimental diets (% dry matter) is presented in Table 3. Diets contained about 38% crude protein and 18.5 MJ/kg gross energy. Dry matter, crude lipid and ash were in the range of 94.4–96.1%, 8.3–8.8% and 10.3–11.1% respectively.

Table 2 Composition of feed ingredients according to proximate and amino acid and non-starch polysaccharides analysis

	Fish meal	*Jatropha* meal (J_a)	*Jatropha* meal (J_b)	Soyabean meal	Soya protein concentrate	Wheat meal
Crude nutrients (g kg $^{-1}$)						
Dry matter	940	941	945	955	940	941
Crude protein	635	646	665	471	900	143
Crude lipid	88	13.2	11.4	11.7	10	16.3
Crude ash	142	125	137	21.4	4.0	1.4
Crude fibre		89	91	38	10	25
Gross energy (KJ/g)	21.1	18.5	18.3	18.2	-	18.7
Essential amino acids (g kg $^{-1}$)						
Arginine	35.3	67.4	69.7	36	67.9	5.4
Histidine	17.7	19.6	21.7	14.4	24.4	3.4
Iso leucine	22.8	24.3	26.7	19.6	36.5	4.2
Leucine	41.6	44.4	46.7	35.7	68.1	9.1
Lysine	40.9	19.7	23.3	29.1	52.1	3.3

Phenylalanine	21.8	27	30.4	24.3	43.2	6.5
Methionine	16	10.2	10.6	6.2	12.1	2
Threonine	23	20.3	22	17.8	31.1	3.7
Tryptophan	4.9	7	7.1	6.4	10.4	1.4
Valine	29.3	28.5	31.6	21.2	37.4	5.1
Non-essential amino acids (g kg $^{-1}$)						
Alanine	43.3	27	29.4	21.4	40.9	4.6
Asparginine	60.5	66.1	68.7	66.6	122.8	7.2
Cystine	4.3	2.2	2.3	6.5	9.8	2.9
Serine	25.5	27.3	30.6	24.4	46	6.3
Glutamine	79.4	99.4	112.1	93.8	174.9	44.9
Glycine	59.8	27.6	31.5	21.3	37.2	5.6
Tyrosine	14.8	15.5	18.8	15.8	31	3.3
Proline	36.9	30.5	32.2	28.2	50.2	14.5
Non-starch polysaccharides (NSP) (g kg $^{-1}$)						
Rhamnose	-	3	3	0	-	-
Fucose	-	1	1	0	-	-
arabinose	-	31	31	24	-	-
Xylose	-	20	20	11	-	-
Mannose	-	5	5	6	-	-
Galactose	-	14	14	42	-	-
Glucose	-	57	57	32	-	-
Glucuronic acid	-	0	0	0	-	-
Galacturonic acid	-	30	30	24	-	-
Total-NSP	-	160	160	140	-	-

J_a and J_b: detoxified *Jatropha* kernel meal obtained from shorter (30 min) and longer (60 min) duration of detoxification process respectively.

Table 3 Composition of experimental diets according to proximate and amino acid analysis (g kg^{-1} feed)

Treatment*	Control	J_{a50}	J_{a75}	J_{b50}	J_{b75}	S_{50}	S_{75}
Proximate (g kg^{-1})							
Dry matter	948	948	954	961	949	944	949
Crude protein	385	384	385	381	382	383	382
Crude lipid	87	86	86	88	87	83	85
Ash	105	108	111	102	100	110	103
Gross energy (kJ/g)	18.4	18.6	18.5	18.2	18.4	18.7	19.4
Essential amino acids (g kg^{-1})							
Arginine	20.09	28.07	32.02	28.10	32.10	24.23	26.21
Histidine	10.35	10.76	10.96	11.13	11.52	10.87	11.10
Iso leucine	13.26	13.58	13.74	13.97	14.34	14.43	14.97
Leucine	24.77	25.34	25.63	25.56	25.99	26.73	27.62
Lysine	22.09	19.23	17.79	19.82	18.73	22.37	22.44
Phenylalanine	13.68	14.90	15.52	15.52	16.47	16.56	17.94
Methionine	8.93	7.41	6.64	7.43	6.69	6.99	6.00
Threonine	13.16	12.42	12.05	12.68	12.44	13.61	13.79
Tryptophan	3.05	3.56	3.82	3.53	3.78	4.04	4.52
Valine	16.92	16.62	16.46	17.16	17.28	16.89	16.82
Non essential amino acids (g kg^{-1})							
Alanine	23.83	19.62	17.51	19.99	18.08	20.45	18.70
Asparginine	33.60	35.02	35.77	35.06	35.88	42.79	47.22
Cystine	3.35	2.78	2.51	2.80	2.54	4.32	4.79
Glycine	32.60	24.32	20.16	25.07	21.29	24.79	20.82
Glutamine	58.37	62.69	64.93	65.19	68.72	68.25	72.99
Proline	24.56	22.68	21.75	22.93	22.13	24.04	23.72
Serine	15.48	15.85	16.04	16.44	16.94	17.54	18.51
Tyrosine	8.84	8.99	9.08	9.66	10.10	10.74	11.65

Amino acid compositions of the experimental diets were calculated from amino acid profile of individual feed ingredients.

*See footnotes to Table 1.

Fish behaviour and feed intake

Based on the visual observation during feeding time, palatability or acceptability of feed was good and the behavior of fish was normal. There was no mortality during the entire experimental period. It was particularly noted that the fish in the groups J_{a50} and J_{a75} fed very slowly on their feed. Sometimes they ingested the feed pellets and spit them out after a few seconds and it was repeated 2 to 3 times before finally ingesting and swallowing the pellets, whereas fish in all other dietary groups fed actively on the experimental diets throughout the experiment.

Growth performance

Weekly body mass gains of fish are given in Figure 1A. The growth performance of the fish at the end of the experimental period is presented in Table 4. Highest body mass gain was observed for the J_{b50} group, which was statistically not different ($P > 0.05$) from that for control group and was higher ($P < 0.05$) than for all other groups.

Table 4 Growth performance of common carp (*Cyprinus carpio* L.) fed experimental diets for eight weeks

Treatment*	IBM (g)	FBM (g)	BMG (g)	SGR	MGR
Control	3.2 ± 0.1	32.0^{a} ± 1.96	28.9^{a} ± 1.95	4.1^{ab} ± 0.11	21.7^{a} ± 0.5
J_{a50}	3.2 ± 0.1	24.9^{d} ± 3.31	21.7d ± 3.24	3.7^{d} ± 0.21	19.3^{b} ± 1.18
J_{a75}	3.3 ± 0.0	20.9^{e} ± 2.04	17.7^{e} ± 2.03	3.3^{e} ± 0.18	17.6^{c} ± 0.93
J_{b50}	3.2 ± 0.1	33.3^{a} ± 0.64	30.1^{a} ± 0.63	4.2^{a} ± 0.05	22.0^{a} ± 0.18
J_{b75}	3.2 ± 0.1	28.3^{bc} ± 1.21	25.1^{bc} ± 1.25	3.9^{cd} ± 0.12	20.1^{b} ± 0.99
S_{50}	3.3 ± 0.1	30.6^{ab} ± 0.72	27.3^{ab} ± 0.68	4.0^{bc} ± 0.05	21.1^{ab} ± 0.20
S_{75}	3.2 ± 0.1	27.7^{c} ± 0.57	24.5^{c} ± 0.64	3.8^{cd} ± 0.08	20.3^{b} ± 0.32
SEM	0.01	0.88	0.88	0.06	0.31

Initial body mass (IBM), final body mass (FBM), body mass gain (BMG), specific growth rate (SGR, % day^{-1}), and metabolic growth rate (MGR, g . $kg^{0.8}$ .day^{-1}).
Values are means (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly (P < 0.05).
*See footnotes to Table 1.

Cholesterol and triglyceride levels in plasma, and cholesterol, phorbol esters and lipid peroxides in muscle

Cholesterol and triglycerides levels in plasma, muscle cholesterol and muscle lipid peroxide of different experimental groups are shown in Table 5. Plasma cholesterol level was highest in control group, which was statistically not different ($P > 0.05$) from those in S_{50} and J_{b50} groups and was higher than in other groups; whereas plasma triglyceride level was statistically lowest ($P < 0.05$) in control group. Muscle cholesterol level was highest in control group, which was statistically not different ($P > 0.05$) from that in S_{50} group and higher than those in other groups. Phorbol ester content in dried whole fish was undetectable. Muscle lipid peroxide value did not differ among the seven groups.

Table 5 Cholesterol and triglyceride in plasma (mg/dl), muscle cholesterol (mg/100 g), and muscle lipid peroxide (nmol malondialdehyde/100 g tissue) in common carp (*Cyprinus carpio*) fingerlings fed different experimental diets

Treatment*	Plasma cholesterol	Plasma triglycerides	Muscle cholesterol	Muscle lipid peroxide
Control	$144.4^{a} \pm 16.3$	$77.2^{b} \pm 7.7$	$142.5^{a} \pm 94.7$	1.60 ± 0.69
J_{a50}	$108.7^{bc} \pm 25.4$	$142.3^{a} \pm 39.9$	$71.8^{b} \pm 7.1$	2.20 ± 1.13
J_{a75}	$107.6^{bc} \pm 19.5$	$133.5^{a} \pm 10.0$	$62.6^{b} \pm 3.8$	2.70 ± 2.12
J_{b50}	$136.4^{ab} \pm 9.0$	$140.4^{a} \pm 8.0$	$66.6^{b} \pm 2.1$	3.83 ± 1.40
J_{b75}	$103.9^{bc} \pm 26.9$	$120.4^{a} \pm 31.1$	$76.8^{b} \pm 5.6$	2.95 ± 2.76
S_{50}	$129.9^{ab} \pm 31.6$	$150.8^{a} \pm 31.8$	$85.3^{ab} \pm 5.2$	1.57 ± 0.55
S_{75}	$93.1^{c} \pm 15.2$	$145.8^{a} \pm 23.7$	$71.3^{b} \pm 5.7$	1.52 ± 0.30
SEM	4.98	6.13	8.71	0.35

Values are means (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).
*See footnotes to Table 1.

Hemato-immunological blood parameters

Red blood cells, WBC, Hb level, Hct content, MCV, MCH, MCHC and spleen index are given in Table 6. White blood cells count and MCHC did not differ among the seven groups. Lowest MCV was observed in S_{75} group. Plant protein in the feed had significant effect on the total RBC count during the experimental periods. As the plant protein content

increased, an increase in the RBC count was observed. Statistically similar ($P > 0.05$) RBC counts were observed in S_{75}, J_{a75} and J_{b75} groups and these values were higher ($P < 0.05$) than those in other groups. Highest MCH was observed in J_{a50} group, which was statistically not different ($P > 0.05$) from those in control, S_{50}, J_{b50}, and J_{b75} groups but was higher ($P < 0.05$) than those in S_{50} and J_{a50} groups. Higher ($P < 0.05$) spleen index was observed in plant protein fed groups than control group.

Metabloic enzymes activities in blood

Highest ALP and ALT activities in blood were observed in J_{a75}, which was statistically not different ($P > 0.05$) from these in J_{a50} groups but were higher ($P < 0.05$) than those in other groups (Table 7).

Albumin, globulin and total protein concentration in the blood and lysozyme activity in serum

Albumin, globulin and total protein in the blood and lysozyme activity in the serum of different experimental groups are shown in Table 8. Highest albumin concentration in blood was observed in control group, which was statistically not different ($P > 0.05$) from that in J_{b50} group but was higher ($P < 0.05$) than those in other groups. Highest globulin concentration in blood was observed in J_{b50} group, which was statistically not different ($P > 0.05$) from that in S_{75} group but was higher ($P < 0.05$) than those in other groups. The concentration of total blood protein was highest in J_{a75} group, which was statistically not different ($P > 0.05$) to those in control, S_{50}, J_{a50}, and J_{b75} groups but was higher ($P < 0.05$) than those in other groups.

Table 6 Effects of experimental diets on haematological parameters [spleen index, RBC (10^6 cells/mm^3), WBC (10^5 cells/mm^3), Hb (g/dl), Hct (%), MCV (fL), MCH (pg), MCHC (g/dl)] of common carp (*Cyprinus carpio*)

Treatment*	*Spleen index*	RBC	WBC	Hb	Hct	MCV	MCH	MCHC
Control	$0.15^{b} \pm 0.01$	$1.58^{b} \pm 0.10$	1.43 ± 0.07	5.00 ± 0.00	29.75 ± 2.73	$189.3^{ab} \pm 4.16$	$31.8^{ab} \pm 1.99$	17.14 ± 2.79
J_{a50}	$0.16^{ab} \pm 0.02$	$1.67^{ab} \pm 0.16$	1.65 ± 0.16	5.48 ± 0.96	30.00 ± 2.57	$179.6^{ab} \pm 13.48$	$33.1^{a} \pm 7.07$	19.30 ± 1.02
J_{a75}	$0.17^{a} \pm 0.02$	$1.87^{a} \pm 0.03$	1.60 ± 0.03	5.00 ± 0.00	31.75 ± 1.71	$170.0^{ab} \pm 8.03$	$26.8^{b} \pm 0.47$	15.78 ± 0.83
J_{b50}	$0.16^{ab} \pm 0.01$	$1.74^{ab} \pm 0.20$	1.50 ± 0.08	5.00 ± 0.00	35.25 ± 5.74	$205.3^{a} \pm 43.06$	$29.1^{ab} \pm 3.37$	14.52 ± 2.73
J_{b75}	$0.16^{ab} \pm 0.02$	$1.85^{a} \pm 0.13$	1.85 ± 0.11	5.22 ± 0.43	31.75 ± 8.54	$172.0^{ab} \pm 7.61$	$28.2^{ab} \pm 0.74$	17.60 ± 1.93
S_{50}	$0.16^{ab} \pm 0.01$	$1.71^{ab} \pm 0.08$	1.73 ± 0.08	5.00 ± 0.00	32.25 ± 1.89	$189.5^{ab} \pm 18.53$	$29.3^{ab} \pm 1.35$	15.54 ± 0.87
S_{75}	$0.18^{a} \pm 0.01$	$1.87^{a} \pm 0.07$	1.75 ± 0.07	5.00 ± 0.00	27.50 ± 5.00	$147.6^{b} \pm 7.32$	$26.8^{b} \pm 0.97$	18.63 ± 1.32
SEM	0.01	0.03	0.13	0.07	1.02	6.61	0.67	0.70

Values are means (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$)

MCV: Mean cell volume (fL)

MCH: Mean corpuscular hemoglobin (pg)

MCHC**:** Mean corpuscular hemoglobin concentration (g/ dl)
*See footnotes to Table 1.

Table 7 Effects of experimental diets on alkaline phosphatase (ALP, U/l) and alanine transaminase (ALT, U/l) in blood of *Cyprinus carpio* L. fingerlings

Treatment*	ALP	ALT
Control	$85^{b} \pm 25.1$	$70^{bc} \pm 5.73$
J_{a50}	$159^{a} \pm 17.5$	$86^{ab} \pm 4.57$
J_{a75}	$162^{a} \pm 24.5$	$92^{a} \pm 6.63$
J_{b50}	$115^{b} \pm 52.1$	$80^{b} \pm 6.21$
J_{b75}	$75^{c} \pm 32.5$	$68^{c} \pm 3.62$
S_{50}	$107^{ab} \pm 21.8$	$77^{b} \pm 4.31$
S_{75}	$61^{b} \pm 24.2$	$71^{b} \pm 3.10$
SEM	5.02	2.61

Mean values in the same column with different superscript differ ($P < 0.05$).
Values are means (n = 4) ± standard deviation.
1 U = 16.66 nKat/l; nKat = Amount of glandular kallikrein which cleaves 0.005 mmol of substrate per minute.
*See footnotes to Table 1.

Table 8 Lysozyme activity (IU/ml) in the serum, albumin (g/dl), globulin (g/dl) and total protein (g/dl) in the blood of common carp (*Cyprinus carpio* L.) fingerlings fed different experimental diets

Treatment*	Lysozyme activity	Albumin	Globulin	Total protein
Control	336 ± 32.0	$1.98^{a} \pm 0.12$	$0.88^{c} \pm 0.17$	$2.85^{ab} \pm 0.13$
J_{a50}	328 ± 100.1	$1.73^{b} \pm 0.11$	$1.03^{bc} \pm 0.19$	$2.75^{b} \pm 0.17$
J_{a75}	287 ± 107.2	$1.43^{bc} \pm 0.28$	$1.18^{bc} \pm 0.17$	$2.63^{b} \pm 0.13$
J_{b50}	448 ± 172.9	$1.35^{c} \pm 0.19$	$1.75^{a} \pm 0.30$	$3.10^{a} \pm 0.20$
J_{b75}	402 ± 186.7	$1.63^{bc} \pm 0.35$	$1.20^{bc} \pm 0.12$	$2.80^{ab} \pm 0.24$
S_{50}	404 ± 101.9	$1.63^{bc} \pm 0.21$	$1.15^{bc} \pm 0.13$	$2.80^{ab} \pm 0.16$
S_{75}	457 ± 107.3	$1.43^{bc} \pm 0.10$	$1.45^{ab} \pm 0.31$	$2.87^{ab} \pm 0.34$
SEM	23.75	0.05	0.06	0.04

Values are means (n = 4) ± standard deviation.

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

IU- The amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25^{0}C, using a suspension of *Micrococcus lysodeikticus* as the substrate.

*See footnotes to Table 1.

Glucose, total bilirubin, blood urea nitrogen, creatinine and ions in blood

Concentration of blood glucose, TBIL, BUN, creatinine and ions (calcium, phosphorus, sodium and potassium) are shown in Tables 9 and 10. Lower ($P > 0.05$) blood glucose concentration was observed in control group than plant protein fed groups. As plant protein content increased in the diet, blood glucose concentration also increased. Highest BUN was observed in J_{a75} group; whereas, TBIL did not differ among the seven groups.

Creatinine concentration in blood was significantly highest in control group**.** Calcium and sodium ions in blood did not differ significantly among the seven groups; whereas, there was significant difference in potassium and phosphorus ions in blood among the different groups. Potassium and phosphorus ion concentrations in blood were higher ($P < 0.05$) in plant protein fed groups than control group.

Table 9 Effects of experimental diets on glucose (mg/dl), total bilirubin, TBIL (mg/dl), blood urea nitrogen, BUN (mg/dl) and creatinine (mg/dl) in blood of common carp (*Cyprinus carpio* L.) fingerlings

Treatment*	Glucose	TBIL	BUN	Creatinine
Control	$72.75^{b} \pm 4.03$	0.30 ± 0.00	$3.00^{c} \pm 0.41$	$1.35^{a} \pm 0.70$
J_{a50}	$86.75^{ab} \pm 8.62$	0.25 ± 0.06	$5.50^{b} \pm 0.29$	$0.25^{b} \pm 0.06$
J_{a75}	$97.50^{a} \pm 2.82$	0.25 ± 0.06	$8.50^{a} \pm 0.29$	$0.23^{b} \pm 0.05$
J_{b50}	$87.75^{ab} \pm 4.03$	0.22 ± 0.05	$4.50^{bc} \pm 0.58$	$0.33^{b} \pm 0.15$
J_{b75}	$97.75^{a} \pm 5.63$	0.22 ± 0.05	$5.00^{b} \pm 0.00$	$0.20^{b} \pm 0.00$
S_{50}	$91.25^{ab} \pm 8.81$	0.22 ± 0.05	$3.00^{c} \pm 0.41$	$0.40^{b} \pm 0.14$
S_{75}	$99.00^{a} \pm 2.46$	0.27 ± 0.05	$3.00^{c} \pm 0.15$	$0.25^{b} \pm 0.06$
SEM	2.95	0.01	0.40	0.09

Values are means (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).
*See footnotes to Table 1.

Table 10 Effects of experimental diets on blood ions [calcium (mg/dl), phosphorus mg/dl, sodium (mmol/l) and potassium (mmol/l)] of common carp (*Cyprinus carpio* L.) fingerlings

Treatment*	Calcium	Phosphorus	Sodium	Potassium
Control	10.43 ± 1.05	5.10^{b} ± 1.69	131.25 ± 3.86	1.50^{c} ± 0.00
J_{a50}	9.98 ± 0.59	7.63^{a} ± 0.23	129.00 ± 1.63	2.18^{b} ± 0.50
J_{a75}	10.60 ± 1.14	5.93^{ab} ± 0.98	129.50 ± 1.91	2.80^{b} ± 0.76
J_{b50}	10.15 ± 0.39	7.78^{a} ± 1.13	131.00 ± 2.45	4.40^{a} ± 0.57
J_{b75}	9.60 ± 0.42	6.30^{ab} ± 1.10	130.75 ± 2.06	2.85^{b} ± 0.17
S_{50}	10.53 ± 0.64	7.93^{a} ± 0.38	130.25 ± 3.30	1.55^{c} ± 0.10
S_{75}	10.35 ± 0.39	7.45^{a} ± 0.87	133.50 ± 4.20	4.23^{a} ± 0.51
SEM	0.14	0.43	0.55	0.31

Values are means (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly (P < 0.05).
*See footnotes to Table 1.

Histopathology

Different experimental diets produced several changes in the intestinal mucosa but not in liver and muscle morphology in common carp. The liver did not show any pathological alteration or signs of steatosis/hepatic lipidosis in any of the experimental groups (Fig 1B). The intestinal loop sections appeared to be normal for fish fed control, S_{50}, S_{75}, J_{b50} and J_{b75} diets. The section of J_{a75} group (Fig 2A) was characterized by reduced size of the intestinal loops compared with control section (Fig 2B). Additionally distinct parts of the anterior and posterior intestine showed severe pathological lesions in J_{a75} group. These lesions can mainly be described as “catarrhal enteritis” which is probably due to presence of PEs in DJ_aKM.

Signs of necrosis characterize numerous enterocytes and denudations of the upper third of the lamina epithelial were accompanied by a massive influx of leucocytes into the lamina propria of the villi in J_{a75} group (Fig 3A). Hyperaemia and inflammation were additional symptoms in this group. There were mild lesions occasionally found in mucosal tissue samples of J_{b50} and J_{b75} groups, which were similar to those found in soybean meal containing diets. In these areas the pathological lesions were minor and all the other intestinal loops of both these groups seem to be unaffected in numerous different samples investigated**.** The intestinal mucosa was well developed and no morphological alteration was found compared to control group (Fig 3B).

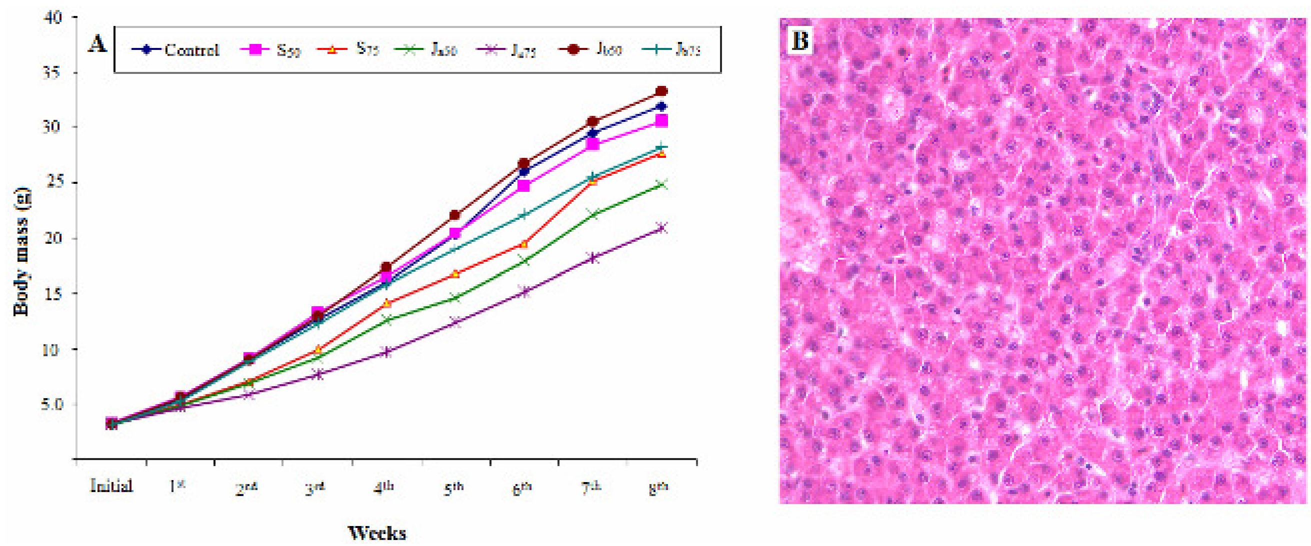

Fig 1 **(A)** Body mass gain of common carp (*Cyprinus carpio* L.) fed experimental diets for eight weeks, **(B)** liver of common carp (*Cyprinus carpio* L.) fed J_{b75} diet showing no pathological signs of steatosis/hepatic lipidosis.

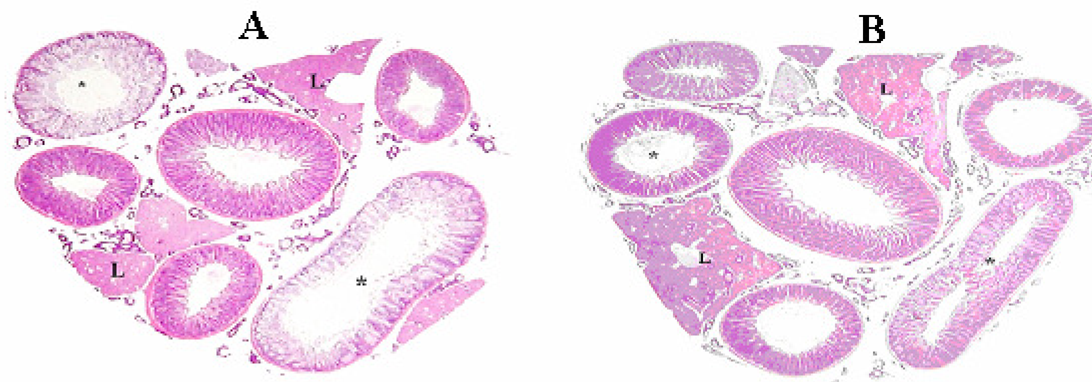

Fig 2 **(A)** Section of liver (L) and intestinal tract of J_{a75} group (reduced size of the digestive system organs and mucosal foldings; and disintegration of villi (*), **(B)** liver (L) and intestinal tract of control group (intestinal loops as well as the liver (L) are well-developed and show normal appearance).

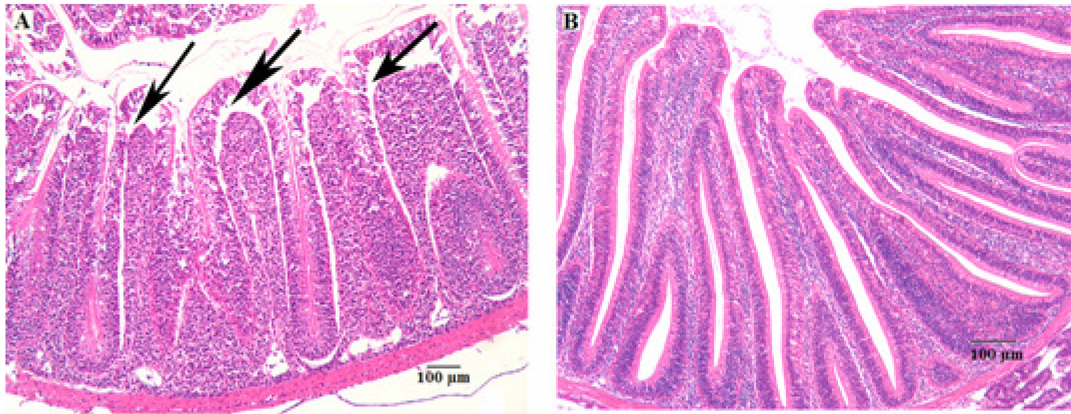

Fig 3 **(A)** Higher magnification of the mucosa of J_{a75} group demonstrates necrosis and denudation (arrows) of enterocytes and massive infiltration of immune-cells, **(B)** higher magnification of the mucosa of control group shows no signs of serious lesions.

Discussion

In the present study *Jatropha curcas* kernel meal was detoxified using heat treatment and solvent extraction. Two duration of detoxification process were investigated: shorter (30 min) and longer (60 min) and the detoxified meals so obtained were designated as J_a and J_b respectively. The results of the present investigation indicate that J_b meal at a lower inclusion level (J_{b50}, 50% of FM protein replaced by DJKM) is a good dietary protein source for carp feed. The performance of SBM fed groups was not as good as of J_b fed groups; however it was better than that of J_a fed groups. Soybean meal was found to be a moderately good protein source for carp at the 50% inclusion level, whereas, it was not promising at a higher inclusion level (75% of FM protein replacement). J_a proved to be unsuitable even at low inclusion (50%) in carp diet.

Several factors such as palatability, acceptability of diets, presence of toxic and antinutritional factors and digestibility of protein and energy in the diets could contribute to the observed variation in the growth responses of carp. Although PEs were not detected by HPLC in J_a meal, the poor growth response, feed intake, haematology and histopathological parameters of carp fed diets containing J_a meal indicate that J_a meal was not completely detoxified and PEs could be present at a concentration below the detection limit of the HPLC. It is possible that PEs might be present in strongly bounded form in the J_a meal, and were not extracted by our protocol for the HPLC determination. Phorbol esters could have been released from J_a meal in the fish intestine during digestion process hindering the growth performance. Lately we have enhanced the sensitivity of the HPLC method for determination

of phorbol esters from 10 ppm to 3 ppm and we found traces of phorbol esters (4 to 9 ppm) in Ja, and hence the effects are due to PEs. Experimental evidence for the toxic action of PEs is well documented in common carp and rat. Feeding trials on common carp and rats with *Jatropha* meal containing PEs have been reported to cause marked reduction in feed intake, diarrhoea and growth depression (Becker and Makkar, 1998; Rakshit et al., 2008).

Growth performance of carp fed J_{b50} diet (50% FM protein replacement by DJKM) was better than of those fed SBM based diets and similar to those fed FM based diets. Slightly lower performance of fish fed the diet where DJ_bKM replaced 75% of FM protein suggested that the capacity of DJKM to fully sustain growth was slightly lower compared to the control diet. This lower growth response of 75% plant protein fed group might be because of several factors such as lower digestibilities of protein and energy in the diets (Kumar et al., 2010), which could lead to lower protein and energy availability from the DJKM and SBM; and/or the presence of antinutrients such as phytate and NSP, which are present in high amounts in the SBM and DJKM and could adversely affect the feed utilization.

Cholesterol and triglycerides in plasma; blood glucose level

Dietary inclusion of DJKM in common carp reduced cholesterol level in plasma and muscle as compared to control group. The decrease in plasma cholesterol levels in fish fed diets with plant proteins has already been reported (Kaushik et al., 1995; Yamamoto et al., 2007). In terrestrial animals, plant products are generally considered to have a hypocholesteromic effect (de Schrijver, 1990), mainly due to the relatively high levels of estrogeno-mimetic isoflavones (Setchell and Cassidy, 1999). In humans, different plant constituents have been reported to lower plasma cholesterol levels (Wester, 2000). Although cholesterol metabolism in mammals and fish could differ, the fish hypocholesterolemia in response to dietary plant protein supply could be due either to an increased excretion of bile salts, to an inhibition of cholesterol intestinal absorption, or just to the withdrawal of FM rather than to the direct effects of plant protein (Kaushik et al., 2004). In any case, the significance of hypocholesterolemia in fish should be studied in depth.

Serum triglycerides act as a short-term indicator of the nutritional status (Bucolo and David, 1973). The increase in whole body fat content in plant protein fed groups (J_{b50} and S_{50}) (Kumar et al., 2010) along with the increase in plasma triglyceride concentrations also suggest significant lipid mobilisation in these groups.

Blood glucose concentration was affected by dietary treatments. Higher blood glucose concentration was observed in plant protein fed groups than in control group. Plant protein based diets contain higher amount of carbohydrates. Usually carbohydrates breakdown into smaller sugar compounds that concur with higher glucose level in blood. Similar trends were shown in fish fed diets containing SBM and corn gluten (Kikuchi et al., 1994; Kikuchi, 1999). On the other hand, Glencross et al. (2004) observed that dietary inclusion of yellow lupin in fish diet did not affect blood glucose level.

Hemato-immunological parameters

The WBC count, Hb content, Hct and MCHC did not differ significantly among the seven groups. As the plant protein content increased, an increase in the RBC count was observed. Higher RBC counts were observed in SBM and DJKM fed groups. Plant ingredients may cause early release of immature erythrocytes (Hemre et al., 2005), increasing the RBC count. Consequently, MCH value was changed at the same time.

Mean cell volume value differs significantly among the groups because RBC count was statistically different among the groups. Lower MCV was observed in plant protein fed groups (except J_{b50} group) than control group. Similarly, significant reduction of MCV on increase in the content of plant proteins in salmon diet was observed (Hemre et al., 2005). As this observation appeared to coincide with increased spleen size (Hemre et al., 2005), it was suggested that some of the plant ingredients might cause early release of immature erythrocytes. This appeared to be the case in the present study, since spleen size increased as the plant protein increased in diet (Table 6).

The Hb and Hct assays are normally used as general indicators of fish health (National Research Council, 1981). The Hb and Hct levels in all groups were within the normal range (Sun et al., 1995).

Blood protein and lysozyme activity in serum

The concentration of total protein in blood is used as a basic index for health and nutrititional status in fish (Martinez, 1976). Among the blood proteins, albumin and globulin are the major proteins, which play a significant role in the immune response. Albumin is used as an indicator of liver impairment (Silverman et al., 1986). Significantly lower blood protein content was observed in J_{a50} and J_{a75} groups that indicate nutritional deficiencies and impaired

protein metabolism in the liver. However, in histopathological study we did not observe any pathological alteration or signs of steatosis/hepatic lipidosis in liver of these groups (Fig 2). Globulin is important for the immunological responses. Higher globulin concentration in blood was observed in DJKM and SBM fed groups compared to control groups. These results are consistent with the higher lysozyme activity observed in plant protein fed groups. This suggests an immunostimulating effect of DJKM and SBM inclusion in the diet of common carp fingerlings.

Lysozyme plays an important role in nonspecific immune response and it is found in mucus, serum and ova of fish. Innate immunity due to lysozyme is caused by lysis of bacterial cell wall and this stimulates the phagocytosis of bacteria. The suppression of the non-specific immune capacity by high concentrations of dietary soybean proteins has been reported in rainbow trout (Burrells et al., 1999). However, other reports wherein SBM was fed to rainbow trout (Rumsey et al., 1994) and Atlantic salmon (Krogdahl et al., 2000) or alginate to Atlantic salmon (Gabrielsen and Austreng, 1998), increased values of different non-specific immune parameters have been reported, which have been interpreted as immunostimulating effects of plant protein sources. Numerically higher lysozyme activity was observed in plant protein fed groups (except Ja50 and Ja75 groups); it might be due to immunostimulating effect of DJ_bKM and SBM in common carp.

Metabolic enzymes

Alkaline phosphatase and ALT are released into blood during organ damage (Racicot et al., 1975). Alkaline phosphatase level rises during bile duct obstruction, and in intrahepatic infiltrative diseases of the liver (Goel et al., 1984). Alanine transaminase also called serum glutamic pyruvate transaminase is an enzyme present in hepatocytes. When liver cell is damaged, it leaks into the blood. Alanine transaminase rises dramatically in acute liver damage (Racicot et al., 1975). Thus, detection of blood level of ALP and ALT gives information on the damage of organs and in particular of liver cells. Significantly higher ALP and ALT activities were observed in J_{a50} and J_{a75} fed groups than other groups indicating toxicity, most probably due to residual PEs present in J_a meal. Phorbol esters could have damaged liver cells and ALP and ALT leaked into blood. Surprisingly we did not observe any liver cell damaged in histopathological study; whereas, we observed severe pathological lesions in mucosa, the first main organ interacting with the toxin. These lesions can mainly be

described as “catarrhal enteritis”. Hemre et al. (2005) and Sanden et al. (2006) also reported similar results on feeding SBM containing diets to Atlantic salmon.

Blood ions

Blood urea nitrogen concentrations were in the normal range (except for J_{a75} group) (Witters, 1986; Wedemeyer, 1996). The normal range for blood urea nitrogen is 2 to 6 mg/dl (Witters, 1986; Wedemeyer, 1996). Blood urea nitrogen levels are thought to be associated with liver or gill dysfunction (Stoskopf, 1993), as these are the sites of urea production and excretion, respectively. Significantly higher (about 2 times) BUN was observed in J_{a75} group than other groups. This indicates liver and/or gill damage in this group. Blood urea nitrogen concentration were within normal range in all groups (except J_{a75} group) suggesting that SBM and DJ_bKM fed groups were normal and healthy.

The concentration of TBIL, an indicator of liver dysfunction (Tietz, 1986) was similar in all groups. Creatinine is used as an indicator of kidney damage or malfunction (Rock et al., 1986; Tietz, 1986), and is a by-product of creatine, which is involved in muscle energy metabolism. Blood creatinine is normally quite stable. The normal range for creatinine level in blood is 0.2 – 1.5 mg/dl (Tietz, 1986). Creatinine was highest in control group but was within the normal range. Creatinine being a degraded product of animal protein and its highest level in control is obviously due to highest content of FM in control diet. The plant protein based diets were supplemented with phytase and higher concentration of phosphorus in blood was observed in plant protein fed groups compared to control group, which could be due to release of phosphorus from bounded phytate and making it available for common carp.

Histology

The necrosis of enterocytes on the tips and sides of villi in J_{a75} group (Fig 3A) appear to affect digestion and absorption of nutrients. The denudation of absorptive cells (enterocytes) and decreased contribution to digestion from the brush border enzymes resulted in overall reduction in absorptive surface. Mucosal lesions were accompanied by varying degrees of inflammatory cell infiltration in the lamina propria and cystic dilation of crypts in the J_a group. These histological results concur with the decrease in nutrient and energy digestibilities and digestive enzyme (amylase, protease and lipase) activities in common carp

intestine fed J_a detoxified kernel meal (Kumar et al., 2010). The results of the present study demonstrate that the J_a meal was not detoxified completely.

Conclusions

The detoxified *Jatropha* kernel meal (DJ_bKM) can replace 50% FM protein in common carp diets, without sacrificing the growth and health of fish. The DJ_bKM can be used as one of the promising FM replacers in the diet of common carp. Phorbol esters, the main toxic principles for *Jatropha* toxicity were not detected in fish muscle tissues, suggesting the fish of DJ_bKM fed groups is safe for human consumption.

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3.1.1.4 Dietary inclusion of detoxified *Jatropha curcas* kernel meal: Effects on growth performance and metabolic efficiency in common carp, *Cyprinus carpio* L.

Introduction

Fish meal (FM) is often utilized in aqua feeds because it offers a balanced source of indispensable amino acids, essential fatty acids, vitamins, minerals, and generally enhances palatability. Current world production of FM is approximately 6–7 million tonnes annually and, while this level of production is expected to remain stable over the next 10 years (New and Wijkström 2002; Mazurkiewicz 2009), rapid expansion of aquaculture during this period will require lowering the inclusion rate of FM in aquafeeds and replacing it with plant-based protein sources (SOFIA 2007). Protein ingredients to substitute for FM, either partially or completely, included terrestrial plant meals and animal by-products readily available on the world markets (Samocha et al. 2004). Reducing the FM content of fish feeds is a strategy to increase the sustainability of carp aquaculture by reducing feed costs as well as reducing the environmental impact. However the studies on FM replacement in carp are sparse compared to other carnivore fishes. Hansan et al. (1997), Hossain and Jauncey (1989), Hossain et al. (2001), Kumar et al. (2008, 2009a ,b, c) and Mazurkiewicz (2009) evaluated use of various oil seed as protein sources in common carp diet. Soybean meal (SBM) is currently the most commonly used plant protein source in fish feeds and amounts to 50% of the diet of freshwater omnivorous fish species (Yue and Zhou 2009). Which indicates that commercial feed depends mostly on SBM as a FM replacer. However the over dependence will cause hike in price of SBM therefore utilization of other inexpensive plant protein source would be beneficial in reducing the feed cost (Yue and Zhou 2009).

Jatropha curcas (L.) (physic nut) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. Its seeds have been extensively investigated as a source of oil. The seed kernel contains about 60% oil that can be converted into biodiesel of high quality upon transesterification and used as a substitute for diesel fuel (Makkar et al. 2007a). The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 58 to 62 % crude protein (Makkar et al. 2008). The levels of essential amino acids (except lysine) are higher in *Jatropha* kernel meal than SBM (Unpublished). However the presences of high levels of antinutrients like trypsin inhibitor, lectin and phytate (Makkar et

al. 2008) and the major toxic components phorbol esters (PE_S) (Makkar and Becker 1997) restrict their use in fish feed. Heat labile antinutrients, like protease inhibitors, and lectins are easy to inactivate by moist heating. A method for detoxification of *Jatropha* kernel meal has been developed in our laboratory. It is based on extraction of PEs using organic solvents and inactivation of trypsin inhibitors and lectin by heat treatment. Our previous study (Kumar et al. 2008) has shown that detoxified *Jatropha* kernel meal (DJKM) is a good protein source and better than SBM for common carp diet.

Growth and production can be described in terms of partition of dietary energy yielding components between catabolism as fuels and anabolism as storage in tissues. Metabolism, which includes all processes where transfer of energy is involved, can be quantified on the basis of the energy expenditure. The energy expenditure can be estimated from the gaseous exchange and the release of nitrogenous compounds (Brouwer, 1965). Many studies have been carried out on the relationship of dietary energy and protein content to growth of carp (Ogino et al. 1976; Schwarz et al. 1983; Gongnet et al. 1987). Some reports deal with the nutritional energetics related to the effect of diets on energy budgets (Cui et al. 1992; Helland and Helland 1998; Francis et al. 2002), but little work has been done to examine the effects of dietary plant proteins on energy allocation in fish (Refstie and Tiestra 2003). The present study was designed to investigate the effects of dietary DJKM on growth performance, nutrient utilization and interrelationships among the major components of the energy budget of common carp, *Cyprinus carpio*, and to compare these parameters with those obtained on dietary inclusion of SBM and FM.

Materials and Methods

Preparation of the Jatropha meal

Jatropha seeds were purchased from India and deshelled manually to obtain kernels. Defatting of *Jatropha* kernels was done using petroleum benzene (b.p. 40–60 ^{O}C) in a Soxhlet apparatus. Organic solvents were used to detoxify defatted *Jatropha* kernel meal (patent application has been filed for the process of detoxification, Makkar and Becker 2008). After removal of PEs, the meal was autoclaved (121 ^{0}C for 15 min) to inactivate heat labile antinutrients, trypsin inhibitor and lectin.

Diet formulation

FM (Seelöwe fishmeal) was procured from Vereinigte Fishmeh werke Cuxhaven GmbH & Co KG, Cuxhaven, Germany; and wheat meal was purchased from a local market. SBM (dehulled, defatted and roasted) was obtained from Institute of Animal Nutrition (450), University of Hohenheim, Germany. Soya protein isolate (SUPRO® 500E IP) was purchased from Solae Europe S.A., 2, Chemin du Pavillon, CH-1218 Le Grand-Saconnex, Geneva, Switzerland. Source of sunflower oil was Thomy, Nestle Deutchland AG, 41415 Neuss; Reg. Trademark of Societe des, Produits Nestle S.A.. Vitamin premix and mineral premix were procured from Altromin Spezialfutter GmbH & Co. KG, Lage, Germany. Source of lysine is Merck KGaA, 64271 Darmstadt, Germany. Phytase (Natuphos 5000 G (EU) 3-phytase (EC 3.1.8)) was purchased from BASF Ludwigshafen, Germany.

Prior to feed formulation, the proximate composition of DJKM, wheat meal, SBM, soya protein concentrate and FM were determined. A total of three isonitrogenous diets were formulated. Experimental diets containing crude protein 38%, crude lipid 9%, vitamin premix 2% and mineral premix 2% were prepared. The inclusion levels of the DJKM and SBM were as follows: Control diet (C_{ontrol}) was prepared with FM and wheat meal, without any DJKM and SBM; *Jatropha*: 75% FM protein replaced by DJKM; and S_{oybean}: 75% FM protein replaced by SBM (Table 1). The final mixture of each diet was made to 2 mm diameter moist pellets by pelletizer (using a Bosch, Type UM60ST 2-M, Robert Bosch Hausgerät GmbH, 70839 Gerlingen, Germany) and then freeze-dried.

Experimental system

During the experiment, the fish were kept individually in respiration chambers 5 l capacity of the fully automated, computer controlled fish respirometer system Focken et al. (1994). Thirty-two measurements of oxygen consumption per individual fish were made every 24 h and recorded on the hard disk of the computer. The system was lit with fluorescent tubes to give a day length of 12 h. The water flow rate through the respirometer chambers (controlled by electronic flow meters connected to each chamber) was adjusted between 0.5 and 0.8 l min^{-1} to keep the oxygen saturation in water above 80% in the chambers. Everyday, about a tenth of the water in the system was replaced. Once a week, when the fish were weighed, the chambers were cleaned and the oxygen electrode calibrated. During the experimental period, all water quality parameters were maintained at optimum range (water temperature: 26.1 –

27.3°C, pH: 7.1 – 7.8, DO: 6.9 – 7.7 mg l^{-1}, NH_3-NH^{4+}: 0.1– 0.2 mg l^{-1}, nitrite: 0.07 – 0.1 mg l^{-1} and nitrate: 1–3 mg l^{-1}).

Table 1 Composition of the experimental diets (g kg^{-1} feed)

Ingredients	Experimental diets		
	Control	*Jatropha*	Soybean
FM	505	126	126
Soyabean meal	-	-	510
Wheat meal	415	368	219
Detoxified *Jatropha* kernel meal	-	381	-
Soya protein isolate	-	8	32
Lysine	-	4	-
Sunflower oil	40	73	73
[1]Vitamin premix	20	20	20
[2]Mineral premix	20	20	20
Total	1000	1000	1000
Phytase (FTU/kg)	-	500	500

[1]Vitamin premix (g or IU kg-1 premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
[2]Mineral premix (g kg-1): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4.H_2O$, 3.12; $ZnSO_4$. $7H_2O$, 4.67; $CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

Experimental animals

Common carp (*Cyprinus carpio* L.) fingerlings obtained from the Institute of Fisheries Ecology and Aquaculture of the Federal Research Center for Fisheries at Ahrensburg, Germany were transferred to the University of Hohenheim, Stuttgart, Germany and kept in two 500 l capacity tanks for acclimatisation. They were fed the Hohenheim standard fish diet (containing approximately 38% protein, 8% lipid, 10% ash and with a gross energy content of 18 kJ g−1 dry matter) until their body weight reached approximately 10 g. Prior to the start of the experiment, 25 carp of comparable body mass (10.9 ± 0.65 g) were selected from a large population, weighed and 15 of them were placed individually in 15 respiration chambers of a

device described by Focken et al. (1994). The remaining 10 fish were sacrificed by a sharp blow to the forehead and preserved for determination of the initial chemical composition. The fish were starved for 24 h prior to the start of feeding the experimental feeds. At the start of the experiment, the three experimental diets namely C_{ontrol}, $S_{oyabean}$ and *Jatropha*, were assigned each to five fish in a random manner.

Feed regime

In a preliminary study, feeds at seven times maintenance requirement were offered to fish. This resulted in substantial presence of uneaten feed in the aquaria. However, no feed was left in the aquaria when feeds at five times maintenance requirement were offered. According to Becker et al. (1983), one time maintenance requirement equals 3.2 g feed per kg metabolic body mass ($kg^{0.8}$). So 16 g feed per kg metabolic body mass ($kg^{0.8}$) was fed for 5 times maintenance requirements, and the feed was split into 5 equal rations per day (at 8:00, 10:30, 13:00, 15:30 and 18:00 h). The feeds were dispensed using an automatic feeder (Graesslin, Rondomatic 400, Graesslin GmbH, St. Georgen/Schwarzw, Germany). Fish were weighed individually at the beginning of the experiment (av. wt. 10.9 ± 0.65 g) and at weekly intervals during the experimental period to adjust the feed amount for the subsequent week. The fish were not fed on the weighing day. At the end of six weeks, the experiment was terminated and the fish were weighed, sacrificed by a sharp blow to the forehead and immediately stored at −20 ^{0}C for chemical composition analysis. Prior to the determination of the proximate composition, the fish were autoclaved at 121 ^{0}C for 20 min, thoroughly homogenised using an Ultra-Turrax T25, frozen overnight and freeze-dried.

Extraction and estimation of phorbol esters by high-performance liquid chromatography, and determination of antinutrients

PEs were determined according to Makkar et al. (2007b), which was based on the method of Makkar et al. (1997). Briefly, 0.5 g of the *Jatropha* meal and dried whole body fish sample were extracted four times with methanol. A suitable aliquot was loaded on a high-performance liquid chromatography (HPLC) reverse-phase C_{18} LiChrospher 100, 5 µm (250 x 4 mm I.D. from Merck (Darmstadt, Germany) column. The column was protected with a head column containing the same material. The separation was performed at room temperature (23

^{o}C) and the flow rate was 1.3 ml/min using a gradient elution (Makkar et al. 2007b). The four-PEs peaks were detected at 280 nm and appeared between 25.5 and 30.5 min. The results were expressed as equivalent to a standard, phorbol-12-myristate 13-acetate. Detection limit of PEs is 3µg/g meal.

Trypsin inhibitor activity was determined essentially according to Smith et al. (1980) except that the enzyme was added last as suggested by Liu and Markakis (1989). Analysis of the lectin content was conducted by haemagglutination assay (Makkar et al. 1997). The haemagglutination activity was expressed as the minimum amount of material (mg mL^{-1} assay medium) that produced agglutination. The minimum amount was the amount of material mL^{-1} assay medium in the highest dilution that was positive for agglutination; the lower this value, the higher the lectin activity. The phytic acid content of samples was determined by a spectrophotometric procedure (Vaintraub and Lapteva 1988). Results were expressed as g phytic acid per 100 g material using phytic acid sodium salt (Sigma, St Louis, MO, USA) as standard. Non-starch polysaccharides were estimated according to Englyst et al. (1994).

Amino acid analysis

The amino acid compositions of FM, DJKM, SBM, soya protein concentrate and wheat meal were determined using an automated amino acid analyser after hydrolysing the samples with 6 M HCl at 110 ^{0}C for 24 h (Bassler and Buchholz 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The tryptophan contents of the above-mentioned samples were determined spectrophotometrically by the method of Pinter-Szakacs and Molnar-Perl (1990).

Proximate analysis

The proximate composition of diet ingredients, diets and whole body of fish was determined using the standard methods of the Association of Official Analytical Chemists (AOAC 1990). Samples of plant and animal origin (fish bodies and FM) were analysed for dry matter (DM), ash, crude protein (CP) and lipid (ether soluble lipid). Gross energy (GE) of diet ingredients, diets and fish bodies was determined with bomb calorimeter (IKA C 7000) using benzoic acid as a standard.

Calculation for growth performance, nutrient utilization and energy budget

All calculations were performed for each fish individually. Growth performance, diet nutrient utilisation and energy budget were assessed in terms of body mass gain (BMG,%), specific growth rate (SGR,%/day), metabolic growth rate (MGR, $gkg^{0.8}$ day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV,%), lipid productive value (LPV,%), energy expenditure (kJ g^{-1}), energy apparently not metabolised (AUE, kJ), energy retention (ER, kJ), average metabolic rate (AMR), metabolisable energy ingested, efficiency of metabolisable energy for growth, metabolisability and energy expenditure (EE, kJ)/g protein fed and retained. These were calculated as follows:

BMG (%) = [(Final body mass - initial body mass) / Initial body mass] X 100; SGR (%/day) = [(ln final body mass in g) - ln initial body mass in g) / number of trial days] X 100; MGR = (Body mass gain in g) / [{(initial body mass in g / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)^$^{0.8}$}/2] / number of trial days (Dabrowski et al. 1986); FCR = dry feed fed (g)/body mass gain (g); PPV (%) = [(final fish body protein in g - initial fish body protein in g) / total protein consumed in g] X 100; LPV(%) = [(final fish body lipid in g - initial fish body lipid in g) / total crude lipid consumed in g] X 100; ER (%) = [(final fish body energy - initial fish body energy)/(gross energy intake)] X 100; PER = fresh body mass gain (g)/crude protein fed (g).

EE (kJ) = Total oxygen uptake (g) X 14.85 (kJ/g) (Huisman 1976); AUE (kJ) = Gross energy – (energy expenditure + energy retention); AMR = Oxygen consumed (mg/h)/[fish wt (g)]^$^{0.8}$; heat released (kJ $kg^{\wedge 0.8}$ d^{-1}) = (AMR X 24 X 14.85)/1000; metabolisable energy intake (kJ) = energy expenditure (kJ) + energy retention (kJ); efficiency of metabolisable energy for growth, metabolizability; EE/g protein fed (kJ g^{-1}) = Total EE (kJ)/ total protein consumed (g); EE/g retained protein (kJ g^{-1}) = Total EE (kJ)/ total protein retained in body (g).

Statistical analysis

All data were subjected to a one-way analysis of variance ANOVA and the significance of the differences between means was tested using Duncan's multiple range test ($P<0.05$). The software used was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). Values are expressed as means ± standard deviation.

Results

Fish behavior and feed intake

Based on the visual observation during feeding time, palatability or acceptability of feed was good and the behavior of fish was normal. There was no mortality during the entire experimental period.

It was particularly noted that the fish of the S_{oybean} group fed slowly on their feed, whereas fish of other dietary groups fed actively throughout the experiment.

Phorbol esters content in defatted *Jatropha* kernel meal and antinutrients contents in detoxified *Jatropha* kernel meal and soybean meal

Phorbol esters content in untreated defatted *Jatropha* kernel meal was 1.8 mg/g. However, phorbol esters in the DJKM and dried whole body fish were undetectable. Lectin and trypsin inhibitor were not detected in DJKM and SBM; and phytate in DJKM and SBM was 9.3% and 2.4% respectively (Table 2). Non-starch polysaccharides level in DJKM and SBM were 16% and 14% respectively (Table 2).

Table 2 Proximate composition, amino acid composition and antinutrient content of feed ingredients

	Fish meal	Detoxified *Jatropha* kernel meal	Soybean meal	Soya protein isolate	Wheat flour
Proximate composition (g kg $^{-1}$)					
Dry matter	940	945	955	940	941
Crude protein	635	665	471	900	143
Crude lipid	88	11.4	11.7	10	16.3
Crude ash	142	137	21.4	40	14
Gross energy (kJ/g)	21.1	18.3	18.2	-	18.7

Essential amino acids composition (g kg $^{-1}$)					
Arginine	35.3	69.7	36	67.9	5.4
Histidine	17.7	21.7	14.4	24.4	3.4
Iso leucine	22.8	26.7	19.6	36.5	4.2
Leucine	41.6	46.7	35.7	68.1	9.1
Lysine	40.9	23.3	29.1	52.1	3.3
Phenylalanine	21.8	30.4	24.3	43.2	6.5
Methionine	16	10.6	6.2	12.1	2
Threonine	23	22	17.8	31.1	3.7
Tryptophan	4.9	7.1	6.4	10.4	1.4
Valine	29.3	31.6	21.2	37.4	5.1
Non-essential amino acids composition (g kg $^{-1}$)					
Alanine	43.3	29.4	21.4	40.9	4.6
Asparginine	60.5	68.7	66.6	122.8	7.2
Cystine	4.3	2.3	6.5	9.8	2.9
Glycine	59.8	31.5	21.3	37.2	5.6
Glutamine	79.4	112.1	93.8	174.9	44.9
Proline	36.9	32.2	28.2	50.2	14.5
Serine	25.5	30.6	24.4	46	6.3
Tyrosine	14.8	18.8	15.8	31	3.3
Antinutrients					
Trypsin inhibitor (mg trypsin inhibited per g sample)	ND	ND	ND	ND	-
Phytate (% dry matter)	-	9.3	2.41	-	-
Lectin[a]	-	ND	ND	-	-
Non-starch polysaccharides (NSP) (g kg $^{-1}$)					
Rhamnose	-	3	0	-	-
Fucose	-	1	0	-	-
arabinose	-	31	24	-	-
Xylose	-	20	11	-	-

Mannose	-	5	6	-	-
Galactose	-	14	42	-	-
Glucose	-	57	32	-	-
Glucuronic acid	-	0	0	-	-
Galacturonic acid	-	30	24	-	-
Total-NSP	-	160	140	-	-

ND: Not detected
[a:]Minimum amount of material (mg mL^{-1} assay medium) that produced agglutination.

Proximate composition and amino acid profile of experimental diets and proximate composition of whole body of fish

Proximate composition, amino acid compositions and antinutrients of feed ingredients are shown in Table 2. Experimental diets contained about 39% crude protein and 19.2 kJ/g gross energy and were isonitrogenous and isocaloric. Dry matter, crude lipid and ash were in the range of 95.8–96.4%, 9.2–9.8% and 10.3–11.1% respectively (Table 3). The quantities of essential amino acids (except methionine) fulfilled the needs of the common carp (NRC 1993) in Table 3.

Table 3 Proximate composition and amino acid composition of the experimental diets (g kg^{-1} feed)

	Control	*Jatropha*	Soybean
Proximate composition (g kg^{-1})			
Dry matter	958	964	959
Crude protein	392	391	390
Crude lipid	94	98	92
Crude ash	108	103	111
Gross energy (kJ/kg)	19.1	19.4	19.0
Essential amino acids composition (g kg^{-1})			
Arginine	20.1	32.1	26.2
Histidine	10.4	11.5	11.1
Iso leucine	13.3	14.3	15.0

Leucine	24.8	26.0	27.6
Lysine	22.1	18.7	22.4
Phenylalanine	13.7	16.5	17.9
Methionine	8.9	6.7	6.0
Threonine	13.2	12.4	13.8
Tryptophan	3.1	3.8	4.5
Valine	16.9	17.3	16.8
Non-essential amino acids composition (g kg^{-1})			
Alanine	23.8	18.1	18.7
Asparginine	33.6	35.9	47.2
Cystine	3.4	2.5	4.8
Glycine	32.6	21.3	20.8
Glutamine	58.4	68.7	73.0
Proline	24.6	22.1	23.7
Serine	15.5	16.9	18.5
Tyrosine	8.8	10.1	11.7

Amino acid composition of the experimental diets were calculated from amino acid profile of individual feed ingredients

The chemical composition of the whole body of fish (wet basis %) is presented in Table 4. Highest whole body moisture content was observed in FM group, and lowest in *Jatropha* group (Table 4). The moisture content of S_{oybean} group was intermediate but was statistically not different ($P > 0.05$) to that of FM and *Jatropha* groups. Whereas opposite trend was observed for whole body lipid content and gross energy content; highest lipid and gross energy content were observed in *Jatropha* group. These parameters for S_{oybean} group and FM group were statistically not different ($P > 0.05$). Highest crude protein and ash were observed in S_{oybean} group and lowest in FM group.

Table 4 Chemical composition of whole body of common carp (*Cyprinus carpio* L.) fingerlings of different experimental groups at the start and at the end of the experiment (% wet basis)

Treatment	Moisture	Crude protein	Crude lipid	Ash	Gross energy (kJ/g)
Intial fish	77.9 ± 0.2	14.6 ± 0.01	3.7 ± 0.11	3.9 ± 0.10	4.0 ± 0.08
C_{ontrol}	76.1^{a} ± 0.8	16.3^{b} ± 0.34	5.0^{b} ± 0.44	2.6^{b} ± 0.08	5.4^{b} ± 0.21
Jatropha	74.3^{b} ± 0.3	16.7^{ab} ± 0.26	6.3^{a} ± 0.25	2.6^{b} ± 0.03	6.0^{a} ± 0.12
S_{oybean}	75.5^{ab} ± 1.3	17.3^{a} ± 0.70	4.3^{b} ± 1.29	3.0^{a} ± 0.14	5.3^{b} ± 0.52
SEM	0.28	0.15	0.29	0.04	0.11

Mean values in the same column with different superscript differ significantly (P < 0.05).
Values are mean (n = 5) ± standard deviation.

Growth performance and feed utilization

Growth performance parameters and daily feed intake are shown in Table 5. Weekly body mass gain indicates that second week onwards there was differential growth among the group, and lower body mass development was observed in S_{oybean} group compared to other groups. This trend was maintained till the end of the experiment. Highest body mass gain, specific growth rate, metabolic growth rate were observed for *Jatropha* group which were statistically not different to that for FM group and significantly higher (P < 0.05) than for S_{oybean} group (Table 5). Whereas opposite trend was observed for FCR. The FCR for *Jatropha* group was statistically not different to that for C_{ontrol} group (Table 6).

PER, PPV and LPV were statistically not different (P>0.05) for C_{ontrol} and *Jatropha* groups and significantly higher (P<0.05) than for S_{oybean} group (Table 6).

Table 5 Growth performance and feed intake per day in common carp (*Cyprinus carpio* L.)

Treatment	IBM (g)	FBM (g)	Feed intake per day (g)	BMG	SGR	MGR
C_{ontrol}	11.2 ± 1.14	49.0^{a} ± 7.9	0.90 ± 0.13	338^{ab} ± 51	3.50^{ab} ± 0.3	15.3^{a} ± 1.3
Jatropha	10.6 ± 0.63	48.3^{a} ± 3.0	0.92 ± 0.05	358^{a} ± 45	3.62^{a} ± 0.2	15.6^{a} ± 0.9
S_{oybean}	10.8 ± 0.97	39.1^{b} ± 8.2	0.84 ± 0.10	262^{b} ± 73	3.02^{b} ± 0.5	13.1^{b} ± 2.2
SEM	0.23	2.02	0.02	17.7	0.05	0.48

Values are mean (n = 5) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).
IBM (g) - Initial body mass, FBM (g) – Final body mass, BMG (%) - Body mass gain, SGR (%/day) – Specific growth rate; and MGR (gkg$^{0.8}$ day^{-1}) - Metabolic growth rate
Energy budget

Table 6 Nutrient utilization in common carp (*Cyprinus carpio*) fed with experimental diets for six weeks

Treatment	FCR	PER	PPV	ER	LPV
C_{ontrol}	1.00^{b} ± 0.05	2.58^{a} ± 0.16	43.1^{a} ± 2.4	33.5^{a} ± 0.7	60.9^{a} ± 4.0
Jatropha	1.03^{b} ± 0.04	2.57^{a} ± 0.15	44.2^{a} ± 2.8	37.3^{a} ± 1.2	75.0^{a} ± 3.9
S_{oybean}	1.30^{a} ± 0.27	2.07^{b} ± 0.41	37.6^{b} ± 5.3	27.3^{b} ± 4.9	43.9^{b} ± 17.9
SEM	0.05	0.09	1.2	1.3	4.3

Mean values in the same column with different superscript differ significantly ($P < 0.05$).
Values are mean (n = 5) ± standard deviation.
PER - Protein efficiency ratio, PPV (%)- Protein productive value and ER (%)- Energy retention, LPV (%)- Lipid productive value

Average metabolic rate and energy budget of fish in different experimental groups are shown in Tables 7 and 8 respectively. Highest ER was observed for *Jatropha* group, which is not statistically different ($P > 0.05$) to that for C_{ontrol}. This value was lowest and significantly different for S_{oybean} group. Gross energy uptake, metabolisable energy intake, metabolisability, EE, AUE, AMR; heat released and EE per g protein fed did not differ significantly among the three groups. Lowest EE per g protein retained in the fish body was observed in *Jatropha* group that was statistically not different from C_{ontrol} group and highest in S_{oybean} groups. Highest ER efficiency and efficiency of ME for growth were observed in

Jatropha group, which is statistically not different to C_{ontrol} group and significantly higher ($P < 0.05$) than S_{oybean} group.

Discussion

In the present study an excellent growth was observed. The fish body mass increased four to five times within six weeks; and growth performance and feed utilization of *Jatropha* group was similar to FM fed group and better than S_{oybean} group. Highest ER was observed for *Jatropha* group, which was statistically not different ($P > 0.05$) to that for C_{ontrol} group and higher than that for S_{oybean} group. Whereas other components of the energy budget and AMR did not differ significantly amongst the groups, suggesting that DJKM is a promising protein source for incorporation in carp feed.

Feed intake, growth performance, nutrient utilization observed in this study was higher than those reported earlier in common carp (Viola et al. 1982; Hasan et al. 1997; Escaffre et al. 1997; Jahan et al. 2003; Kumar et al. 2008,2009a; Mazurkiewicz 2009). Although feed GE uptake did not differ significantly among the three groups, numerically, substantially higher (approximately 10%) feed GE uptake was observed in *Jatropha* and FM groups compared to S_{oybean} group. This concurs with the higher growth performance of fish in *Jatropha* and C_{ontrol} groups, and indicates that higher energy (feed GE uptake) was available for growth in these groups compared to SBM fed groups. A similar observation was recorded for energy allocation in European minnow, *Phoxinus phoxinus* (L.) (Cui 1987).

High and statistically not different feed efficiency, PER, LPV, ER and PPV values of fish in C_{ontrol} and *Jatropha* groups suggest that digestion and absorption of nutrients from DJKM and FM were similar. Nutrient utilization values found in this study signify very good utilization of the diets. These results were similar to those observed in our earlier study (Kumar et al. 2008) wherein DJKM fed groups were better than SBM in common carp.

Table 7 Average metabolic rate, derived heat release and energy expenditure per g protein fed and retained in common carp (*Cyprinus carpio* L.)

	C_{ontrol}	*Jatropha*	S_{oybean}	SEM
Average metabolic rate ($mgO_2kg^{\wedge0.8}\ h^{-1}$)	363 ± 83.3	442 ± 120.9	372 ± 60.6	23.9
Heat released ($kJ\ kg^{\wedge0.8}\ d^{-1}$)	129.3 ± 29.7	157.6 ± 43.1	132.7 ± 21.6	8.5
Energy expenditure (EE)/g protein fed (kJ)	19.6 ± 3.9	18.9 ± 1.1	20.0 ± 3.2	0.8
Energy expenditure (EE)/g protein retained (kJ)	45.3^{ab} ± 7.7	42.9^{b} ± 2.4	53.4^{a} ± 5.8	1.88

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Mean values in the same row with different superscript differ significantly ($P < 0.05$).
Values are mean ($n = 5$) ± standard deviation.
Metabolic rate = Oxygen consumed (mg/h)/[fish wt (g)]$^{\wedge0.}$

Table 8 Energy budget of common carp (*Cyprinus carpio* L.) in different experimental groups

	Control	*Jatropha*	Soybean	SEM
Initial GE content of carcass (kJ)	44.7 ± 4.6	41.9 ± 3.0	43.3 ± 3.9	1.06
Final GE content of carcass (kJ)	260.5 ± 32.7	285.1±25.0	208.1±44.1	12.95
Feed GE uptake (kJ)	645 ± 90.7	658 ± 45.2	600 ± 71.0	19.46
Energy expenditure (kJ)	289 ± 88.2	273 ± 17.2	270 ± 48.9	16.06
Energy expenditure (EE; % of GE fed)	44.3 ± 8.4	41.5 ± 0.9	45.1 ± 6.2	1.73
Energy retention (kJ)	216^{ab} ± 29.5	243^{a} ± 26.0	165^{b} ± 43.8	12.78
Energy retention (ER; % of GE fed)	33.5^{ab} ± 0.7	36.9^{a} ± 1.5	27.3^{b} ± 4.9	1.39
Metabolizable energy (ME) ingested (kJ)	505 ± 109	516 ± 42	435 ± 89	23.60
Metabolizability (ME % of diet)	77.8 ± 8.2	78.4 ± 1.1	72.3 ± 10.1	2.08
Efficiency of ME for growth	43.4^{a} ± 4.4	47.1^{a} ± 1.4	37.6^{b} ± 3.1	1.31
Apparently unmetabolised energy (AUE) (kJ)	140 ± 50.8	142 ± 3.9	164 ± 58.4	12.84
Apparently unmetabolised energy (AUE; % of GE fed)	22.2 ± 8.2	21.6 ± 1.10	27.7 ± 10.1	2.23
Efficiency of energy retention (ER/EE)	0.77^{a} ± 0.13	0.89^{a}± 0.05	0.61^{b}± 0.08	0.04

Mean values in the same row with different superscript differ significantly ($P < 0.05$).
Values are mean (n = 5) ± standard deviation.
EE = Oxygen uptake (g) X 14.85 kJ/g
AUE = Energy fed - energy expenditure - energy retention.

A comparison was made of the growth rate and energy budget in the common carp fed different experimental diets. Throughout the experiment, oxygen consumption of fish in FM, DJKM and SBM fed groups did not differ significantly, indicating no difference in metabolic activity, from start to end of the experiment, among the groups. Similar results have been reported by Suárez et al. (2009), wherein for white shrimp (*Litopenaeus vannamei*) juveniles fed soybean-canola meal (at an 80% substitution level) energy budget did not differ significantly for plant protein and FM protein fed groups.

In our study, lowest ER in SBM fed group conforms to the lowest growth performance of this group. Whereas, ER in *Jatropha* group was statistically similar to that in C_{ontrol} group and, reflecting higher growth performance in both groups. Lower growth performance and ER in SBM fed group compared to FM fed groups might be because of higher crude fibre content in this diet that could have led to losses of nitrogen and lipid through faeces (Hajra et al. 1987). Another reason could be due to lower protein and other nutrient availability from the SBM than from DJKM and FM. In another study (Kumar et al. 2009a) fractional digestibility of protein of SBM was lower than that of DJKM.

Cui and Liu (1990) constructed average energy budgets for six teleost species fed *ad libitum* and found that heat loss was always the largest component, 50 – 69% of consumed energy, whereas the energy used for growth was much smaller; 21 – 35%. In the present study, this was the case for fish fed all experimental diets for which the energy retained for growth was between 27.5 and 37%, whereas energy expenditure was between 41.4 and 45.1%. Metabolizable energy of the diet ranges from 72 – 78% and utilization of metabolisable energy for growth was 38 – 47%. Highest metabolisable energy for growth was registered in *Jatropha* group which is not statistically different from C_{ontrol} group that indicate utilization of DJKM in carp was better than SBM. Whereas highest EE per g protein retained in fish body was observed in S_{oybean} group, which exhibit that utilization of SBM in carp require higher energy than DJKM and FM (Table 7).

Kumar et al. (1986) demonstrated that the toxic substance (di Nitro benzene, DNB) progressively decrease O_2 consumption as the concentration of DNB wastewater increase. It is reported that aromatic nitro compounds (for e.g. DNB) combine with haemoglobin and cause methaemoglobinemia in higher vertebrates (Hamblin 1963). It may also be the case in fish. Fish have also been shown to exhibit stress reactions due to the presence of antinutrients like phorbol esters, trypsin inhibitors, and saponins through dietary (Makkar et al. 1998; Makkar and Becker 1997; Becker and Makkar 1998). Saponin can also increase O_2

consumption in common carp (Francis et al., 2002) and perch, *Anabas testudineus* (Roy and Munshi 1989). The present study indicates that DJKM does not contain any toxic substance (for e.g. phorbol esters) since it did not effect on O_2 consumption. Similar conclusion was derived in our previous studies (Kumar et al. 2008, 2009a) based on determination of various blood enzymes and metabolites and organ enzymes and on histopathological investigations.
We observed a significant increased in fat content in *Jatropha* group. This consequently resulted in increase in whole body energy content. Detoxified *Jatropha* kernel meal contains almost three times higher phytate content than soybean meal. Because of high phytate content, it might be that the availability of phosphorus and other minerals was low in the *Jatropha* group. Deficiency of phosphorus has been shown to increase body fat content (Sugiura et al. 2004). The whole body moisture content was lower and crude protein higher in plant protein fed groups, which is supported by Hasan et al. (1997); they observed that carp fed with plant protein (mustard, sesame and linseed oil cake) exhibited lower moisture and higher crude protein content compared to FM fed groups.

Conclusions

The results of this study showed that the detoxified *Jatropha* kernel meal fed group exhibited best growth performance (almost five times increased in fish body mass within six week) and good feed conversion ratio (about one). Overall, growth performance, feed utilization and energy budget of fish fed detoxified *Jatropha* kernel meal and fish meal were similar. This plant based protein-rich meal is a promising new ingredient for the aqua feed industry that could replace 75% of fish meal protein in the diets of common carp.

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3.1.1.5 Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus mykiss*) juveniles fed detoxified *Jatropha curcas* kernel meal

Introduction

Rainbow trout (*Oncorhynchus mykiss*) have been the most extensively studied aquaculture species for nutritional research. Intensive rainbow trout farming has undergone spectacular growth in the world especially in Europe in recent years due to its increase in demand for human consumption. This activity requires feeds with high levels of protein, which taxes finite global sources of fish meal (FM) supplies (SOFIA 2007). Therefore, alternative feed ingredients are required to provide the essential nutrients for the growth and quality of aquaculture production. Several compounds have been tested as alternative protein sources, such as animal by-products, single cell proteins including micro algae (*Spirulina maxima*), bacterial single cell protein and yeast (El-Sayed 1994; Perera *et al.* 1995) and plant proteins (Guillaume & Métailler 2001, Hasan *et al.* 1997). Among the potential substitutes, plant ingredients appear to be the best candidates. Substantial progress has been made towards the replacement of FM with a number of different plant ingredients, including soyabean meal (SBM), lupin, peas, rapeseed meal and sunflower (Kaushik *et al.* 1995; Vielma *et al.* 2002). Among plant ingredients SBM is currently the most commonly used plant protein source in fish feeds (Yue & Zhou 2009). However, SBM competes with human food and hence there is a need to identify other protein rich plant resources that could be used in fish diets.

Many plant protein sources can be used to partially or almost totally replace dietary FM (Kaushik *et al.* 2004), provided that the essential amino acid requirements of the fish species is met, the palatability of the diets is improved and the levels of anti-nutritional factors are reduced (Guillaume & Métailler 2001). Our previous studies demonstrate that plant protein sources (*Moringa* oleifera leaf meal, *Sesbania aculeate* seed meal and detoxified *Jatropha curcas*

kernel meal) could partially replace FM in the diet of tilapia, *Oreochromis niloticus* and common carp, *Cyprinus carpio* (Hossain *et al.* 2001; Richter *et al.* 2003a; Dongmeza *et al.* 2006; Kumar *et al.* 2008, 2010).

Jatropha curcas (L.) (physic nut) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. It is a hardy plant, thrives on degraded land and requires limited amounts of nutrients and water. Its seeds have been extensively investigated as a source of oil. The seed kernel contains about 60% oil that can be converted into biodiesel of high quality upon transesterification and used as a substitute for diesel fuel (Makkar *et al.* 2007a). The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 58 to 62 % crude protein. The levels of essential amino acids (except lysine) are higher in *Jatropha* kernel meal than SBM (Kumar *et al.* 2010). However the presences of high levels of antinutrients like trypsin inhibitor, lectin and phytate (Makkar *et al.* 2008) and the major toxic components phorbol esters (PE_S) (Makkar & Becker 1997) restrict their use in fish feed. Heat labile antinutrients, like protease inhibitors, and lectins are easy to inactivate by moist heating. A method for detoxification of *Jatropha* kernel meal has been developed in our laboratory. It is based on extraction of PEs using organic solvents and inactivation of trypsin inhibitors and lectin by heat treatment.

The International *Jatropha* Organization has claimed that in 2017 there will be around 32.72 million hectares of land cultivated worldwide producing 160 million tons of seeds and 95% of its total production will be concentrated in Asia (mostly in China and India). The total projected annual *Jatropha* oil production in Asian countries will be 46.88 million tons (Siang 2009). *Jatropha* plant can yield up to 5 tons seed per year from one hectare of plantation, which can produce approximately one ton of kernel meal rich in protein (Makkar & Becker 1997). This means that there is possibility of producing enough *Jatropha* seed meal to meet growing aquaculture industry demand. Our previous study (Kumar *et al.* 2008, 2010) has shown that detoxified *Jatropha* kernel meal (DJKM) is a good protein source and better than SBM for carp diet. There is no study on the inclusions of DJKM into the diet of rainbow trout. This study reports the nutritional, physiological and haematological responses of adding DJKM in rainbow trout.

Material and methods

Preparation of the Jatropha meal

Jatropha seeds were purchased from India and deshelled manually to obtain kernels. Defatting of *Jatropha* kernals was done using petroleum benzene (b.p. 40–60 ^{O}C) in a Soxhlet apparatus. Organic solvents were used to detoxify defatted *Jatropha* kernel meal (patent application has been filed for the process of detoxification). After removal of PEs, the meal was autoclaved (121 ^{0}C for 15 min) to inactivate heat labile antinutrients, trypsin inhibitor and lectin.

Diet formulation

FM and wheat meal were purchased from Kurt Becker GmbH, Bremen, Germany, at a local market respectively. Source of fish oil (Menhaden, batch # 102k0126) and gluten (wheat, lot number # 100K0191) from Sigma-Aldrich, Chemie GmbH Steinheim Getmany. Source of sunflower oil was Thomy, Nestle Deutchland AG, 41415 Neuss; Reg. Trademark of Societe des, Produits Nestle S.A.. Vitamin premix and mineral premix were procured from Altromin Spezialfutter GmbH & Co. KG, Lage, Germany. Source of lysine is Merck KGaA, 64271 Darmstadt, Germany. Prior to feed formulation, the proximate composition of defatted *Jatropha* meal, wheat meal, wheat gluten and FM were determined. Three isonitrogenous and isoenergetic diets were formulated. Experimental diets containing crude protein 45%, crude lipid 24%, vitamin premix 2%, mineral premix 2% and Titanium oxide (TiO_2) 1% were prepared. TiO_2 was added for digestibility measurement. Lysine was supplemented at the rate of 1.5% of DJKM inclusion in the diet. Each experimental feed, except the control, contained 500 FTU phytase (NATUPHOS 5000G, BASF, Ludwigshafen) per kg. The inclusion levels of the DJKM were as follows:

Control diet (C_{ontrol}) was prepared with fishmeal and wheat meal, without DJKM. J_{50}: 50% FM protein replaced by DJKM; and $J_{62.5}$: 62.5% FM protein replaced by DJKM. The final mixture of each diet was made into 2 mm diameter moist pellets (using a Bosch, Type UM60ST 2-M, Robert Bosch Hausgerät GmbH, 70839 Gerlingen, Germany) and then freeze-dried (Table 1).

Table 1 Composition of the experimental diets (g kg^{-1} feed) for rainbow trout (*Oncorhynchus mykiss*) juveniles

Ingredients	Experimental diets		
	C_{ontrol}	J_{50}	$J_{62.5}$
Fish meal	687	339	255
[1]Wheat meal	93	53	57
Jatropha meal	-	343	433
Wheat gluten	-	10	5
Sunflower:Fish oil (1:1)	180	209	202
[2]Vitamin premix	20	20	20
[3]Mineral premix	20	20	20
Lysine (g)	-	5	7
Total	1000	1000	1000
Phytase (FTU/kg)	-	500	500
TiO2	10	10	10

[1]Whole wheat meal.
[2]Vitamin premix (g or IU kg-1 premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
[3]Mineral premix (g kg-1): $CaCO_3$, 336; KH_2PO_4, 50_2; MgSO4. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67; $CuSO_4.5H_2O$, 0.62; KI, 0.16; $CoCl_2.6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

Experimental system and animals

Rainbow trout (*Oncorhynchus mykiss*) juveniles (about 3 g) obtained from the Fischzucht, Peter Störk, Wagenhausen, Bad Saulgau, Germany were transferred to University of Hohenheim, Stuttgart and kept in 1000 l capacity tanks for acclimatisation. They were fed the Hohenheim standard fish diet containing approximately 45% protein, 24% lipid, 12% ash and with a gross energy content of 22 kJ g^{-1} dry matter.

In our initial trial, six fish per aquarium (45 l) were kept so as to have six fish per replicate. However this resulted in high mortality due to aggressive/hierarchical behaviour of rainbow trout. Therefore, after the acclimatisation, 36 fish were randomly distributed into three groups with 12 replicates (av. wt. 4.2 ± 0.4 g). Two fish kept in an aquarium (45 l capacity) were separated from each other by a perforated plastic sheet. All the aquaria were supplied with water at 11 ± 2.0 ^{O}C from a recirculatory system. The system was subjected to a photoperiod of 12 h light : 12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 9.0 – 13.0 ^{O}C, pH 7.0 – 7.5, dissolved oxygen 6.2 – 7.6 mg l^{-1}, total NH_3 0.08– 0.18 mg l^{-1}, nitrite 0.06 – 0.09 mg l^{-1} and nitrate 1–2 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation above 80%. One day before start of the experiment, the fish were starved. According to Becker et al. (1983), one time maintenance requirement equals 3.2 g feed per kg metabolic body mass ($kg^{0.8}$). So 16 g feed per kg metabolic body mass ($kg^{0.8}$) was fed for 5 times maintenance requirements, and the feed was split into 5 equal rations per day (at 8:00, 10:30, 13:00, 15:30 and 18:00 h). The feeds were dispensed using an automatic feeder (Graesslin, Rondomatic 400, Graesslin GmbH, St. Georgen/Schwarzw, Germany). Fish were weighed individually at the beginning of the experiment and at weekly intervals during the experimental period to adjust the feed amount for the subsequent week. The fish were not fed on the weighing day. During last two weeks of the experiment, fish were fed with a diet containing a marker (TiO_2) for digestibility measurement (Mamun et al., 2007). The faeces collection was qualitative, as the experimental diets contained an inert marker (TiO2). During last two weeks of the experiment, faeces were collected daily.

After each feeding the aquaria were controlled for remaining feed; generally, there were no feed residues left. Every day prior to the faeces collection, aquaria were siphoned out to clean any residues. Faeces subsequently excreted by the fish were collected in separate beakers for each aquarium by siphoning with a short small pipe (Mamun *et al.* 2007). The collected mixture of water and faeces was centrifuged at 4000 × g for 10 min, the supernatant discarded and the faeces were then stored at -20°C until analysis. For the analysis, faeces from all the experimental periods from the same fish were pooled. During the experiment there was no mortality. At the start of the experiment, 12 fish of the same population were killed and preserved at −20 ^{O}C for analysis of the initial body composition.

The experiment was terminated after 12-week and the fish were killed. At the end of experiment, fish were anesthsized by tricaine methanesulfonate (MS222) at 250 ppm in water. Blood was drawn near caudal peduncle from four fish from each group and transferred into a heparinized tube for hematological study. Blood was drawn from another four fish from each group and divided into two equal part, one part was centrifuged at 1500×*g* for 5 min at room temperature (24 ^{O}C) to obtain plasma, which was then stored at −20 ^{O}C for determination of cholesterol and triglycerides. Another part of blood was kept outside at room temperature for few minutes to collect serum. Serum was stored at −20 ^{O}C for lysozyme determination. Four fish per group were carefully dissected to isolate intestine and stored in liquid nitrogen for digestive enzymes assay. Muscle was isolated from same fish and stored at −20 ^{O}C for determination of cholesterol and muscle lipid peroxides value. Those four fish from which blood was drawn for haematological study were stored at −20 ^{O}C for chemical composition analysis. Prior to determination of the proximate composition, the fish were autoclaved at 121 ^{O}C for 20 min, thoroughly homogenised using an Ultra-Turrax T25, frozen overnight and freeze-dried.

The University animal welfare committee (University of Hohenheim Germany) approved all experimental procedures involving rainbow trout.

Determination of phorbol esters, trypsin inhibitor, lectin, phytate and non startch polysaccharides

Phorbol esters (PEs) were determined according to Makkar *et al.* (2007b), which was based on the method of Makkar *et al.* (1997). Briefly, 0.5 g of the *Jatropha* meal and dried whole body fish sample were extracted four times with methanol. A suitable aliquot was loaded on a high-performance liquid chromatography (HPLC) reverse-phase C_{18} LiChrospher 100, 5 µm (250 x 4 mm I.D. from Merck (Darmstadt, Germany) column. The column was protected with a head column containing the same material. The separation was performed at room temperature (23 ^{O}C) and the flow rate was 1.3 ml/min using a gradient elution (Makkar *et al.* 2007b). The PEs peaks were detected at 280 nm and appeared between 25.5 and 30.5 min. The results were expressed as equivalent to a standard, phorbol-12-myristate 13-acetate. Detection limit of PEs is 3 µg/g meal.

Trypsin inhibitor activity was determined essentially according to Smith *et al.* (1980). except that the enzyme was added last as suggested by Liu & Markakis (1989). Analysis of the lectin content was conducted by haemagglutination assay (Makkar *et al.* 1997). The haemagglutination activity was expressed as the minimum amount of material (mg mL^{-1} assay medium) that produced agglutination. The minimum amount was the amount of material mL^{-1} assay medium in the highest dilution that was positive for agglutination; the lower this value, the higher the lectin activity. Phytate content of samples was determined by a spectrophotometric procedure (Vaintraub & Lapteva 1988). Results were expressed as g phytic acid per 100 g material using phytic acid sodium salt (Sigma, St Louis, MO, USA) as standard. Non-starch polysaccharides (NSP) were estimated according to Englyst *et al.* (1994).

Amino acid analysis

Amino acid composition of FM, DJKM and wheat meal was determined using an automated amino acid analyser after hydrolysing the samples with 6 M HCl at 110 ^{0}C for 24 h (Bassler & Buchholz 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. Tryptophan content of

the above-mentioned samples was determined spectrophotometrically by the method of Pinter-Szakacs & Molnar-Perl (1990).

Proximate analysis

The proximate composition of diet ingredients, diets and whole body of fish was determined using the standard methods of the Association of Official Analytical Chemists (1990). Samples were analysed for dry matter (DM), ash, crude protein (CP) and lipid (ether soluble lipid). Gross energy (GE) of diet ingredients, diets and fish bodies was determined with bomb calorimeter (IKA C7000) using benzoic acid as a standard.

Growth parameters

Growth performance and diet nutrient utilization were assessed in terms of body mass gain (BMG), specific growth rate (SGR), metabolic growth rate (MGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), apparent lipid conversion (ALC) and energy retention (ER). These were calculated as follows:
BMG (%) = [(Final body mass - initial body mass) / Initial body mass] X 100; SGR = [(ln final body mass in g) - ln initial body mass in g) / number of trial days] X 100; MGR = (Body mass gain in g) / [{(initial body mass in g / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)$^{\wedge 0.8}$}/2] / number of trial days (Dabrowski *et al.* 1986); FCR = dry feed fed (g)/body mass gain (g); PER = body mass gain (g)/crude protein fed (g); PPV (%) = [(final fish body protein in g - initial fish body protein in g) / total protein consumed in g] X 100; ALC (%) = [(final fish body lipid in g - initial fish body lipid in g) / total crude lipid consumed in g] X 100; ER (%) = [(final fish body energy - initial fish body energy)/(gross energy intake)] X 100.

Digestibility measurement

Titanium dioxide in the feed and faeces was determined according to the method described by Richter *et al.* (2003b). The percentage of apparent dry matter digestibility of diets was calculated according to Maynard *et al.* (1981).

Apparent dry matter digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces)}] X 100

Apparent digestibility coefficients (ADCs) of nutrients and energy of the experimental diets were calculated according to Maynard & Loosli (1969).

The nutrient and energy digestibility were obtained using the following formula:

Apparent nutrient digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces) X (% Nutrient or energy in faeces) / (% Nutrient or energy in feed)}] X 100

Efficiency of digestible nutrients and gross energy = (Nutrient and energy retained in the whole body/Digestible nutrient and digestible energy) x 100

Digestible nutrients and energy = Total offered of nutrients and gross energy through feed X digestibility coefficient.

Relative intestinal length (RIL), spleen Index (SI), hepatosomatic index (HSI), and gastro somatic index (GSI)

RIL was measured excluding pyloric caeca and is expressed in relation to each animal weight and expressed in mm g^{-1}.

RIL, SI, HSI, and GSI are calculated as indicated below:

RIL = Intestine length (mm)/ body mass (g)

SI = Spleen mass (g) / body mass (g)

HSI = Liver mass (g) X 100 / body mass (g)

GSI = Intestine mass (g) X 100 / body mass (g)

Digestive enzymes assay

The reducing sugars produced due to the action of glucoamylase and α- amylase on carbohydrate was estimated using dinitro-salicylic-acid (DNS) method (Rick & Stegbauer 1974). Amylase activity was expressed as mmol of maltose released from starch per min at 37 ^{O}C. Protease activity was determined by the casein digestion method of Drapeau (1974), and one unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to $\Delta 0.001A_{280}$ per minute at 37 ^{O}C and pH 7.8. Lipase activity was assayed by the method of Cherry & Crandell (1932), and one unit of enzyme was the amount of enzyme that hydrolysis 1.0 microequivalent of fatty acid from a triglyceride in 24 h at pH 7.7 at 37 ^{O}C.

Cholesterol and triglyceride estimation and assay of lipid peroxides

The determinations of the plasma cholesterol and triglycerides were using enzymatic colorimetric kits: Hitado Diagnostic system, Nobiflow cholesterin (kit lot number 60041889) and Nobiflow triglyceride-GPO (kit lot number 60040710) respectively. Muscle cholesterol content was determined using a kit (lot no. 10139050035) (Boehringer Mannheim, Germany). The color intensity was determined photometrically and was directly proportional to the concentration of cholesterol and triglycerides in the plasma sample. Lipid peroxides in fish muscle were determined using the procedure of Utley et al. (1967).

Haematological parameters

Total erythrocyte count (RBC) and total leucocyte count (WBC)

RBC and WBC were counted by Neubauer's counting chamber of haemocytometer. Care was taken to avoid trapping of air bubbles. The RBC lying inside the five small squares were counted under high power (40X) of light microscope.

The following formula was used to calculate the number of RBC per mm^3 of the blood sample:

Number of RBC/mm^3 = (N x dilution)/area counted x depth of fluid

Haemoglobin (Hb) and hematocrit (Hct) content

The Hb content of blood was analyzed by Reflotron Hemoglobin test (REF 10744964, Roche diagnostic GmbH, Manheim Germany). Hct was determined on the basis of sedimentation of blood. Heparinised blood (50 µl) was taken in the hematocrit capillary (Na-heparinised) and centrifuged in the Hematocrit 210 Hettich Centrifuge (Tuttlingen Germany) to obtain hematocrit value.

From analyses of Hct, Hb and RBC, the following parameters were calculated: mean cell volume, MCV (fL) = (Haematocrit [in L/L] x 1000) ÷ (RBC count [in millions/µL]); mean corpuscular haemoglobin, MCH (pg) = (Hemoglobin [g/dL] x 10 ÷ (RBC count [in millions/µL]) and mean cell haemoglobin concentration, MCHC [in g/dL] = Haemoglobin [in g/dL] ÷ Haematocrit [in L/L].

Lysozyme activity in serum

Lysozyme activity of serum was measured by EnzChek Lysozyme Assay Kit (E-22013) Leiden, The Netherlands. The assay measures lysozyme activity on *Micrococcus lysodeikticus* cell wall, which is labeled to such a degree that the fluorescence is quenched. Lysozyme action relieves this quenching, yielding a dramatic increase in fluorescence that is proportional to lysozyme activity. The fluorescence increase was measured by using spectrofluorometer that detects fluorescein. Lysozyme hydrolyzes β-(1-4)-glucosidic linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues present in the mucopolysaccharide cell wall of the microorganism.

Blood parameters analysis by Vet Scan

VetScan Chemistry Analyzer (Scil Animal care company GmbH, Technischer Service, Germany) was used for determinations of alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total calcium (Ca^{++}), creatinine, globulin, glucose, phosphorus, potassium (K^{+}), sodium (Na^{+}), total bilirubin (TBIL), total protein, and urea nitrogen (BUN) in heparinized blood.

Histopathological studies

Immediately after killing the fish a total of 45 histological samples of different segments of the intestinal tract were fixed in Bouin´s fluid or in methanol/acetic acid for 48 h or 24 h respectively. The intestine was thereby subdivided in fore-, mid- and hindgut. After dehydration and embedding in paraffin serial sections of 5 µm thickness were prepared and processed for conventional histopathological studies. Haematoxylin and eosin (H&E) was used for staining the tissue sections.

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) and the significance of the differences between means was tested using Duncan's multiple range test ($P<0.05$). The software used was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). Values are expressed as means ± standard deviation.

Results

Phorbol esters and antinutrients content in defatted Jatropha kernel meal

PE content in untreated defatted *Jatropha* kernel meal was 1.8 mg/g. However, PEs in DJKM was undetectable. Trypsin inhibitor and lectins were also not detected in DJKM; whereas phyate and NSP levels in DJKM were 9.1% and 16% respectively (Table 2).

Table 2 Proximate composition, antinutrients content and amino acid composition of feed ingredients (dry matter basis)

	Fish meal	*Jatropha* meal	Wheat gluten	Whole wheat meal
Proximate composition (g kg $^{-1}$)				
Dry matter	940	945	937	941
Crude protein	635	665	856	143
Crude lipid	88	11.4	13.4	16.3
Crude ash	142	137	8.7	14
Gross energy (KJ/g)	21.1	18.3	21.1	18.7
Antinutrients				
Trypsin inhibitor (mg trypsin inhibited per g sample)	ND	ND	ND	-
Lectin[a]	-	ND	ND	-
Phytate (% dry matter)	-	9.3	-	-
Non-starch polysaccharides (NSP) (g kg $^{-1}$)				
Rhamnose	-	3	-	-
Fucose	-	1	-	-
arabinose	-	31	-	-

Xylose	-	20	-	-
Mannose	-	5	-	-
Galactose	-	14	-	-
Glucose	-	57	-	-
Glucuronic acid	-	0	-	-
Galacturonic acid	-	30	-	-
Total-NSP	-	160	-	-
Essential amino acids composition (g kg $^{-1}$)				
Arginine	35.3	69.7	4.3	5.4
Histidine	17.7	21.7	2.1	3.4
Iso leucine	22.8	26.7	4.3	4.2
Leucine	41.6	46.7	6.9	9.1
Lysine	40.9	23.3	1.6	3.3
Phenylalanine	21.8	30.4	4.9	6.5
Methionine	16	10.6	1.7	2
Threonine	23	22	2.4	3.7
Tryptophan	4.9	7.1	1.0	1.4
Valine	29.3	31.6	4.3	5.1
Non-essential amino acids composition (g kg $^{-1}$)				
Alanine	43.3	29.4	2.0	4.6
Asparginine	60.5	68.7	3.4	7.2
Cystine	4.3	2.3	1.7	2.9
Glycine	59.8	31.5	32.5	5.6
Glutamine	79.4	112.1	1.7	44.9
Proline	36.9	32.2	11.6	14.5
Serine	25.5	30.6	4.3	6.3
Tyrosine	14.8	18.8	2.8	3.3

ND: Not detected

[a:] Minimum amount of material (mg mL $^{-1}$ assay medium) that produced agglutination.

Fish behaviour and feed intake

Based on the visual observation during feeding time, palatability or acceptability of feed was good and the behaviour of fish was normal. No left feed was observed in the aquaria. There was no mortality during the entire experimental period.

Proximate composition of feed ingredient, experimental diets and of whole body of fish and amino acid profile of feed ingredients and experimental diets

Proximate composition and amino acid profiles of feed ingredients and diets are shown in Tables 2 and 3. Diets contained about 45% crude protein and 21.0 kJ/g gross energy and were isonitrogenous and isoenergetic. Crude lipid and ash were in the range of 23.9–24.7% and 13.6–14.1% respectively. All experimental diets had almost similar amino acid composition.

Table 3 Proximate and amino acid composition of experimental diets (dry matter basis) for rainbow trout (*Oncorhynchus mykiss*) juveniles

	C_{ontrol}	J_{50}	$J_{62.5}$
Proximate (g kg^{-1})			
Dry matter	947	948	944
Crude protein	467	464	461
Crude lipid	247	240	239
Crude ash	141	138	136
Gross energy (KJ/g)	21.0	21.2	20.8
Essential amino acids (g kg^{-1})			
Arginine	24.75	36.81	39.96
Histidine	12.48	13.92	14.31

Iso leucine	16.05	17.64	17.95
Leucine	29.43	31.49	31.93
Lysine	28.41	27.33	27.96
Phenylalanine	15.58	18.77	19.48
Methionine	11.18	9.39	8.94
Threonine	16.15	15.88	15.84
Tryptophan	3.50	4.30	4.49
Valine	20.60	21.60	21.82
Non-essential amino acids (g kg^{-1})			
Alanine	30.17	25.36	24.33
Asparginine	42.23	45.07	46.09
Cystine	3.22	2.59	2.37
Glycine	41.60	34.82	31.09
Glutamine	58.72	68.39	71.98
Proline	26.70	25.65	24.96
Serine	18.10	20.03	20.48
Tyrosine	10.47	11.99	12.33

Highest moisture content of the whole body was observed in C_{ontrol} group, which was statistically not different ($P > 0.05$) to that in $J_{62.5}$ group and was lowest in J_{50} groups; whereas, crude lipid in whole body had reverse trend (Table 4). Gross energy content of the whole body did not differ significantly among the groups. Highest crude protein and ash deposition were observed in J_{50} groups and these values were statistically not different ($P > 0.05$) for those in $J_{62.5}$ groups and were higher than those in control group (Table 4).

Table 4 Chemical composition of whole body of rainbow trout (*Oncorhynchus mykiss*) juveniles of different experimental groups at the start and at the end of the experiment (g kg^{-1}, wet basis ± SD)

Treatment	Moisture	Crude protein	Crude lipid	Ash	Gross energy (KJ/g)
Initial fish	736 ± 2.0	173 ± 0.50	56 ± 1.20	30 ± 1.10	59 ± 1.50
C_{ontrol}	745^{a} ± 2.70	139^{b} ± 4.90	92^{ab} ± 4.40	23^{b} ± 1.50	67 ± 1.60
J_{50}	720^{b} ± 4.80	159^{a} ± 4.20	96^{a} ± 1.60	25^{a} ± 0.80	70 ± 3.10
$J_{62.5}$	736^{a} ± 8.10	153^{a} ± 6.30	86^{b} ± 5.80	25^{a} ± 1.00	66 ± 2.90
SEM	3.50	2.90	1.70	0.50	0.90

Values are mean (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly (P < 0.05).

Growth performance and feed utilization

Weekly body mass gains of fish are given in Figure 1. The growth performance of the fish at the end of the experimental period and the nutrient utilization are presented in Table 5. Weekly body mass gain indicates that second week onwards there was differential growth among the group, and lower body mass development was observed in J_{a2} group compared to other groups. This trend was maintained till the end of the experiment. The BMG, SGR, MGR and ALC were statistically similar (P>0.05) for C_{ontrol} and J_{50} groups and significantly higher (P<0.05) compared to those for $J_{62.5}$. FCR, PER, PPV, and ER did not differ significantly among the three groups (Table 5).

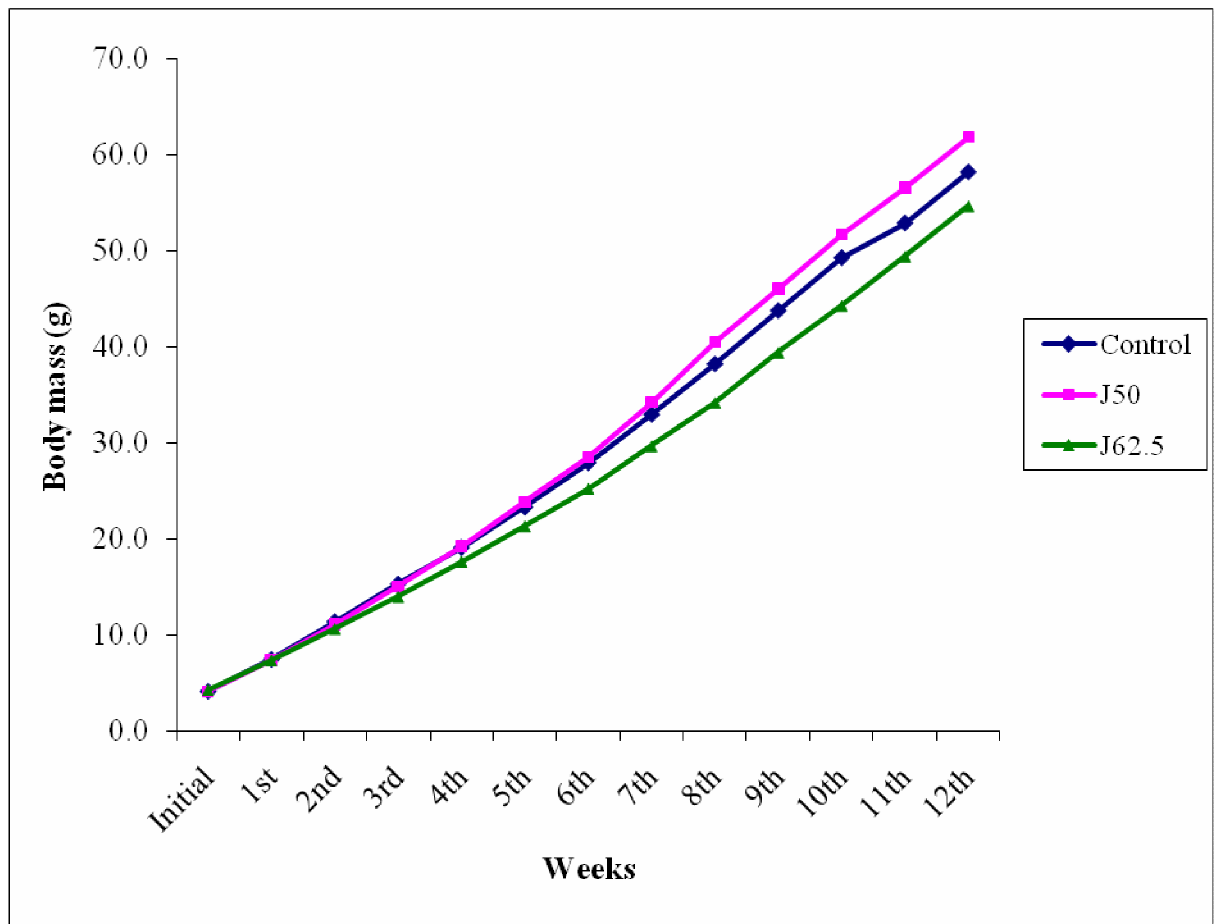


Figure 1: Weekly body mass gain (g) of rainbow trout (*Oncorhynchus mykiss*) juveniles fed with experimental diets for 12 weeks.

Table 5 Initial body mass (IBM), growth performance and nutrient utilization of rainbow trout (*Oncorhynchus mykiss*) juveniles fed with experimental diets for 12 weeks

Treatment	IBM (g)	BMG (%)	SGR (%)	MGR (g kg$^{0.8}$ day^{-1})	FCR	PER	PPV (%)	ALC (%)	ER (%)
C_{ontrol}	4.12 ± 0.26	1385^{a} ± 157	3.2^{a} ± 0.12	11.4^{a} ± 0.37	1.3 ± 0.10	1.6 ± 0.10	22.8 ±1.10	31.0^{a} ± 2.30	24.8 ± 0.45
J_{50}	4.17 ± 0.54	1394^{a} ± 185	3.2^{a} ± 0.15	11.4^{a} ± 0.42	1.2 ± 0.10	1.7 ± 0.11	26.3 ±2.37	31.9^{a} ±1.80	25.5 ± 1.49
$J_{62.5}$	4.31 ± 0.49	1178^{b} ± 257	3.0^{b} ± 0.24	10.8^{b} ± 0.75	1.3 ± 0.20	1.6 ± 0.40	25.2 ± 4.59	27.5^{b} ±1.89	23.2 ± 2.18
SEM	0.07	38.66	0.03	0.10	0.02	0.04	0.91	0.80	0.49

BMG - Body mass gain, SGR – Specific growth rate and MGR - Metabolic growth rate; FCR – Feed conversion ratio, PER - Protein efficiency ratio, PPV - Protein productive value, ALC - Apparent lipid conversion and ER - Energy retention

Values are mean (n = 12) ± standard deviation for BMG, SGR, MGR, FCR and PER; Values are mean (n = 4) ± standard deviation for PPV, ALC and ER

Mean values in the same column with different superscript differ significantly (P < 0.05).

Dry matter and nutrient digestibility and efficiency of digestible nutrients and energy

The digestibility of dry matter and nutrients of different experimental diets are given in Table 6. The dry matter protein, lipid and energy digestibility were highest for control group and statistically not different (P>0.05) from J_{50} group but significantly higher (P<0.05) than $J_{62.5}$ group.

Efficiency of digestible nutrients and energy of experimental diets are shown in Table 6. Efficiency of digestible nutrients and energy of experimental diets did not differ significantly among the three groups.

Digestive enzymes activities

Amylase, protease and lipase activities were highest in control group, followed by J_{50} and $J_{62.5}$ groups; all being significantly different (Table 6).

Relative intestinal length (RIL), spleen Index (SI), hepatosomatic and gastro somatic index (GSI)

RIL, SI, hepatosomatic and GSI of different experimental groups are given in Table 7. Highest HSI and SI were observed in control group and statistically (P>0.05) lowest value in $J_{62.5}$ groups. Whereas RIL exhibited opposite trend. An increase in the RIL was noticed in J_{50} and $J_{62.5}$ with the longest length in the latter. There was no significant difference in GSI among the different groups.

Table 6 Effects of experimental diets on the apparent digestibility coefficient of dry matter, nutrient and energy digestibility (%); utilization of digestible protein, lipid and energy; and digestive enzymes activities (U/mg protein) in rainbow trout (*Oncorhynchus mykiss*) juveniles

Treatment	Dry matter digestibility	Protein digestibility	Lipid digestibility	Energy digestibility	DPE (%)	DLE (%)	DEE (%)	Amylase	Protease	Lipase
C_{ontrol}	$78.6^{a} \pm 0.81$	$89.8^{a} \pm 0.55$	$95.2^{a} \pm 0.43$	$86.8^{a} \pm 0.83$	25.4 ± 1.08	32.6 ± 2.55	28.7 ± 2.15	$4.6^{a} \pm 0.40$	$50.3^{a} \pm 3.59$	$13.9^{a} \pm 1.02$
J_{50}	$79.5^{a} \pm 0.42$	$89.7^{a} \pm 0.83$	$95.2^{a} \pm 0.80$	$86.1^{a} \pm 0.71$	29.3 ± 2.58	33.5 ± 2.10	29.4 ± 4.12	$3.2^{b} \pm 0.20$	$41.0^{b} \pm 2.16$	$10.8^{b} \pm 0.38$
$J_{62.5}$	$73.3^{b} \pm 2.11$	$84^{b} \pm 1.41$	$89.8^{b} \pm 0.86$	$81.8^{b} \pm 1.13$	29.9 ± 5.05	30.6 ± 1.87	26.2 ± 3.43	$2.5^{c} \pm 0.15$	$32.5^{c} \pm 1.29$	$8.6^{c} \pm 0.48$
SEM	0.90	0.85	0.79	0.71	1.06	0.68	0.98	0.27	2.28	0.68

DPE: digestible protein efficiency; DLE: digestible lipid efficiency; DEE: digestible energy efficiency
Values are mean (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Cholesterol and triglyceride levels in plasma, and cholesterol, phorbol esters (PEs) and lipid peroxides in muscle

Cholesterol and triglycerides levels in plasma, muscle cholesterol and muscle lipid peroxide of different experimental groups are shown in Table 7. Plasma cholesterol and muscle cholesterol levels were highest in control group, and lowest in $J_{62.5}$ groups. PEs content in dried whole fish was undetectable. Muscle lipid peroxide value did not differ significantly among the three groups.

Blood chemistry (Hemato-immunological parameters)

Haematological, biochemical and metabolic response parameters are presented in Tables 8 - 9. RBC and WBC counts; hemoglobin, hematocrit level, MCV, MCH, MCHC, globulin, total bilirubin, BUN, calcium and potassium contents; and ALP and ALT activities in blood and lysozyme activities in serum did not differ significantly among the three groups.

Glucose and creatinine level in blood were statistically similar for C_{ontrol} and J_{50} and significantly higher than those for $J_{62.5}$ group. On the other hand, opposite trend was observed for the phosphorus and sodium ion, albumin, and total protein in blood.

Histopathology

In the present study we **did** not observe any signs of pathological lesions in rainbow trout. Rainbow trout was shown in Figure 2. As presented in (Fig. 3), serial sections made at level 1 include: liver (L), cranial part of the stomach (S), pyloric appendices of mid gut (P) and hindgut (E). At level 2 the stomach, loop formation of pyloric appendices and the hindgut are sectioned and at level 3 the caudal flexure of the stomach, a few distal parts of pyloric appendices and the most distal part of the hindgut are shown.

Table 7 Cholesterol and triglyceride (mg/dl) level in plasma; and muscle cholesterol (mg/100 g) level and muscle lipid peroxide (nMol MDA (malondialdehyde) /100g tissue) level, relative intestinal length (RIL) (mm g^{-1}), spleen index (SI), hepatosomatic index (HSI) and gastro somatic index (GSI) of rainbow trout (*Oncorhynchus mykiss*) juveniles

Treatment	Plasma cholesterol	Plasma triglycerides	Muscle cholesterol	Muscle lipid peroxide	*RIL*	SI	*HSI*	GSI
Control	246^{a} ± 23.9	162^{b} ± 3.5	93^{a} ± 2.0	3.70 ± 2.12	0.47^{a} ± 0.02	0.19^{a} ± 0.01	1.3^{a} ± 0.20	2.25 ± 0.65
J_{50}	182^{b} ± 11.2	235^{a} ± 3.9	85^{b} ± 5.5	4.23 ± 1.40	0.56^{b} ± 0.02	0.22^{b} ± 0.01	1.2^{ab} ± 0.10	1.95 ± 0.34
$J_{62.5}$	171^{b} ± 3.4	255^{a} ± 6.4	75^{b} ± 4.8	3.95 ± 2.76	0.63^{c} ± 0.01	0.24^{c} ± 0.00	1.1^{b} ± 0.10	1.74 ± 0.14
SEM	12.7	14.3	2.8	0.55	0.02	0.01	0.04	0.13

Values are mean (n = 4) ± standard deviation.
Mean values in the same column with different superscript differ significantly (P < 0.05).

Table 8 Effects of experimental diets on the haematological parameters (RBC (10^6 cells/mm^3), WBC (10^3 cells/mm^3), Hb (g/dl), Hct (%), MCV (fL), MCH (pg), MCHC (g/dl), albumin (g/dl), globulin (g/dl) and total protein (g/dl) in the blood and lysozyme activity (IU/ml) in the serum of rainbow trout (*Oncorhynchus mykiss*) juveniles

Treat	RBC	WBC	Hb	Hct	MCV	MCH	MCHC	Albumin	Globulin	Total protein	Lysozyme activity
C_{ontrol}	0.96 ± 0.05	19.7 ± 3.69	4.5 ± 0.5	45.6 ± 5.32	466 ± 30.8	46.6 ± 3.08	10.18 ± 1.41	2.18^b ± 0.28	1.64 ± 0.30	3.8^b ± 0.20	494 ± 52
J_{50}	0.97 ± 0.07	19.8 ± 3.18	4.3 ± 0.4	45.2 ± 4.10	442 ± 24.6	44.2 ± 2.46	9.54 ± 0.95	2.66^a ± 0.15	1.40 ± 0.32	4.10^a ± 0.30	505 ± 45
$J_{62.5}$	1.05 ± 0.08	19.4 ± 2.94	4.5 ± 0.5	47.2 ± 8.04	432 ± 67.1	43.2 ± 6.71	10.57 ± 3.07	2.36^b ± 0.53	1.46 ± 0.34	3.8^b ± 0.50	564 ± 94
SEM	0.02	0.79	0.10	2.49	11.41	1.14	0.69	0.15	0.08	0.10	23.02

Values are mean (n = 4) ± standard deviation.

Mean values in the same column with different superscript differ significantly ($P < 0.05$)

MCV: Mean cell volume (fL); MCH: Mean corpuscular hemoglobin (pg); MCHC**:** Mean corpuscular hemoglobin concentration (g/dl)

IU- The amount of enzyme required producing a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25^0C, using a suspension of *Micrococcus lysodeikticus* as the substrate.

Table 9 Effects of experimental diets on alkaline phosphatase (ALP, U/l), alanine transaminase (ALT, U/l), glucose (mg/dl), total bilirubin (TBIL, mg/dl), blood urea nitrogen (BUN, mg/dl) and creatinine (mg/dl) in blood, blood ions (calcium (mg/dl), phosphorus mg/dl, sodium (mMol/l) and potassium (mMol/l) of rainbow trout (*Oncorhynchus mykiss*) juveniles

Treatment	ALP	ALT	Glucose	TBIL	BUN	Creatinine	Calcium	Phosphorus	Sodium	Potassium
C_{ontrol}	101 ± 8.3	58.4 ± 13.1	117^{a} ± 10.4	0.22 ± 0.04	2.0 ± 0.01	1.70^{a} ± 0.86	12.4^{b} ± 0.49	14.9^{b} ± 0.98	141^{b} ± 4.04	1.48 ± 0.36
J_{50}	96 ± 25.0	75.2 ± 41.8	90^{b} ± 6.8	0.20 ± 0.01	2.2 ± 0.45	0.98^{ab} ± 0.22	13.3^{a} ± 0.35	17.3^{a} ± 1.57	143^{ab} ± 3.16	1.5 ± 0.31
$J_{62.5}$	84 ± 30.7	71.0 ± 35.5	86^{b} ± 2.75	0.24 ± 0.05	2.0 ± 0.01	0.34^{b} ± 0.21	12.2^{b} ± 0.82	16.7^{a} ± 0.62	146^{a} ± 2.68	1.6 ± 0.32
SEM	5.91	8.40	5.57	0.01	0.01	0.19	3.26	0.38	1.01	0.08

Values are mean (n = 4) ± standard deviation.

Mean values in the same column with different superscript differ significantly (P < 0.05).

1 U = 16.66 nKat/l; nKat = Amount of glandular kallikrein which cleaves 0.005 mmol of substrate per minute).

The stomach of all investigated rainbow trout appears as a tube of slightly varying diameter. Depending on which levels sections were cut, luminal folds are designed differently and the thickness of the muscle layers varies. Gastric glands in all three feeding experiments are well developed, and the epithelium lining the luminal surface consisting of highly columnar cells, which produce protective mucous, are not altered or even damaged. The same is true for the branched tubular glands at the bottom which are also well developed (Fig. 4 and 5). Shape and cellular morphology of pepsin and hydrochloric acid producing cell-type (oxyntopeptidic cell) remain unchanged and no signs of inflammation or increased leucocyte immigration were found in all three experimental groups.

The development of intestinal loops, pyloric appendices and terminal intestine (hind gut) remains largely unchanged (Fig. 6) and the villi of appendices or terminal intestine are well constructed. The surface epithelium layer is thereby composed of unchanged slender highly columnar enterocytes (Fig. 7) and region-specific with different numbers of goblet cells. Apopotic cell death of enterocytes is found in rather infrequent numbers in all three experiments, which is roughly the same for immune cell infiltration. No pathological alterations can also be seen in the hindgut or terminal gut segment.

Regarding liver and pancreatic morphology, the typical arrangement of parenchymal cells in both glands (dissiminated pancreas in trout) remains unchanged. No signs of hepatic steatosis or lipidosis were found in rainbow trout fed with DJKM (Fig. 8).

Figure 2 Rainbow trout (*Oncorhynchus mykiss*)

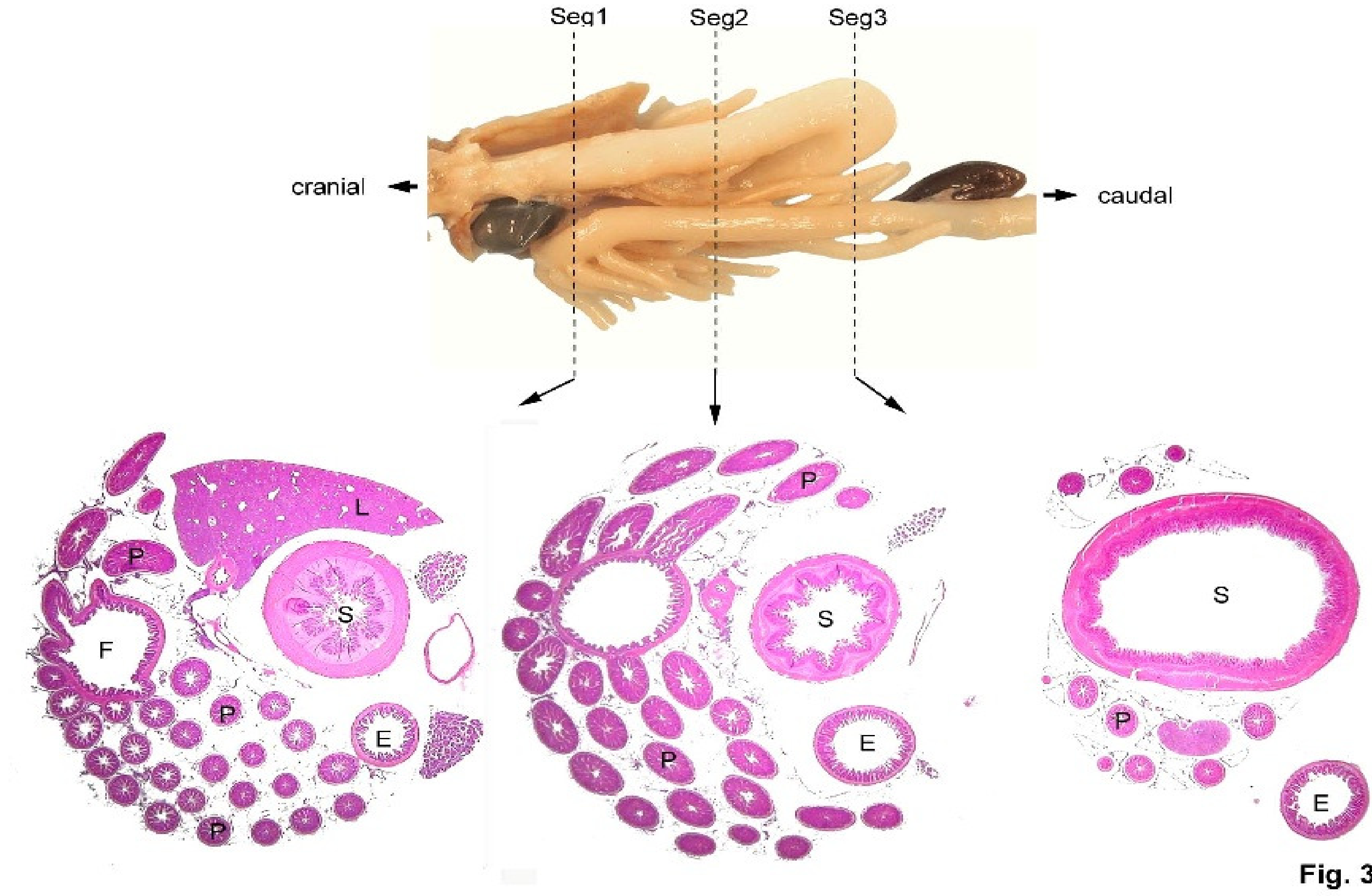
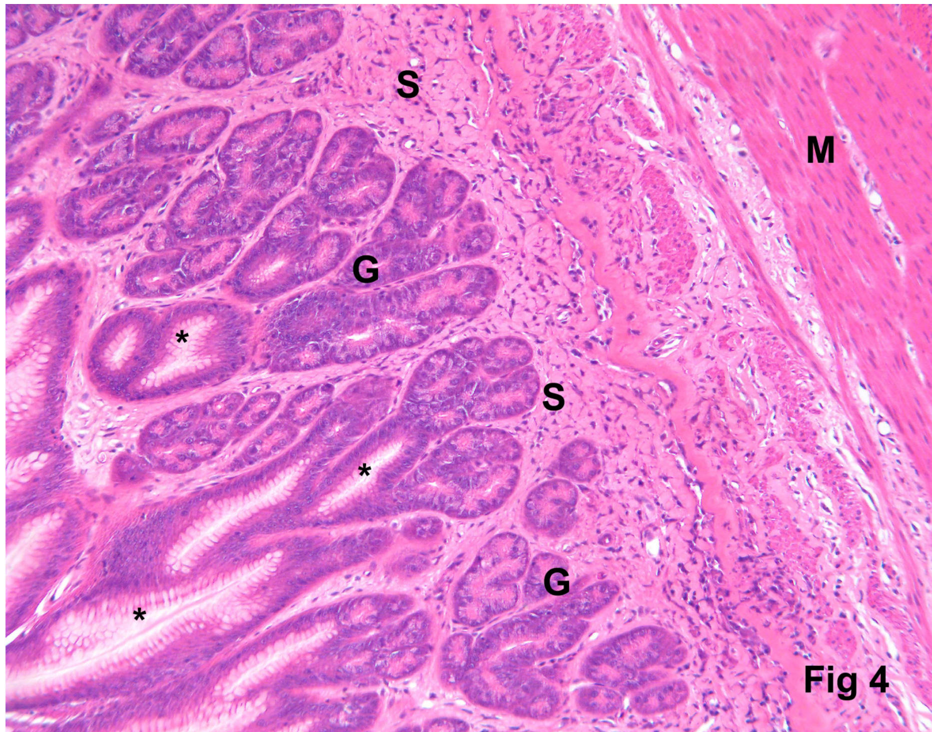


Fig. 3 On top: different section-planes through the digestive tract of the trout. Below: sections through segment 1, 2 and 3 consisting of: liver (L), stomach (S), foregut (F) with pyloric appendices (P), hind gut/rectum (E).

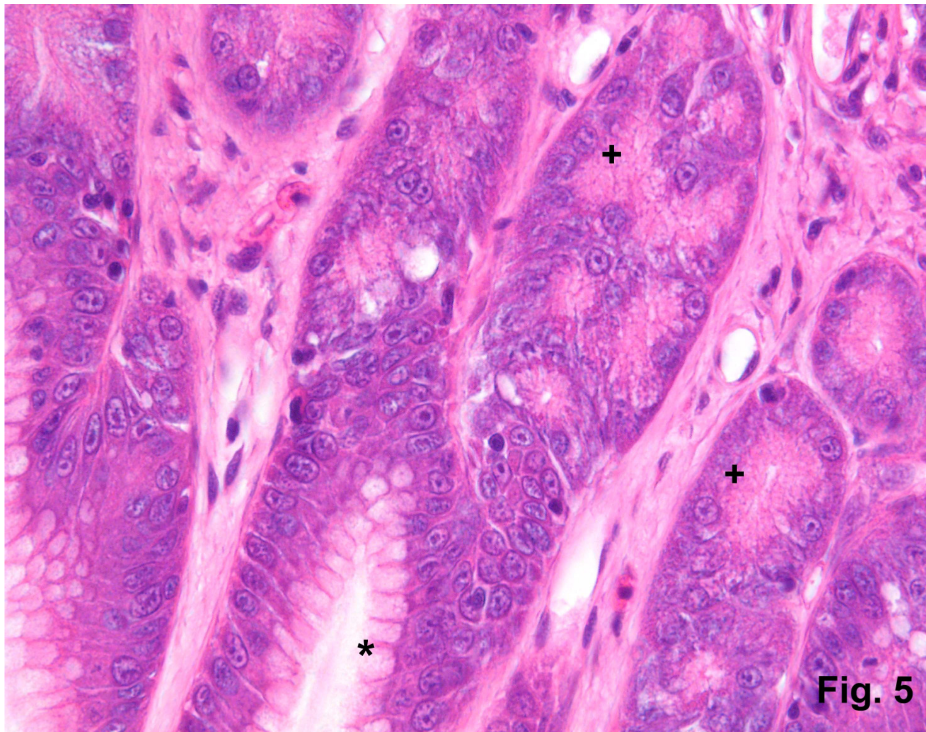
1



2 **Fig. 4** Trout stomach showing well developed gastric glands (G), submucosa (S) and

3 the tunica muscularis (M) of $J_{62.5}$ group**.**

4

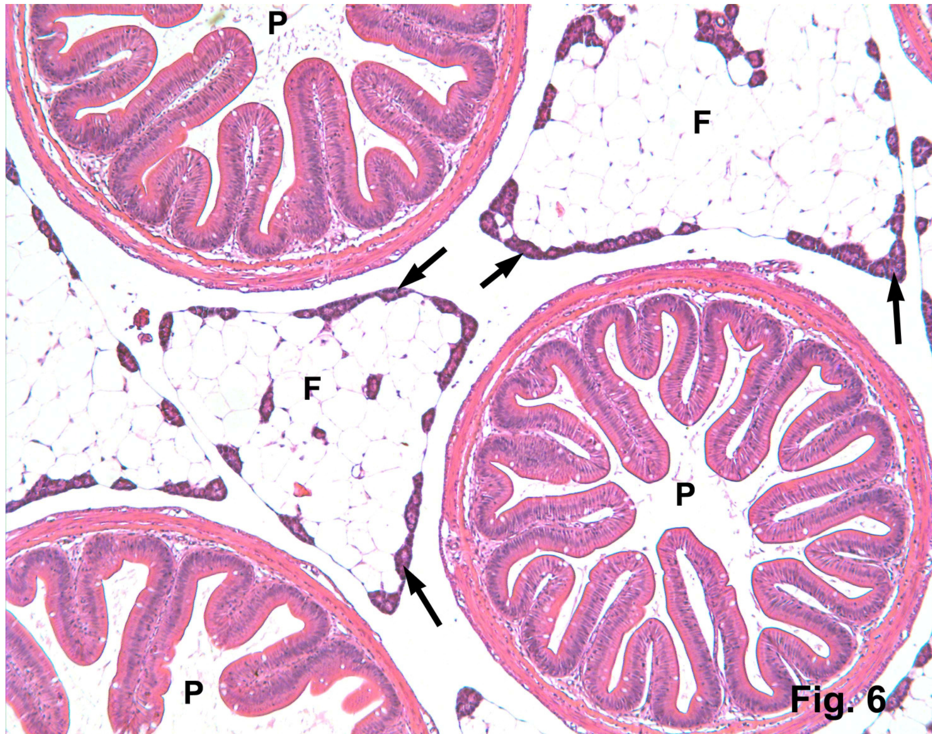


5

6 **Fig. 5** Higher magnification of branched tubular gastric glands. Shape and cellular
7 morphology of mucous (*) and pepsin and hydrochloric acid producing cell-type (+)
8 (oxyntopeptidic cells) of $J_{62.5}$ group did not show any signs of pathological
9 alterations compared with Control or J_{50} groups.

10

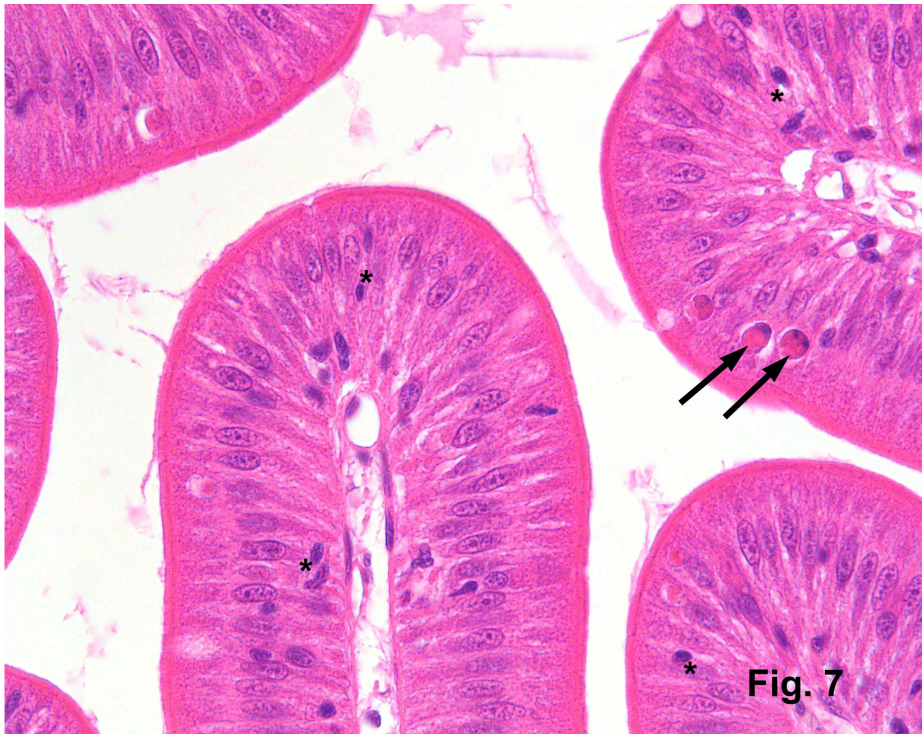
11



12 **Fig. 6** Cross section through pyloric appendices **(P)**, disseminated pancreatic cells

13 (arrows) and fat tissue (F) of $J_{62.5}$ group.

14

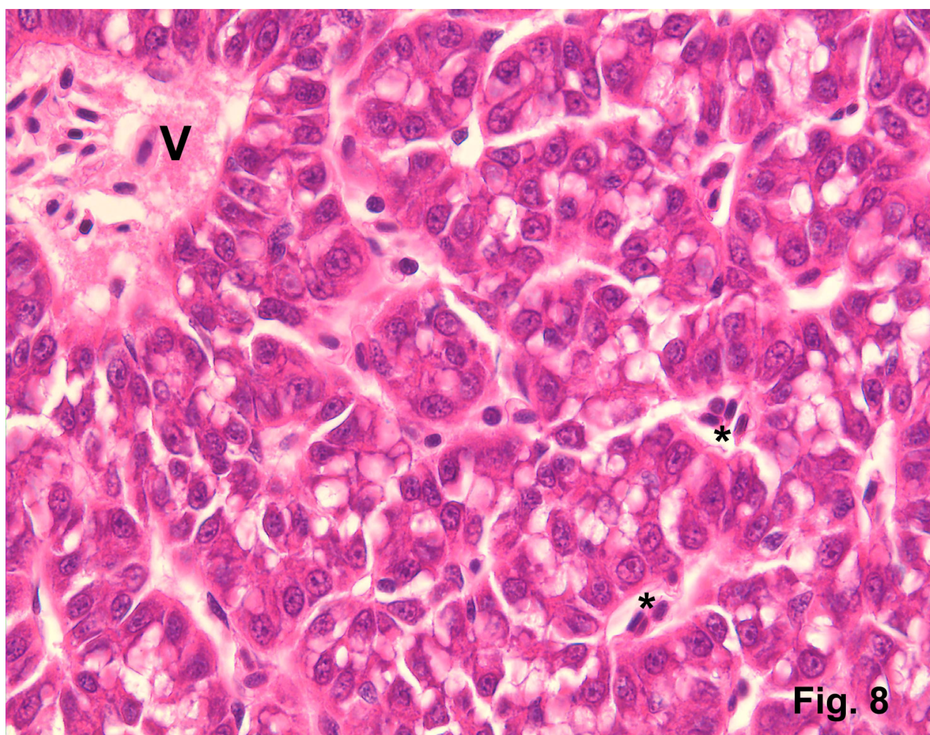


15

16 **Fig. 7** Detail of the previous figure showing several villus tips and enterocytes.
17 Typical and unchanged appearance of absorbing cells provided with a well
18 developed brush border lining the cell surface ($J_{62.5}$ group). Also visible single
19 penetrating lymphocytes (*) and apoptotic enterocytes (arrows).

20

21



22 **Fig. 8** The liver of $J_{62.5}$ group did not show any pathological alterations or signs of

23 steatosis (hepatic lipidosis). Cords of hepatocytes separated by sinusoids containing

24 erythrocytes.

25

26 **Discussion**

27 A large number of investigations have been conducted on the replacement of FM

28 with SBM as a protein source in feeds for rainbow trout (Sanz *et al.* 1994; Kaushik

29 *et al.* 1995; Barrows *et al.* 2008). Similarly, other plant sources such as peas

30 (Gouveia *et al.* 1993; Pfeffer *et al.* 1995), lupin (Gouveia *et al.* 1993; Bangoula *et al.*

31 1993), faba beans (Gouveia *et al.* 1993; Bangoula *et al.* 1993), rapeseed, canola

32 (Bangoula *et al.* 1993; Stickney 1996), and sunflower meal (Sanz *et al.* 1994) have

33 been used in trout feeds. To our knowledge this is the first study on the replacement

34 of FM by DJKM in rainbow trout. Results of this study showed that DJKM could

35 replace 50% of dietary FM without affecting growth performance, nutrient

36 utilization, and metabolic and hematological parameters.

37

38 *Growth performance and nutrient utilization*

39 Growth performance and nutrient utilization of rainbow trout fed J_{50} diet (50% FM
40 protein replacement by DJKM) were better than that of $J_{62.5}$ group and similar to the
41 control group (FM based diet). These results are in concurrence with other studies
42 on SBM wherein SBM protein could substitute 50% of FM protein without
43 evidencing a negative effect on the feed intake and growth performance (Refstie *et*
44 *al.* 2000; Francesco *et al.* 2004; Aksnes *et al.* 2006; Refstie *et al.* 2006). Since there
45 were no refusals for any of the diets, the observed differences in growth in this
46 experiment were not due to different palatability of the diets. Significantly lower
47 growth response of $J_{62.5}$ group might be because of several factors such as lower
48 digestibilities of protein and energy in the diets (Table 6), which could lead to lower
49 protein and energy availability from the DJKM (plant protein structures in general
50 are much more compact than FM protein, so digestive enzymes act slowly on DJKM
51 proteins), and/or the presence of antinutrients such as phytate and NSP, which are
52 present in high amounts in the DJKM and could affect adversely the feed utilization
53 at higher level of its incorporation. Another constrain related to the digestion of
54 plant sources is their relatively high carbohydrate content that is generally not well
55 digested by salmonids (Singh & Nose 1967). The negative effects at high inclusion
56 levels of plant protein sources such as sunflower meal, SBM and maize gluten (> 75
57 % replacement of FM protein) on growth performance are well documented in
58 earlier work on trout (Kaushik *et al.* 1995; Sanz *et al.* 1994; Refstie *et al.* 2000;
59 Morris *et al.* 2005).

60 We did not observe any significant difference in FCR, PER, PPV and ER
61 among the groups. Our results are in contrast with those obtained in earlier studies
62 wherein SBM has been evaluated in trout diets and diets of several other species by
63 many researchers (Refstie *et al.* 2000; Refstie *et al.* 2006; Opstvedt *et al.* 2003).
64 They observed slightly reduced nutrient utilization (PER and PPV) when 50 - 75%
65 of FM protein was replaced by SBM. These differences could due to the inherent

66 differences between the nature of the two plant protein sources, soyabean meal and
67 detoxified *Jatropha* kernel meal.

68

69 *Biochemical composition of whole body of fish*

70 In the present study the decrease in protein content in the whole body of fish fed
71 control diet was associated with increase in whole body lipid content. Moisture
72 content exhibited inverse relationship with crude lipid in the whole body of rainbow
73 trout. Highest lipid concentration was observed in J_{50} group, which was similar to
74 that in control group, and lowest deposition was observed in $J_{62.5}$ group. Similarly,
75 Francesco *et al.* (2004) observed higher lipid content in rainbow trout fed plant
76 protein. There is evidence that replacement of FM by plant protein sources such as
77 corn gluten meal and soy protein concentrates affects increaes hepatic lipogenic
78 enzyme activities in seabass (Dias 1999; Kaushik *et al.* 2004) that leads to higher
79 whole body lipid. In salmonids, increases found in whole body fat content with the
80 use of dietary plant proteins, were explained by imbalances in amino acid
81 concentrations (Kaushik *et al.* 2004; Bjerkeng *et al.* 1997). Furthermore, it is
82 suggested that unbalanced amino acid composition influences energy metabolism.
83 Vilhelmsson *et al.* (2004) found an up-regulation of several proteins involved in
84 energy metabolism in rainbow trout liver when fed plant (maize gluten meal, wheat
85 gluten, extruded whole heat, extruded peas and rapeseed meal) protein and
86 concluded that the plant protein increase the energy demands of fish. Possible reason
87 could be higher supply of some of the dispensable amino acids such as glutamic acid
88 in excess by the plant protein fed diets that could have lead to higher lipid retention
89 (Barrows *et al.* 2008). These authors also indicate the involvement of possible
90 metabolic or endocrine mechanisms in eliciting such differences in whole body lipid
91 deposition. It may be noted that in our study as well, glutamic acid concentration in
92 DJKM based diets was higher.

93 Efficient protein synthesis requires sufficient availability of all essential
94 amino acids (Dabrowski & Guderly 2002). Unbalanced amino acid concentrations in
95 a diet resulted in increased protein degradation (Langar *et al.* 1993; von der Decken
96 & Lied 1993), and thereby increased protein turnover (Martin *et al.* 2003). Many
97 researchers (Refstie *et al.* 2000; Pack *et al.* 1995; Cheng *et al.* 2003) reported that
98 the plant protein (SBM) based diets lower nitrogen retention in salmon and trout
99 because these diets have less digestible energy and an amino acid profile that is
100 suboptimal for muscle growth. Interestingly in our study crude protein content in
101 whole body was higher in DJKM fed groups. Similarly Barrows *et al.* (2008) and
102 Cheng *et al.* (2003) also found that the body protein content increased significantly
103 when SBM replaced FM in trout diet. This indicates that DJKM contain optimum
104 digestible energy and balanced amino acid profile optimal for trout growth.

105 DJKM based diets (J_{50} and $J_{62.5}$) were supplemented with phytase and that
106 could have released minerals bound to phytate. Consequently, leading to increase in
107 body minerals contents, as observed in J_{50} and $J_{62.5}$ groups. On the other hand,
108 Elangovan & Shim (2000) and Barrows *et al.* (2008) observed that SBM containing
109 diets up to (35%) inclusion (without the addition of phytase) had no effect on body
110 ash content. The effects of phytate or phyatse are not expected to be apprehensive
111 for SBM containing diets because it contains lower ash and phytate contents than
112 DJKM.

113 Similar values of HSI in control and J_{50} groups and these values being higher
114 than that in $J_{62.5}$ group suggest higher lipid deposition in liver of control and J_{50}
115 group. It may be noted that the whole body lipid content followed a similar pattern.
116 HSI values of above 1, as observed here, are common in rainbow trout and European
117 seabass (Francesco *et al.* 2004; Dias 1999; Ballestrazzi *et al.* 1998) where hepatic fat
118 deposition is very high (Dias 1999).

119

120 *Digestibility measurement and efficiency of digestible nutrients and energy*

121 Detoxified *Jatropha* kernel meal in combination with FM protein showed excellent
122 dry matter, crude protein, lipid and energy digestibilities in the present study.
123 Generally, oil seed meal proteins have digestibilities of 80-95% for fish (Jauncey &
124 Ross 1982). Dry matter, protein, lipid and energy digestibilities of experimental
125 diets were 73-80%, 84-90%, 90-95% and 82-87% respectively, which indicate
126 excellent utilization of feed ingredients. The protein digestibility is a key factor in
127 the evaluation of the quality of a diet for fish and its potential for the synthesis of
128 new tissues.

129 The ADCs observed are comparable to values obtained for good quality
130 proteins in fish diets (Cho & Kaushik 1990). Dry matter digestibilities were above
131 73% for all diets and these values were higher than those (56-60%) observed by
132 Hilton & Slinger (1986) and Abdou *et al.* (1990). Higher ADCs for protein of J_{50}
133 diet than $J_{62.5}$ diet implies that a greater amount of protein would have been available
134 to the fish from the J_{50} diets. While ADCs of proteins was little affected by dietary
135 treatments, inclusion of DJKM at 62.5% replacement of FM protein, decreased dry
136 matter, lipid and energy digestibilities. The low-energy digestibility of the plant
137 (DJKM) based diet ($J_{62.5}$) can be attributed to their high-carbohydrate content and its
138 poor digestibility by rainbow trout, although the energy digestibility values were
139 higher (82-87%) than those reported by many researchers for fish (Gomes *et al.*
140 1983; Gouveia *et al.* 1993).

141 In our study higher inclusion of DJKM in rainbow trout diet decreased lipid
142 digestibility. Several studies have concluded that dietary plant protein lower the lipid
143 digestibility in salmonids (Storebakken *et al.* 1998; Romarheim *et al.* 2006;
144 Yamamoto *et al.* 2007). The lower lipid digestibility of the fish fed the $J_{62.5}$ diet may
145 be associated with the increase in the NSP content, which reduces fat absorption by
146 disturbing micelle formation in the gastro intestinal tract; and another reason could

147 be that NSP entrap bile salts, thereby reduce their effectiveness in solubilizing fats
148 (Krogdahl *et al.* 2003; Gatlin *et al.* 2007; Øverland *et al.* 2009).

149 Interestingly it was found that J_{50} diet had similar ADC of dry matter, protein
150 and lipid and apparent energy digestibility as for control diet. These results
151 demonstrate that the rainbow trout were efficient in digesting protein, lipid and
152 energy from the DJKM meal at 50% replacement level, while fish fed at higher level
153 (62.5% replacement of FM) in general exhibited lower digestibilities. Crude protein
154 digestibilities of DJKM diets were high (above 84%) in rainbow trout, suggesting
155 DJKM to be an excellent protein source for this species.

156 Efficiency values of digestible nutrients and energy indicate retained
157 nutrients and energy in whole body relative to the total digestible nutrients and
158 digestible energy. Efficiency of digestible nutrients and energy of diets did not differ
159 significantly among the three groups, indicating that rainbow trout has utilized
160 DJKM well and retained nutrients in the body maximally and similar to control
161 group. The value for the efficiency of digestible protein, lipid and energy were in the
162 range of 25-30%, 31-34% and 26-29% respectively.

163

164 *Digestive enzyme activities and relative intestinal length (RIL)*

165 Heat labile antinutrients such as trypsin inhibitors and lectins were not detected in
166 the autoclaved DJKM, whereas heat stable antinutrient (phytate) was present in the
167 DJKM. Phytate is known to inhibit activities of digestive enzymes such as pepsin,
168 trypsin and alpha-amylase (Robaina *et al.* 1995; Alarcon *et al.* 1999), or to form
169 complexes with minerals (Teskeredzic *et al.* Sugiura *et al.* 1999) and proteins
170 (Moyano *et al.* 1999), thereby modifying digestion processes and impairing
171 intestinal absorption. In our study digestive enzymes activities decreased on
172 inclusion of DJKM in the rainbow trout diet. Decrease in digestive enzyme
173 (amylase, protease and lipase) activities in trout intestine might be because of
174 phytate present in the DJKM based diets. For plant based feeds, phytase has been

175 added in the feed at a level of 500 FTU per kg (Forster *et al.* 1999; Cheng *et al.*
176 2004). We used 500 FTU phytase per kg feed, which might not be sufficient because
177 of the high phytate content in DJKM. For any new feed resource such as *Jatropha*
178 kernel meal separate systematic studies on optimization of phytase level in the diet
179 need to be undertaken. In our laboratory, work is in progress to optimize phytase
180 level in the DJKM based diets for trout, common carp (*Cyprinus carpio* L.) and
181 tilapia (*Oreochromis niloticus*).

182 Lower protease activities corresponded to decrease in protein availability
183 from DJKM and the presence of unutilised phytate, as DJKM level increased in
184 rainbow trout diets. Similar results were observed by Santigosa *et al.* (2008),
185 Sandholm *et al.* (1976) and Krogdahl *et al.* (1994). They found that protein digesting
186 enzyme (for example trypsin) activity decreases as plant protein inclusion increases
187 in trout diet, and they concluded that trypsin is highly sensitive to plant antinutrients.
188 Reduced nutrient digestibility and morphological changes in the distal intestinal
189 mucosa leading to digestive and absorptive dysfunctions, with digestive enzyme
190 activities decreasing in a dose-dependent manner with increasing SBM inclusion
191 levels in Atlantic salmon (Krogdahl *et al.* 2003) have been reported. The lower
192 activity of digestive enzymes in DJKM fed groups was correlated with lower
193 nutrient digestibility.

194 It is known that carnivorous and omnivorous fish require longer time to
195 digest plant protein based diets (Buddington *et al.* 1997). Direct relationship
196 between the amount of dietary plant protein and RIL has been reported earlier in fish
197 (Kramer & Bryant 1995). In rainbow trout, DJKM based diets exhibited higher RIL
198 than the control group. RIL value increases as the plant protein inclusion increases
199 in the trout diets (Øverland *et al.* 2009). From a physiological view point, a longer
200 RIL would facilitate an increase in digestibility and retention time by enhancing
201 contact time of the digestive enzymes and the feed components, resulting in increase
202 in their digestion and absorption. Carnivorous fish like rainbow trout species showed
203 compensation mechanisms, such as an increase in RIL and as a result increase in

204 digestive activity, to achieve a digestive balance and growth rates similar to those
205 observed for FM fed group.

206 *Cholesterol and triglycerides; and blood glucose level*

207 Dietary inclusion of DJKM in the trout reduced cholesterol level in plasma and
208 muscle as compared to control group. The decrease in plasma cholesterol levels in
209 fish fed diets with plant proteins has already been reported in rainbow trout
210 (Kaushik *et al.* 1995; Romarheim *et al.* 2006;Yamamoto *et al.* 2007). In terrestrial
211 animals, plant products are generally considered to have a hypocholesteromic effect
212 (De Schrijver 1990), mainly due to the relatively high levels of estrogeno-mimetic
213 isoflavones (Setchell & Cassidy 1999). In humans, different plant constituents have
214 been reported to lower plasma cholesterol levels (Wester 2000). Although
215 cholesterol metabolism in mammals and fish could differ, the fish
216 hypocholesterolemia in response to dietary plant protein supply could be due to an
217 increased excretion of bile salts, an inhibition of cholesterol intestinal absorption, or
218 just the withdrawal of FM rather than to the direct effects of plant protein (Kaushik
219 *et al.* 2004). In any case, the significance of hypocholesterolemia in fish should be
220 studied in depth. Plasma triglycerides increased in concentrations with increased
221 dietary DJKM level. The increased in whole body lipid content in J_{50} group along
222 with the increased in plasma triglyceride concentrations. Whereas Shimeno *et al.*
223 (1993) observed opposite trend in the yellowtail (*Seriola dumerilii*).

224 Blood glucose level was affected by dietary treatments. Lower blood glucose
225 level was observed in DJKM fed groups than control group. DJKM based diets
226 contain higher amount of NSP. Usually NSP in monogastric animals can delay
227 intestinal absorption of glucose, possibly through a reduced rate of gastric emptying,
228 leading to delayed absorption (Knudsen Bach 2001). Opposite trends were shown in
229 fish fed diets containing SBM and corn gluten as a substitute (Kikuchi *et al.* 1994;
230 Kikuchi 1999), but Glencross *et al.* (2004) observed that dietary inclusion of yellow
231 lupin in trout diet did not affect blood glucose level.

232 *Blood chemistry*

233 Red blood cells and WBC counts; and Hct and Hb level did not differ significantly
234 among the groups and their ranges were in the normal range reported by Blaxhall &
235 Daisley (1973) for healthy trout. A corresponding similar Hb and Hct content
236 accompanied the similar number of red blood cells. Consequently, MCV, MCH and
237 MCHC value was not changed. MCV did not differ significantly among the groups
238 because RBC counts and Hct values were not different among the groups. Whereas
239 in another study (Hemre *et al.* 2005) significant reduction of MCV on increase in the
240 content of SBM in salmon diet was observed. As reduction of MCV appeared to
241 coincide with increased spleen size (Hemre *et al.* 2005), it was suggested that some
242 of the plant ingredients might cause early release of immature erythrocytes. In our
243 study spleen size increased as the plant protein increased in the diet.

244 The hematocrit assay is normally used as a general indicator of fish health
245 (NRC 1981). Hematocrit level in all groups was within the normal range and did not
246 differ significantly among the groups (Blaxhall & Daisley 1973; Sun *et al.* 1995). In
247 another study Soltan *et al.* (2008) observed that FM protein replaced by mixture of
248 plant proteins in Nile tilapia diets that lead to the lower hematocrit levels could be
249 attributed to the binding of phytate to minerals (iron) and/or amine group of amino
250 acids causing their low availabilities in the body and increase in erythrocyte
251 fragility.

252 *Blood protein and lysozyme activity in serum*

253 Total blood protein content is taken as an index of nutritiional status (Martinez
254 1976). Among the blood protein, albumin and globulin are the major proteins, which
255 play a significant role in the immune response. Total blood protein concentration in
256 fish fed J_{50} diet, was significantly elevated over that of the control group, suggesting
257 an immunostimulating effect of DJKM at low inclusion level in rainbow trout
258 juveniles. The blood protein content values are similar to those found for rainbow
259 trout (Martinez 1976).

260 Lysozyme plays an important role in nonspecific immune response and it is
261 found in mucus, serum and ova of fish. Innate immunity due to lysozyme is caused
262 by lysis of bacterial cell wall and this stimulates the phagocytosis of bacteria. The
263 suppression of the non-specific immune capacity by high concentrations of dietary
264 soybean proteins has been reported in rainbow trout (Burrells *et al.* 1999). However,
265 other reports wherein SBM was fed to rainbow trout (Rumsey *et al.* 1994) and
266 Atlantic salmon (Krogdahl *et al.* 2000) or alginate was fed to Atlantic salmon
267 (Gabrielsen *et al.* 1998), increased values of different non-specific immune
268 mechanisms, which have been interpreted as immunostimulating effects. In the
269 present study lysozyme activity was statistically not different amongst the groups
270 but numerically it was higher in DJKM fed groups, indicating an immunostimulating
271 effect of DJKM in trout.

272

273 *Metabolic enzymes*

274 Alkaline phosphatase and ALT are released into blood during organ damage
275 (Racicot *et al.* 1975). Thus, detection of high levels of ALP and ALT in blood gives
276 information on the damage of organs and in particular of liver cells. Levels of ALP
277 and ALT were similar in all the diets, indicating normal organ function on feeding
278 of DJKM. Hemre *et al.* (2005) and Sanden *et al.* (2006) also reported similar results
279 on feeding SBM containing diets to Atlantic salmon.

Blood ions

Blood urea nitrogen levels are associated with liver or gill dysfunction (Stoskopf 1993) and in our study these levels were in the normal ranges (Witters 1986; Wedemeyer 1996). Furthermore, these values did not differ significantly among the groups. These results show that DJKM fed groups were normal and healthy.

TBIL, an indicator of liver dysfunction (Tietz 1986) was similar for all groups. Creatinine was highest in control group. Creatinine is a metabolite of animal protein and its highest level in control is due to highest content of FM in the diet. Creatinine level reflects kidney dysfunction, and its level in all the experimental group were in the normal range. Detoxified *Jatropha* kernel meal based diets were supplemented with phytase and higher phosphorus and other ions in blood observed in DJKM fed fish could be due to increased release of phosphorus, sodium and calcium from feed and making them available for trout.

Our histological findings demonstrate that trout did not show any abnormal changes in intestine and liver.

Conclusions

Rainbow trout can efficiently use DJKM as a source of protein. DJKM could replace 50% FM protein in rainbow trout diets, without sacrificing growth and nutrient utilization; and without affecting physiological and haematological parameters. The results of this study enlarge the portfolio of plant protein sources that can be used in fish diets, and open a new market opportunity for use of a new feed resource in the feed industry.

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656 **3.1.1.6 Substitution of fish meal by *Jatropha curcas* kernel meal: Effects on**
657 **growth performance and body composition of white leg shrimp (*Penaeus***
658 ***vannamei*)**

659

660 **Introduction**

661

662 The current trend in intensification of shrimp farming is towards increased
663 strengthening of culture systems that made it essential to develop suitable diets for
664 shrimp using alternative protein sources. Shrimp feed is still highly dependent upon
665 marine capture fisheries and the production of important dietary constituents like
666 fish meal (FM) and fish oil. This dependency is particularly strong within
667 compounded aqua feeds for farmed marine shrimp (Tacon and Metian, 2008),
668 mainly because it offers a balanced source of amino acids, essential fatty acids,
669 vitamins and minerals, and generally has good palatability (Suárez et al., 2009).
670 According to most estimates, modern fish farming is now a net drain on
671 world's seafood supply (Halweil, 2008). As the captured fish productions have
672 remained stagnant for the last few decades and aquaculture has had phenomenal
673 growth rates and this growth is projected to continue, the FM demand is expected to
674 outstrip the world's supply by 2050 (Halweil, 2008). These conflicting developments
675 are the main reasons for rising and fluctuating FM prices.

676 Alternative protein rich feed ingredients will reduce the levels of FM in diets
677 (Samocha et al., 2004). Many studies have achieved notable successes in substituting
678 FM by plant protein sources such as soybean meals and canola or rapeseed
679 (Gauquelin et al., 2007). In shrimp feed formulation, soybean meal is considered the
680 most widely used plant protein source amongst the many available plant protein
681 feedstuffs, owing due to its nutritional quality, favorable cost, and consistent
682 availability (Akiyama, 1991). The utilization of soybean protein, however, in human
683 food and terrestrial animal feeds as well, may imply future problems such as restricted

684 availability, higher production costs and risks associated with the strong dependence
685 on one ingredient. Thus, research should be conducted to assess the suitability of
686 alternative protein sources to soybean meal in formulated shrimp diets.

687 In quest for indigenous sources of renewable liquid fuels, *Jatropha curcas*
688 (Physic nut) has received increasing interest since the beginning of the 21st century
689 (Ye et al., 2009). Its seeds are rich in oil (25-35%) that can be used as fuel directly
690 or after transesterification, as a substitute for diesel fuel (Makkar et al., 2008). *J.*
691 *curcas* is especially interesting because of its ability to grow quickly under adverse
692 environmental conditions like stony or degraded soils or in severe drought
693 conditions. It is therefore, used to reclaim degraded land that would otherwise
694 remain underutilized. This also enables biofuel production in a manner that does not
695 compete with food crops for water, nutrients and land. Large plantations of *J. curcas*
696 have already taken place in India, Myanmar, Indonesia and China. This has resulted
697 in production of an increasing quantity of by-products, rich in nutrients that could be
698 used as livestock feed. This was impeded so far, because of the toxicity of *J. curcas*
699 oil, seedcake or kernel meal, which is mainly ascribed to the presence of phorbol
700 esters (PEs) and other antinutritive factors such as trypsin inhibitors, lectin and
701 phytate (Makkar and Becker, 2009).

702 Recently a process of detoxification for *J. curcas* kernel meal has been
703 developed in our laboratory (Makkar and Becker, 2008). The crude protein content
704 in the detoxified kernel meal is approximately 60 - 66 % and the protein has a good
705 amino acid balance. The levels of essential amino acids (except lysine) are higher in
706 *Jatropha* kernel meal than soybean meal (Makkar and Becker, 2009). This new
707 development offers an array of opportunities for using the detoxified kernel meal in
708 animal nutrition; substitution of FM in shrimp feeds being one of them.

709 Our previous study (Kumar et al. 2010a,b) has shown that detoxified
710 *Jatropha* kernel meal (DJKM) is a good protein source and better than soybean meal
711 for common carp diets. The objective of the present study was to investigate the
712 potential of substituting FM by DJKM in shrimp diets. Growth rate and nutrient

713 utilization in shrimp fed a diet replacing 25 and 50 % FM protein by the detoxified
714 kernel meal have been reported.
715
716 **Material and methods**

717

718 *Preparation of the Jatropha meal*
719
720 *Jatropha* seeds were purchased from India and deshelled and defatted in
721 Germany. Organic solvents were used to detoxify defatted *Jatropha* kernel meal
722 (patent application has been filed for the process of detoxification, Makkar and
723 Becker, 2008). After removal of PEs, the meal was autoclaved (121 ^{o}C for 15 min) to
724 inactivate heat labile antinutrients, trypsin inhibitors and lectin.
725
726 *Diet formulation*
727
728 Fish meal (Seelöwe fishmeal) was procured from Vereinigte
729 Fischmehlwerke Cuxhaven GmbH & Co. KG, Cuxhaven, Germany. Source of fish
730 oil was Menhaden, batch # 102k0126) and source of sunflower oil was Thomy,
731 Nestle Deutchland AG, 41415 Neuss; Reg. Trademark of Societe des Produits
732 Nestle S.A.. Vitamin premix and mineral premix were procured from Altromin
733 Spezialfutter GmbH & Co. KG, Lage, Germany. Prior to feed formulation, the
734 proximate composition of DJKM and FM was determined. A total of three diets
735 were formulated. All three diets were calculated to be isonitrogeneus, isoenergetic
736 and isolipidic. The formulation implied 35% of crude protein and 9% crude lipid.
737 Soy lecithin and cholesterol were added at the rate of 1.0% and 0.5% of diets
738 respectively to meet the unique needs of shrimps (Kaushik and Cuzon, 2001). To
739 equalise the lipid levels, the three diets were formulated with different amounts of
740 fish oil and sunflower oil. Due to the high oil content of FM, there was no fish oil

741 added to the Control feed. This was meant to ensure, that the only factor changed,
742 was the protein source. The inclusion levels of the DJKM were as follows:

743 Control diet was prepared with FM as protein source, without any DJKM.
744 JC_{25}: 25% of FM protein replaced by DJKM. JC_{50}: 50% of FM protein replaced by
745 DJKM. The final mixture of each diet was made into 3 mm diameter moist pellets
746 (using a Bosch, Type UM60ST 2-M, Robert Bosch Hausgerät GmbH, 70839
747 Gerlingen, Germany) and then dried in oven at 40 ^{o}C for overnight. Compositions of
748 the experimental diets are listed in Table 1.

749

750 **Table 1** Composition of the experimental diets (g kg^{-1} feed) for white leg shrimp
751 (*Penaeus vannamei*)

Ingredients	**Experimental diets**		
	Control	**JC_{25}**	**JC_{50}**
Fish meal	492.3	369.2	246.2
Wheat starch	312.5	292.0	271.5
Jatropha meal	—	124.6	249.2
Dextrose	50	50	50
Cellulose	69.3	74.2	79.1
Sunflower oil	20.9	20.2	19.4
Fish oil	—	14.8	29.5
[1]Vitamin premix	20	20	20
[2]Mineral premix	20	20	20
Cholesterol	5.0	5.0	5.0
Soy lecithin	10	10	10
Total	1000	1000	1000

752 [1]Vitamin premix (g or IU kg-1 premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic
753 acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5;
754 menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
755 [2]Mineral premix (g kg-1): $CaCO_3$, 336; KH_2PO_4, 50_2; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9;
756 $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67;$CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate,
757 0.06; $NaSeO_3$, 0.02.
758

759 *Experimental system and animals*

760

761 *P. vannamei* juveniles (average weight 2 – 4 g) were obtained from the
762 northern German aqua farm EAP (Ecological Aquaculture Production AG) in
763 Strande/Kiel Germany. About 1000 individuals were stocked into a recirculation
764 system in the "Experimental Unit" at the University of Hohenheim, Stuttgart,
765 Germany. The system was stocked at a density of 320 animals per m^2, while water
766 quality and relevant parameters were monitored frequently. The shrimp were fed a
767 standard fish feed (35% crude protein, 10% lipids, 11 - 14% ash and 19 MJ $kg^{-1}DM^{-}$
768 ^{1} gross energy). After an acclimatization period of five weeks, 60 shrimp juveniles
769 were randomly distributed into three groups with four replicates; each replicate
770 contained five shrimp (av. wt. 4.46 ± 0.65 g) per aquarium (45 l capacity).

771 All the aquaria were supplied with water from a recirculatory system. The
772 system was subjected to a photoperiod of 12 h light : 12 h darkness. Water quality
773 was monitored throughout the experiment. All the water parameters were in the
774 optimum range (temperature 26.2 – 27.5°C, salinity 16 – 19‰, pH 7.0 – 7.5,
775 dissolved oxygen 6.9 – 7.4 mg l^{-1}, total NH_3 0.1– 0.2 mg l^{-1}, nitrite 0.07 – 0.1 mg l^{-1}
776 and nitrate 1–3 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation
777 above 80%. One day before start of the experiment, the shrimp were starved and
778 during the experimental period shrimp were fed at 5% of animal biomass per day
779 and this was adapted according to Kaushik and Cuzon (2001), after each weighing.
780 Feeding frequency was set to five times per day (Cavalho and Nunes, 2006). Two of
781 the feedings were set to night time, to better suit the animal's natural feeding
782 behaviour (Pontes et al., 2001).

783

784 *Survival*

785

786 Survival was calculated by using the following formula:

787 Survival (%) = (Number of shrimp at the end of experiment/Number of shrimps
788 stocked initial)*100
789
790 *Feed pellet stability*
791
792 Briefly, 0.5 g of each feed samples with duplicate kept in plastic tubes and
793 added 10 ml distilled water. Tubes were exposed to an ultrasonic bath (47 kHz, 105
794 W). Two repetitions were performed for each feed. Time was measured until about
795 90% of the pellets had disintegrated. This percentage was chosen merely to imply
796 that pellets did not have to dissolve completely till measurement.
797
798 *Sampling*
799
800 The experiment was terminated after eight weeks and the shrimps were killed.
801 At the end of experiment, shrimps were anaesthetized by tricaine methanesulfonate
802 (MS222) at 250 ppm in water. Haemolymph was drawn from two shrimps from each
803 group and transferred into a tube, which was centrifuged at 1500×*g* for 5 min at room
804 temperature (24 ^{o}C) to obtain plasma, which was then stored at −20 ^{o}C for
805 determination of cholesterol. Shrimp were stored at −20 ^{o}C for chemical composition
806 analysis. Prior to determination of the proximate composition, the shrimp were
807 autoclaved at 121 ^{o}C for 20 min, thoroughly homogenised using an Ultra-Turrax T25,
808 frozen overnight and then freeze-dried.
809
810 *Determination of phorbol esters, trypsin inhibitor, lectin, phytate and non starch*
811 *polysaccharides*
812
813 Phorbol esters were determined according to Makkar et al. (2007), which was
814 based on the method of Makkar et al. (1997). Briefly, 0.5 g of the untreated *Jatropha*
815 meal sample was extracted four times with methanol. A suitable aliquot was loaded

816 on a high-performance liquid chromatography reverse-phase C_{18} LiChrospher 100, 5
817 µm (250 x 4 mm I.D. from Merck (Darmstadt, Germany) column. The column was
818 protected with a head column containing the same material. The separation was
819 performed at room temperature (23 ^{o}C) and the flow rate was 1.3 ml/min using a
820 gradient elution (Makkar et al., 2007). The PEs peaks were detected at 280 nm and
821 appeared between 25.5 and 30.5 min. The results were expressed as equivalents to the
822 standard, phorbol-12-myristate 13-acetate. Detection limit of PEs was 3 µg/g meal.
823 Trypsin inhibitor activity was determined essentially according to Smith et al.
824 (1980) except that the enzyme was added last as suggested by Liu and Markakis
825 (1989). Analysis of the lectin content was conducted by haemagglutination assay
826 (Makkar et al., 1997). The haemagglutination activity was expressed as the minimum
827 amount of material (mg mL^{-1} assay medium) that produced agglutination. The
828 minimum amount was the amount of material mL^{-1} assay medium in the highest
829 dilution that was positive for agglutination; the lower this value, the higher the lectin
830 activity. Phytate content of samples was determined by a spectrophotometric
831 procedure (Vaintraub and Lapteva, 1988). Results were expressed as g phytic acid per
832 100 g material using phytic acid sodium salt (Sigma, St Louis, MO, USA) as standard.
833 Non-starch polysaccharides (NSP) were estimated according to Englyst et al. (1994).
834
835 *Amino acid analysis*
836
837 Amino acid composition of FM and DJKM was determined using an
838 automated amino acid analyser after hydrolysing the samples with 6 M HCl at 110 ^{o}C
839 for 24 h (Bassler and Buchholz, 1993). The sulphur-containing amino acids were
840 oxidised using performic acid before the acid hydrolysis. Tryptophan content of the
841 above-mentioned samples was determined spectrophotometrically by the method of
842 Pinter-Szakacs and Molnar-Perl (1990).
843

844 *Proximate analysis*

845

846 The proximate composition of diet ingredients, diets and whole body of
847 shrimp was determined using the standard methods of the Association of Official
848 Analytical Chemists (1990). Samples were analyzed for dry matter, ash, crude protein
849 and lipid (ether soluble lipid). Gross energy of diet ingredients, diets and shrimp
850 bodies was determined with a bomb calorimeter (IKA C7000) using benzoic acid as a
851 standard.

852

853 *Feed intake, growth performance and nutrient utilization parameters*

854

855 Feed intake: Feed offered was used as feed intake since no leftovers were
856 observed. The leaching losses were considered as negligible.
857 Growth performance and nutrient utilization were assessed in terms of body mass
858 gain (BMG), specific growth rate (SGR, % day^{-1}), metabolic growth rate (MGR, g
859 $kg^{0.8}$ day^{-1}), feed conversion ratio (FCR) and protein efficiency ratio (PER).
860 BMG (%) = [(Final body mass - initial body mass) / Initial body mass] X 100; SGR
861 (% day^{-1}) = [(ln final body mass in g) - ln initial body mass in g) / number of trial
862 days] X 100; MGR (g $kg^{0.8}$ day^{-1}) = (Body mass gain in g) / [{(initial body mass in g
863 / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)^$^{0.8}$}/2] / number of trial days; FCR = dry
864 feed fed (g)/body mass gain (g) and PER = body mass gain (g)/crude protein fed (g).

865

866 *Cholesterol estimation*

867

868 The determination of the plasma cholesterol was using enzymatic colorimetric
869 kits: Hitado Diagnostic system, Nobiflow cholesterin (kit lot number 60041889). The
870 color intensity was determined photometrically and was directly proportional to the
871 concentration of cholesterol in the plasma sample.

872

873 *Statistical analysis*

874 All data were subjected to a one-way analysis of variance (ANOVA) and the
875 significance of the differences between means was tested using Tukey HSD test ($P <$
876 0.05). The software used was Statistica 7.0 (Statsoft, Inc. 1984-2004). Values are
877 expressed as means ± standard deviation.

878

879 **Results and discussion**

880 *Phorbol esters and antinutrients content in defatted Jatropha kernel meal*

881

882 Phorbol esters content in untreated defatted *Jatropha* kernel meal was 1.8 mg
883 g^{-1}. However, PEs in DJKM was undetectable. Trypsin inhibitors and lectins were
884 also not detected in DJKM; whereas phytate and NSP levels in DJKM were 9.5%
885 and 16% respectively (Table 2).

886

887 **Table 2** Proximate composition and amino acid composition of feed ingredients

	Fish meal	***Jatropha* meal**
Proximate composition (g kg $^{-1}$)		
Dry matter	940	945
Crude protein	650	665
Crude lipid	88	11.4
Crude ash	142	82
Gross energy (KJ/g)	21.1	18.3
Antinutrients		
Trypsin inhibitor (mg trypsin inhibited per g sample)	–	ND
Lectin[a]	–	ND
Phytate (% dry matter)	–	9.5
Essential amino acids composition (g kg $^{-1}$)		
Arginine	35.3	69.7
Histidine	17.7	21.7

Iso leucine	22.8	26.7
Leucine	41.6	46.7
Lysine	40.9	23.3
Phenylalanine	21.8	30.4
Methionine	16	10.6
Threonine	23	22
Tryptophan	4.9	7.1
Valine	29.3	31.6
Non-essential amino acids composition (g kg $^{-1}$)		
Alanine	43.3	29.4
Asparginine	60.5	68.7
Cystine	4.3	2.3
Glycine	59.8	31.5
Glutamine	79.4	112.1
Proline	36.9	32.2
Serine	25.5	30.6
Tyrosine	14.8	18.8
Non-starch polysaccharides (NSP) (g kg $^{-1}$)		
Rhamnose	-	3
Fucose	-	1
arabinose	-	31
Xylose	-	20
Mannose	-	5
Galactose	-	14
Glucose	-	57
Glucuronic acid	-	0
Galacturonic acid	-	30
Total-NSP	-	160

888 ND: Not detected

889 [a:]Minimum amount of material (mg mL^{-1} assay medium) that produced agglutination.

890

891

892 **Table 3** Proximate composition and amino acid composition of the experimental

893 diets (g kg^{-1} feed)

	Control	**JC_{25}**	**JC_{50}**
Proximate composition (g kg $^{-1}$)			
Dry matter	955	961	962
Crude protein	356	353	346
Crude lipid	95	96	99
Crude ash	142	127	113
Gross energy (KJ/g)	19.3	19.3	19.5
Crude fibre	35	59	78
Acid detergent fibre	41	64	83
Essential amino acids composition (g kg $^{-1}$)			
Arginine	17.38	21.72	26.06
Histidine	8.71	9.24	9.77
Iso leucine	11.22	11.74	12.27
Leucine	20.48	21.18	21.88
Lysine	20.14	18.00	15.88
Phenylalanine	10.73	11.84	12.94
Methionine	7.88	7.23	6.58
Threonine	11.32	11.23	11.15
Tryptophan	2.41	2.69	2.98
Valine	14.42	14.75	15.09
Non-essential amino acids composition (g kg $^{-1}$)			
Alanine	21.32	19.65	17.99
Asparginine	29.78	30.90	32.02
Cystine	2.12	1.87	1.63
Glycine	29.44	26.00	22.57
Glutamine	39.09	43.28	47.48
Proline	18.17	17.64	17.11
Serine	12.55	13.23	13.90
Tyrosine	7.29	7.81	8.33

894 *Proximate composition of feed ingredient, experimental diets and amino acid profile*
895 *of feed ingredients and experimental diets; and feed pellet stability*
896
897 Proximate composition and amino acid profiles of feed ingredients and diets
898 are shown in Tables 2 and 3. Diets contained about 35% crude protein, 9.5 – 9.9%
899 crude lipids and 19.0 kJ/g gross energy and were isonitrogenous, isolipidic and
900 isoenergetic. Ash content was in the range of 11.3 –14.2%. All experimental diets had
901 almost similar amino acid composition. Highest feed stability was observed in JC_{50}
902 groups compared to other two groups (Table 4).
903 Amino acid compositions of the experimental diets were calculated from
904 amino acid profile of individual feed ingredients
905
906 **Table 4** Time (min) required for pellet disintegration of different experimental diets

Diets	Disintegration time (min)
Control	$4.32^{b} \pm 0.03$
JC_{25}	$1.23^{b} \pm 0.11$
JC_{50}	$18.08^{a} \pm 1.31$

907 Values are mean (n = 2) ± standard deviation.
908 Mean values in the same column with different superscript differ significantly ($P < 0.05$).
909
910 *Shrimp behaviour, feed intake and survival*
911 Based on visual observation during feeding time, palatability or acceptability
912 of feed was good and the behaviour of shrimp was normal. No left over feed was
913 observed in the aquaria. In hatcheries survival rate for *P. vannamei* is not higher than
914 50–60% (Jory and Cabrera, 2003) because of their cannibalistic behaviour, especially
915 at high stocking densities. The process of moulting is also a critical period in shrimp
916 development. At this time they are not only incapable of defending, but are also
917 highly susceptible to stress factors. Furthermore, it can occur that animals cannot
918 moult completely and asphyxiate in their exuvia. In the present study survival rates
919 were very high. There was no mortality during the entire experimental period. This

920 implies that environmental conditions were well suited for *P. vannamei*. Furthermore,
921 it can be assumed, that there where no serious nutritional deficiencies or
922 incompatibilities generated by any of the offered experimental diets.
923
924 *Growth performance and nutrient utilization*
925
926 Weekly body mass gains of white leg shrimp are given in Figure 1. These
927 indicate that from second week onwards there was differential growth among the
928 groups and lower body mass development was observed in control group than in other
929 groups. This trend was maintained till the end of the experiment. Growth
930 performances and nutrient utilization parameters were affected by dietary treatments
931 (Table 5, Figures 2 and 3). Our previous studies indicate that 50% FM protein
932 replaced by DJKM exhibited similar growth performance and nutrient utilization to
933 FM fed group in common carp and rainbow trout (Kumar et al., 2009, 2010a,b).
934 Interestingly in the present study growth performance and nutrient utilization of
935 shrimp fed DJKM based groups were better than that of FM fed group. Many
936 researchers (Colvin and Brand, 1977; Davis and Arnold, 2000; Fox et al., 2004;
937 Gonzalez-Rodriguez and Abdo de la Parra, 2004) reported that fish meal protein
938 could be replaced at a range of 40 – 80% by plant protein (soybean meal and
939 cottonseed meal) in shrimp diets, and higher inclusion (more than 80% replacement of
940 fish meal) of plant protein in shrimp diets leads to lower growth because of lower feed
941 intake. Paripatananont et al. (2001) reported that soy protein concentrate can replace
942 50% FM protein without hindering the growth performance and nutrient utilization.
943 Amaya et al. (2007) observed that FM could be replaced with plant protein sources
944 (solvent extracted soybean meal, corn gluten meal and corn fermented solubles) in
945 shrimp diets including 16% poultry by-product meal without affecting growth and
946 production. Suárez et al. (2009) also reported that FM substitutions as high as 80% for
947 a mixture of soybean meal and canola meal and proves, that diets based mainly on
948 plant protein are feasible for *P. vannamei*. In the present study the higher growth

949 response of DJKM fed groups could be due to higher protein availability from the
950 DJKM than FM, which enhances the feed utilization. There is a possibility of
951 synergistic effects between the used feed ingredients (FM and DJKM); both were
952 complementary to each other in their amino acid composition. Therefore, DJKM
953 protein in combination with FM protein probably induces excellent nutrient and
954 energy digestibility and lead to higher growth performance and nutrient utilizations in
955 shrimp. Amino acid composition of the diets tested indicates that the requirements of
956 shrimp for these nutrients were met in all the dietary treatments, thus growth of the
957 shrimp was better in DJKM fed groups.

958 Results from this study provide important information regarding the potential
959 of white leg shrimp (*P. vannamei*) to utilize alternative feed formulations with low
960 levels of animal protein under experimental conditions.

961

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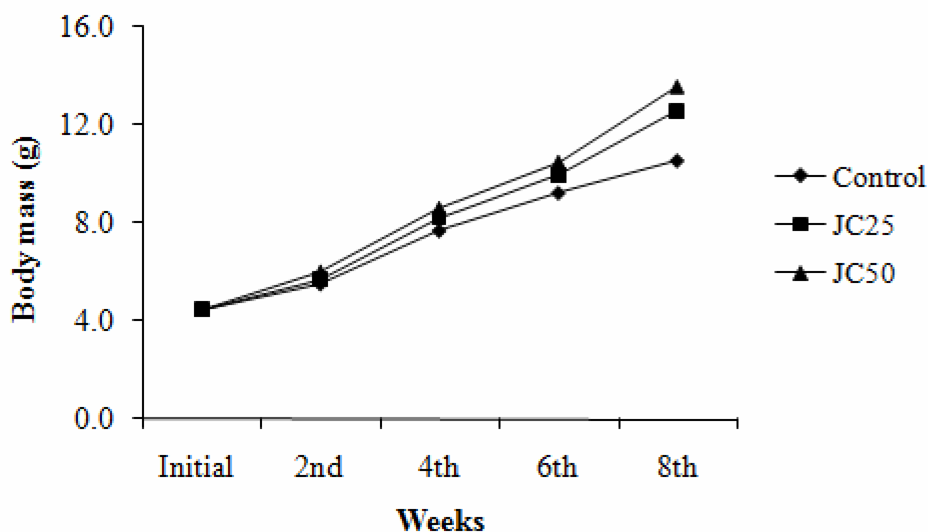
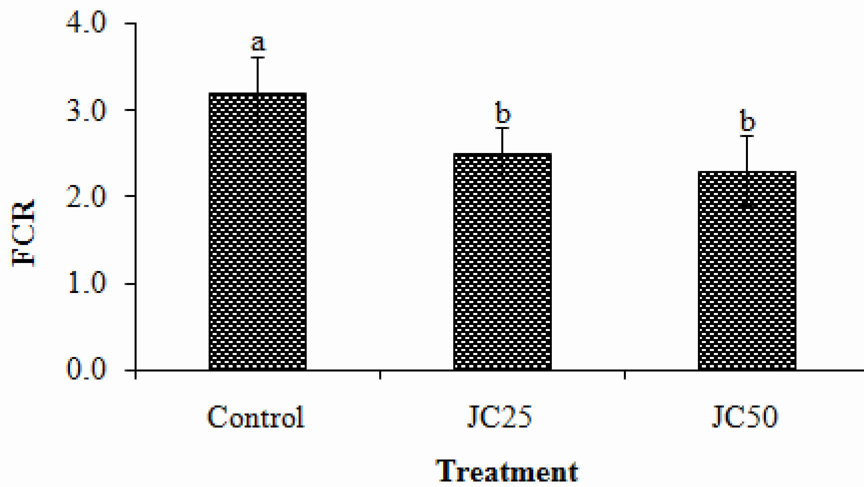


963 **Figure 1** Body mass gain of white leg shrimp (*Penaeus vannamei*) fed experimental
964 diets for eight weeks

965

966

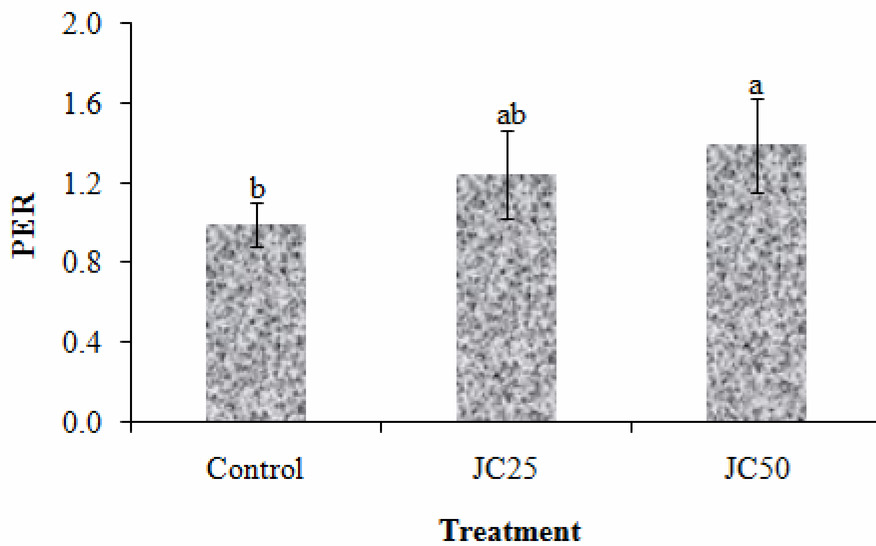


967 **Figure 2** Feed conversion ratio (FCR) in white leg shrimp (*Penaeus vannamei*) of

968 different experimental groups

969

970



971 **Figure 3** Protein efficiency ratio (PER) in white leg shrimp (*Penaeus vannamei*) of

972 different experimental groups

973

974 **Table 5** Growth performance of white leg shrimp (*Penaeus vannamei*) fed with
975 experimental diets for eight weeks

Treatment	IBM (g)	FBM (g)	BMG (%)	SGR (%)	MGR (g $kg^{0.8}$ day^{-1})
Control	4.46 ± 0.60	10.54^b ± 3.17	138^b ± 29.23	1.54^b ± 0.21	5.51^b ± 0.70
JC_{25}	4.47 ± 0.64	12.59^a ± 3.98	182^a ± 13.53	1.85^a ± 0.09	6.67^a ± 0.38
JC_{50}	4.45 ± 0.69	13.60^a ± 3.18	209^a ± 40.88	2.00^a ± 0.24	7.22^a ± 0.75

976 IBM- Initial body mass, FBM- Final body mass, BMG - Body mass gain, SGR – Specific growth rate and MGR
977 - Metabolic growth rate.
978 Values are mean (n = 4) ± standard deviation.
979 Mean values in the same column with different superscript differ significantly ($P < 0.05$).
980
981 *Chemical composition of whole shrimp body*
982
983 Whole body chemical composition is shown in Table 6. Moisture contents of
984 whole shrimp body did not differ significantly among the three groups. A similar
985 trend has been observed by Paripatananont et al. (2001). In their study tiger shrimp
986 (*Penaeus monodon*) fed with soy protein concentrate substituting 25–100% FM
987 protein, did not differ significantly in moisture content of whole shrimp body. In the
988 same study they observed that soy protein concentrate inclusion in shrimp diets lead
989 to higher crude lipid and gross energy content in whole body than FM protein based
990 diets. In the present study crude lipid content of whole body of shrimp did not differ
991 significantly among the three groups whereas highest gross energy content of whole
992 body of shrimp was observed in Control group, followed by JC_{25} and JC_{50} groups; all
993 being significantly different.
994 Detoxified *Jatropha* kernel meal contained 9.5% phytate. Detoxified *Jatropha*
995 kernel meal based diets (JC_{25} and JC_{50}) contained bound phytate and most of the
996 minerals bound to phytate. Consequently, lower availability of minerals in body that
997 leads to lower ash contents, as observed in JC_{25} and JC_{50} groups. On the other hand,
998 Paripatananont et al. (2001) observed that soy protein concentrate substituting 25–
999 100% FM protein did not differ significantly in ash content of whole body of shrimp.

1000

1001 **Table 6** Chemical composition of whole body of white leg shrimp (*Penaeus*

1002 *vannamei*) of different experimental groups (% wet basis weight ± SD)

Treatment	Moisture	Crude protein	Crude lipid	Ash	Gross energy (kJ/g)
Control	65.5^{a} ± 8.81	27.05^{a} ± 0.53	1.70^{a} ± 0.11	5.64^{a} ± 0.27	6.75^{a} ± 0.17
JC_{25}	67.2^{a} ± 3.82	25.62^{b} ± 0.60	1.77^{a} ± 0.13	4.92^{b} ± 0.20	6.26^{b} ± 0.13
JC_{50}	69.3^{a} ± 5.84	24.01^{b} ± 0.43	1.69^{a} ± 0.27	4.30^{b} ± 0.27	5.99^{c} ± 0.13

1003 Values are mean (n = 4) ± standard deviation.

1004 Mean values in the same column with different superscript differ significantly (P < 0.05).

1005

1006 *Cholesterol level in plasma*

1007

1008 Cholesterol is a vital component of cell membranes and is the precursor of bile

1009 acids, steroids, and molting hormones. It is reported to be an essential nutrient for

1010 growth and survival of all crustacean species (Kanazawa et al., 1971). Experimental

1011 diets were supplemented with cholesterol, because shrimp, like other crustaceans,

1012 cannot synthesize cholesterol de novo (Teshima and Kanazawa, 1971). Previous

1013 researches have demonstrated that cholesterol supplementation in diets improves

1014 biological performance of prawns (*P. japonicus*; Teshima and Kanazawa, 1986), tiger

1015 shrimp (*P. monodon*; Sheen et al., 1994), and Pacific white shrimp (*L. vannamei*;

1016 Duerr and Walsh, 1996).

1017 Cholesterol levels in plasma of shrimp were affected by dietary treatments and

1018 lower (P > 0:05) cholesterol level was observed in JC_{25} and JC_{50} group (Figure 4).

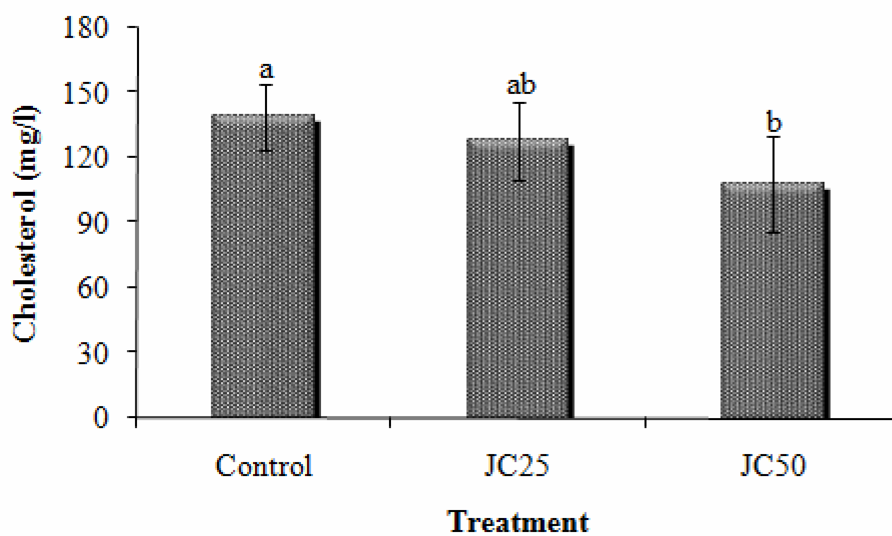
1019 Detoxified *Jatropha* kernel meal reduced cholesterol level in plasma compared to the

1020 control group. The decrease in plasma cholesterol concentrations in shrimp fed diets

1021 with plant protein is in accordance with the results of Cheng and Hardy (2004). In

1022 terrestrial animals, plant products are generally considered to have a

1023 hypocholesteromic effect, mainly due to the relatively high levels of estrogeno-
1024 mimetic isoflavones (Setchell and Cassidy, 1999). In humans, different plant
1025 constituents have been reported to lower plasma cholesterol concentration (Wester
1026 2000). Although cholesterol metabolism in animals, fish and shrimp could differ, the
1027 shrimp hypocholesterolemia response to dietary plant protein supply could be due
1028 either to an increased excretion of bile salts, to an inhibition of cholesterol intestinal
1029 absorption or just to the withdrawal of FM rather than to the direct effects of plant
1030 protein.
1031



1032
1033 **Figure 4** Cholesterol level in plasma of white leg shrimp (*Penaeus vannamei*) of
1034 different experimental groups
1035
1036 **Conclusions**
1037
1038 This study demonstrates that fish meal can be removed partially from
1039 commercially manufactured shrimp diets using detoxified *Jatropha* kernel meal
1040 (DJKM) as protein source. Growth performance and nutrient utilization in white leg
1041 shrimp (*P. vannamei*) for DJKM fed groups were better than fish meal fed groups
1042 indicating that shrimp can efficiently use DJKM, which indicates a good protein
1043 quality. Although the DJKM based diets showed better shrimp performance than FM

1044 based diets, these diets may be marginal and further studies are recommended to
1045 evaluate potential limiting nutrients. The results of this study enlarge the portfolio of
1046 plant protein sources that can be used in shrimp feeding, and open a new market
1047 opportunities for the use of a new feed resource. Additional studies with DJKM
1048 based diets at a larger scale and under commercial pond conditions are suggested.

1049

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1166

1167 **3.1.1.7 Effects of replacing soybean meal by detoxified *Jatropha curcas* kernel**
1168 **meal in the diet of growing pigs on their growth, serum biochemical parameters**
1169 **and visceral organs (this was done in China and workers responsible from**
1170 **China:** Hai-feng Wang, Yi Chen, Yi-nuo Zhao, and Jian-xin Liu)
1171
1172
1173 **Introduction**
1174 *Jatropha curcas* L. is an oil-bearing shrub belonging to the family of
1175 *Euphorbiaceae*, widely distributed in many Latin American, Asian, and African
1176 countries (Gübitz et al., 1999). Nowadays oil prices are fluctuating unpredictably,
1177 but are likely to stay higher in the future (Makkar and Becker, 2009), which will
1178 encourage the cultivation of oilseed crops including *J. curcas* to produce biofuel.
1179 *Jatropha* has considerable potential in China, as it is a versatile oil plant with many
1180 economical and ecological attributes (Ye at al., 2009). Crude protein account for 27-
1181 30% of raw *Jatropha* seeds (Makkar et al., 1998b). The residual protein-rich seed
1182 cake or kernel meal, remaining after extraction of the oil, could form a protein-rich
1183 ingredient in animal diets. Increased production of *J. curcas* as a fuel source will
1184 increase production of the by-products, *J. curcas* seed meal and kernel meal in the
1185 future.
1186 At present, soybean, a major protein source in pig rations also serves as a food
1187 ingredient in human diets. Consequently, pig production competes heavily with
1188 humans for dietary proteins. The shortages of protein resources highlight an urgent
1189 need for a search for a suitable complement or substitute to soybean. The contents of
1190 essential amino acids (except lysine) in *Jatropha* kernel proteins are higher than the
1191 FAO reference protein (Makkar et al., 2008). Therefore, *J. curcas* kernel meal,
1192 which contains 58 to 60% crude protein and 90% of this is true protein, has high
1193 potential to complement and or substitute for soybean meal as a protein source in the
1194 diets for monogastric animals, pigs and poultry. However, *J. curcas* seeds are highly
1195 toxic to a number of animal species (Makkar et al., 1998b). The toxicity is ascribed
1196 to the presence of phorbol esters (Goel et al., 2007). Other antinutrients present in
1197 high amounts in the kernel meal include trypsin inhibitor, lectin and phytate
1198 (Makkar et al., 1997).

Phorbol esters, the main toxin present in the kernel meal can not be destroyed by heat treatment because they are heat stable and withstand even roasting temperatures (Makkar et al., 1998b). However, its content in the meal could be reduced by chemical treatments (Makkar and Becker, 2009). The kernel meal from the non-toxic genotype of *J. curcas*, devoid of phorbol esters, did not show any toxicity to humans in Mexico and to fish on experimental feeding (Makkar et al, 1998a; Makkar and Becker, 2009). After removing the toxic and heat-stable factors from the toxic genotype, *Jatropha* kernel meal was found to be non-toxic to fish (Makkar and Becker, 2009). The aim of the present study was to investigate the effects of substituting soybean meal with detoxified *Jatropha curcas* kernel meal (DJM) as a protein source on growth, serum biochemical parameters, visceral organ weight and histopathological change in liver and kidney in growing pigs.

Material and methods

Animals and diets

Thirty-six pigs (Duroc × Landrace × Yorkshire), with an average weight of about 21 kg, were randomly assigned to three dietary treatments, with three replicates per treatment. Four animals with 2 castrated males and 2 females were kept in a pen as an experimental unit. The DJM, prepared at Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim, Germany (Patent Number: PCT/EP2010/051779), was included in the diets at levels to replace 0, 25 or 50% of soybean meal protein, and designated as DJM0, DJM25 and DJM50, respectively. Because the DJM had a higher content of nitrogen than soybean meal, total amount of DJM and soybean meal in diets, DJM25 and DJM50 was slightly lower than diet DJM0 to make all the diets isonitrogenous (Table 1). The pigs had free access to feed and drinking water.

Dry matter (DM) and ash contents of feeds were determined according to method No. 942.05 (AOAC, 1995). The sample was analyzed for Kjeldahl N (No 954.01), ether extract (No. 920.39) and crude fiber (No. 978.10, AOAC, 1995). The ingredients and chemical composition of different diets are presented in Table 1.

1232 *Growth performance*

1233 The experiment lasted for 28 days. Feed intake and weight gain of pigs were
1234 recorded at the start) and at 2 (middle) and 4 weeks (final) of the feeding. Daily feed
1235 intake, average daily gain and feed conversion ratio were calculated.

1236

1237

1238 *Blood Sampling and analysis*

1239 On the last day of the experiment, 10 ml of blood samples were collected in the
1240 morning before the animals were offered the feed. Blood was sampled from the
1241 jugular vein and centrifuged at 3000 g for 15 min. Serum was frozen at -10°C for later
1242 analysis of total protein, albumin, urine nitrogen, glucose, triglyceride, superoxide
1243 dismutase, lactic dehydrogenase, lysozyme, glutamic-pyruvic transaminase, glutamic-
1244 oxalacetic transaminase, alkaline phosphatase and acid phosphatase by automatic
1245 biochemistry analyzer (HITACHI 7020). Test kits were purchased from Diasys
1246 diagnostic systems (Shanghai Co. Ltd.).

1247

1248 **Table 1** Ingredients and chemical composition of different diets

	DJM0	DJM25	DJM50
Ingredients (%)			
Corn grain	65.3	66.6	67.9
Soybean meal	23.0	16.3	10.3
Jatropha curcas kernel meal	0.0	5.4	10.2
Fish meal	5	5	5
Powder fat	3	3	3
$NaHCO_3$	0.5	0.5	0.5
Lysine	0.20	0.30	0.35
Lime stone	1.1	1	0.9
Bicalcium phosphate	0.5	0.5	0.5
Salt	0.3	0.3	0.3
Choline chloride	0.1	0.1	0.1
Premix	1	1	1
Nutrient level [1] (%DM)			
Crude protein	17.2	17.2	17.2
Acid detergent fiber	4.1	4.2	4.3
Ether extracts	4.0	3.9	4.0
Ca	0.93	0.93	0.92
P	0.59	0.60	0.61
Lysine	1.19	1.21	1.20
Digestible energy (Mcal/kg)	3.41	3.41	3.41

1249 [1] Determined values except digestible energy and lysine, which are the calculated
1250 values.

1251 DJM0, DJM25 and DJM50: diets included detoxified *Jatropha curcas* kernel meal
1252 to replace 0, 25 and 50% of soybean meal protein, respectively.
1253

1254 *Slaughter and histopathological studies*

1255 A total of 12 pigs, 6 each from control and DJM50, were slaughtered at the end of
1256 experiment to examine the visceral organs. The pigs had free access to water, but
1257 were fasted for 16 h prior to slaughter by electrical stunning using operator-handled
1258 tongs (250V, 10s). The specimens of tissues from liver and kidneys were taken for
1259 histopathological examination. The tissues were immediately rinsed with
1260 physiological saline, fixed overnight in 4% paraformaldehyde, and then dehydrated in
1261 a graded series of ethanol and embedded in paraffin for later slicing and heamatoxylin
1262 and eosin staining. The eviscerated carcass was split longitudinally through the
1263 vertebrae midline, and carcass weight and length were recorded. The liver, heart,
1264 lung, spleen and kidneys were weighed.

1265

1266 *Statistics*

1267 For feed intake and feed conversion ratio, pen was considered as the experimental
1268 unit. Data were analyzed as a completely randomized design using the General Linear
1269 Models (GLM) procedure of SAS (1996). Differences among means for the three
1270 treatments were tested using Duncan's new multiple range test. For weight gain,
1271 dressing percentage, and internal organ weight, the pig was considered as the
1272 experimental unit.

1273

1274 **Results**

1275 The feed intake of diet DJM25 was significantly lower than those of DJM0 and
1276 DJM50 during the first stages ($P<0.05$), but no significant difference was observed
1277 during the second stage ($P>0.05$, Table 2). No significant differences were observed
1278 in initial, middle and finial weights among pigs on different diets ($P>0.05$, Table 2).
1279 Average weight gain and feed to gain ratio were also not different among pigs on
1280 different diets ($P>0.05$, Table 2).

1281 The pigs fed diets DJM25 and DJM50 did not show difference in serum
1282 biochemical parameters, compared to those fed DJM0 ($P>0.05$, Table 3). However,
1283 the pigs on diet DJM50 had significantly higher total protein, albumin and superoxide
1284 dismutase content than those on DJM25 ($P<0.05$, Table 3).

1285 The carcass weight, dressing percentage and backfat thickness of pigs fed DJM50
1286 were not different from those on DJM0 ($P>0.05$, Table 4). No significant differences

1287 were observed in visceral organ weight and its ratio to body weight ratio between two
1288 the treatments (P>0.05, Table 4). No histopathological change was observed in liver
1289 (Fig.1.) and kidney (Fig.2) between pigs on diets DJM0 and DJM50. The liver and
1290 kidney from both treatments were normal in histomorphology.
1291

1292 **Table 2** Effect of replacing soybean protein with detoxified *Jatropha curcas* kernel
1293 meal on growth performance of growing pigs

	DJM0	DJM25	DJM50	SEM
First stage (1-13 d)				
Feed intake (kg)	1.09 ^{A}	1.00 ^{B}	1.15 ^{A}	0.024
Initial weight (kg)	21.45	21.43	21.44	0.244
Middle weight (kg)	29.17	28.00	29.42	0.511
Average weight gain (kg)	0.594	0.506	0.613	0.0404
Feed to gain ratio	1.86	1.99	1.87	0.113
Second stage (14-27 d)				
Feed intake (kg)	1.68	1.67	1.68	0.007
Middle weight (kg)	29.17	28.00	29.42	0.511
Final weight (kg)	38.76	38.40	39.24	0.695
Average weight gain (kg)	0.685	0.743	0.702	0.0312
Feed to gain ratio	2.46	2.25	2.42	0.116
Whole period (1-27 d)				
Feed intake (kg)	1.39 ^{A}	1.35 ^{B}	1.42 ^{A}	0.011
Initial weight (kg)	21.45	21.43	21.44	0.244
Final weight (kg)	38.76	38.40	39.24	0.695
Average weight gain (kg)	0.641	0.629	0.659	0.0232
Feed to gain ratio	2.18	2.14	2.16	0.065

1294 DJM0, DJM25 and DJM50: diets included detoxified *Jatropha curcas* kernel meal
1295 to replace 0, 25 and 50% of soybean meal protein, respectively.

1296 ^{A,B} Means with different superscripts within the same rows differ (P<0.01).

1297
1298
1299 **Table 3** Effect of replacing soybean protein with detoxified *Jatropha curcas* kernel
1300 meal on serum characteristics of growing pigs

	DJM0	DJM25	DJM50	SEM
Total protein (g/L)	60.5 ^{AB}	58.0 ^{B}	63.6 ^{A}	1.46
Albumin (g/L)	34.6 ^{AB}	33.3 ^{B}	36.3 ^{A}	0.94
Urine nitrogen (mmol/L)	2.89	2.80	3.04	0.146
Glucose (mmol/L)	5.40	4.99	4.95	0.350
Triglyceride (mmol/L)	0.39	0.35	0.36	0.026
Superoxide dismutase (U/ml)	148.6 ^{AB}	138.7 ^{B}	157.3 ^{A}	4.25
Lactate dehydrogenase (U/ml)	14.0	15.1	14.7	1.02
Lysozyme (U/ml)	70.0	62.8	70.3	4.22
Glutamic-pyruvic transaminase (U/L)	11.7	12.3	12.5	0.81
Glutamic-oxalacetic transaminase (U/L)	10.8	11.4	10.9	1.02
Alkaline phosphatase (U/100ml)	20.9	18.1	20.6	1.15
Acid phosphatase (U/100ml)	11.6	10.8	11.7	0.81

1301 DJM0, DJM25 and DJM50: diets included detoxified *Jatropha curcas* kernel meal
1302 to replace 0, 25 and 50% of soybean meal protein, respectively.
1303 ^{A,B} Means with different superscripts within the same rows differ (P<0.01)

1304 **Table 4** Effect of replacing soybean protein with detoxified *Jatropha curcas* kernel
1305 meal on slaughter characteristics and internal organs of growing pigs

	DJM0	DJM50	SEM
Body weight (kg)	39.4	39.6	0.44
Carcass weight (kg)	26.7	27.3	0.38
Dress percentage (%)	67.7	68.9	0.76
Backfat thickness (cm)	1.30	1.15	0.09
Visceral organ weight (g)			
Heart	206	190	13.1
Live	851	862	26.4
Lung	789	823	21.9
Spleen	83	76	4.2
Kidney	204	201	6.5
Ratio to body weight (‰)			
Heart	5.2	4.8	0.34
Live	21.6	21.7	0.56
Lung	20.0	20.8	0.54
Spleen	2.1	1.9	0.10
Kidney	5.2	5.1	0.15

1306 DJM0, DJM25 and DJM50: diets included detoxified *Jatropha curcas* kernel meal
1307 to replace 0, 25 and 50% of soybean meal protein, respectively.

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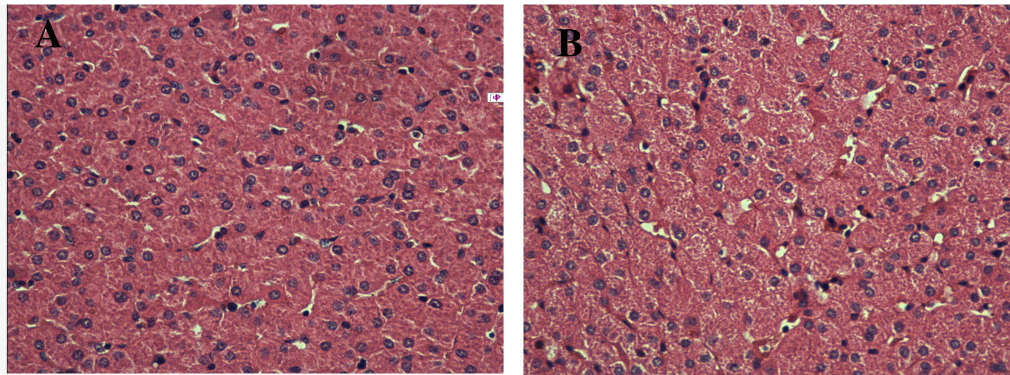

1319 Fig.1. Histopathological change in liver of pigs fed on diets containing *Jatropha*
1320 *curcas* kernel meal to replace 0 (A) and 50% (B) of soybean meal protein.
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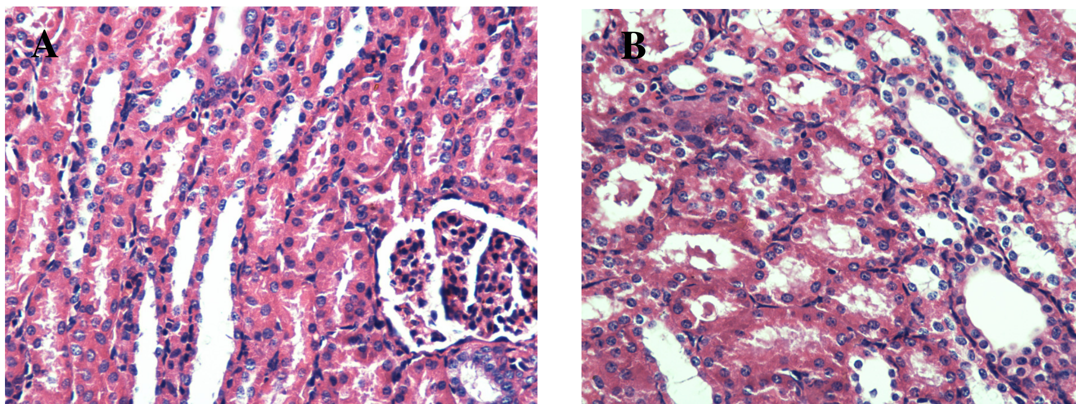

1341 Fig.2. Histopathological change in kidney of pigs fed on diets containing *Jatropha*
1342 *curcas* kernel meal to replace 0 (A) and 50% (B) of soybean meal protein.
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1347 **Discussion**

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1349 The *Jatropha* seed meal left after extraction of oil is presently used as a fertilizer or
1350 disposed of and not suitable for animal feed, because of the presence of high levels of
1351 phorbol esters and antinutrients (Achten, 2008). *Jatropha* seeds and oil have been found
1352 to be toxic to mice, rats, calves, sheep and goats, human and chickens (Goel et al., 2007;
1353 Li et al, 2010). Makkar and Becker (1999) considered that the residual protein-rich
1354 kernel meal, remaining after extraction of the oil, could form a protein-rich ingredient in
1355 feeds for poultry, pigs, cattle and even fish if it could be detoxified. After removing the
1356 toxic factor (phorbol esters) and heat-labile antinutrients (such as trypsin inhibitor and
1357 lectins), the *Jatropha* kernel meal was non-toxic to fish (Makkar and Becker, 2009).
1358 *Jatropha* kernel meal contains good nutrient profile, comparable to commercially
1359 available soybean meal.A comparison in amino acid profiles between *Jatropha* seed
1360 protein and soybeans (Vasconcelos et al., 1997) revealed a similar pattern for most
1361 essential amino acids, except lysine and sulphur amino acids. The content of lysine and
1362 methionine were 1.9 and 1.1% in *Jatropha* kernel meal used in our work (data not
1363 reported), and 2.7 and 0.6% for common soybean meal. Level of lysine was lower in the
1364 *Jatropha* kernel meals (Martinez-Herrera et al., 2006), but methionine content was
1365 higher than that in soybean meal. Supplementation of *Jatropha* kernel meal with lysine,
1366 the only deficient amino acid, is likely to improve the nutritive value of the meal.
1367 Therefore, lysine was supplemented in the diet with the inclusion of DJM in this study.
1368 No significant difference was observed in growth performance with detoxified *Jatropha*
1369 kernel meal substituting 25 or 50% of soybean meal protein in the diet of growing pigs.
1370 No significant difference shown in daily weight gain and feed utilization indicated that
1371 the nutrient value of *Jatropha* kernel meal is comparable with that of soybean meal as a
1372 protein source for growing pigs.
1373 No difference in total protein, albumin and urea nitrogen in serum of pig fed diets
1374 with detoxified *Jatropha* kernel meal when compared to those on the control diet (Table
1375 3), indicate that the amino acid balance was similar among the three diets. Similarly, no
1376 difference in total protein, urea, albumin, glucose, triglyceride, lysozyme activity and
1377 alkaline phosphatase level in common carp (*Cyprinus carpio*) fingerlings fed a diet
1378 containing detoxified *Jatropha* kernel meal (detoxified by the same method as used in

1379 this study) at a level of 50% replacement of fish meal protein in the diet (Kumar et al.,
1380 2010). Decrease in serum glucose level and an increase in serum concentration of
1381 arginase, glutamate, and oxaloacetate transaminase were observed in goats fed on
1382 undetoxified *Jatropha* seed meal, with lack of appetite, reduced water intake, diarrhea,
1383 dehydration, and other hemorrhagic effects in different organs (Adam and Magzoub,
1384 1975). Several cases of *J. curcas* nut poisoning in humans after accidental consumption
1385 of seeds have been recorded. Symptoms such as giddiness, emesis and diarrhea have
1386 been reported (Makkar and Becker, 1999). However, the pigs fed DJM diet not show
1387 any signs of toxicity. There was no significant difference in content of serum parameters
1388 including glucose and glutamic-oxalacetic transaminase among pigs fed DJM or control
1389 diet ($P>0.05$, Table 3), which could manifest the good growth performance of pig fed
1390 *Jatropha* kernel meal-containing diet.

1391 No adverse histopathological changes were observed in liver or kidney at a
1392 replacement of 50% soybean meal protein by the detoxified *J. curcas* meal (Fig 1 and
1393 2) in the diet. In evaluation of toxicity of *J. curcas* phorbol esters, Li et al. (2010)
1394 found that the mice had no significant abnormal changes in the organs at the lowest
1395 dose (21.26 mg/kg body mass), but prominent lesions in lung and kidney in the form of
1396 diffused haemorrhages in lung and glomerular sclerosis and atrophy in kidney were
1397 observed at higher doses (32.40 mg/kg body mass). The histophathological results (no
1398 adverse effects) indicated *Jatropha* kernel meal we used was detoxified completely,
1399 suggesting that detoxified *Jatropha* kernel meal is safe for animal feeding. the blood
1400 parameters were also in the normal ranges.

1401 The above results demonstrate that the nutritive value of the detoxified *Jatropha*
1402 kernel meal is comparable with that of soybean meal for growing pigs and it is safe to
1403 replace 50% of soybean meal with the detoxified kernel meal. Further studies by
1404 replacing more than 50% of soybean meal protein by detoxified *Jatropha* kernel meal
1405 should be conducted.

1406

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3.1.1.8 Amino acid digestibility of detoxified *Jatropha curcas* L. kernel meal and protein isolate in turkeys (responsible institute 8 and 1)

Introduction

Jatropha curcas L. is a drought-resistant shrub or small tree belonging to the genus *Euphorbiaceae*, which is mainly cultivated for the production of oil and biodiesel. De-oiling the kernel yields a meal of high protein content with an excellent amino acid composition. Detoxified *Jatropha* kernel meal (**DJKM**) produced by the Institute for Animal Nutrition in the Tropics and Subtropics (480b) at Hohenheim University showed good growth response in carp, tilapia, trout and shrimp, and therefore may be a valuable protein source for growing poultry.

Precaecal (**pc**) amino acid (**AA**) digestibility has become most relevant in evaluating feed protein sources for poultry. Samples are taken at the end of ileum. Therefore, effects of postileal fermentation on digestibility are not relevant. One approach for measuring digestibility is to include the protein under test into the diet at different inclusion levels. When linear regression analysis is applied to quantitative data for both AA intake and AA flow at the terminal ileum, the slope describes the proportion of incremental intake from the test protein feed that did not reach the terminal ileum[1]. Digestibility determined in this way does not need any correction for endogenous amino acid losses.

The objective of this study was o determine the pc AA digestibility of DJKM and a detoxified protein isolate produced from *Jatropha* (**DJPI**) in young turkeys.

1480 ## Material and methods

1481 *Diets*

1482 The basal diet was mainly based on maize, solvent-extracted soybean meal from
1483 dehulled seed, wheat gluten and soybean oil (Table 1). In addition, free amino acids,
1484 monocalcium phosphate, calcium carbonate, sodium chloride, cholinchloride, sodium
1485 bicarbonate and a vitamin and mineral mix were included. The diet contained TiO_2 as
1486 indigestible marker at a level of 5 g/kg of diet.

1487 The test protein feeds (DJKM and DJPI) were supplied by the Animal Nutrition
1488 in the Tropics and Subtropics Group of Hohenheim University. They were included in
1489 concentrations of 10 and 20 % (DJKM) or 7.5 and 15 % of the diet (DJPI). Inclusions
1490 were made at the expense of maize starch. Hence changes in the AA concentrations of
1491 the experimental diets resulted from the respective protein source only. The analysed
1492 crude nutrient and amino acid concentrations are summarized in Table 2. It can be
1493 noted that the concentrations of some AA, especially arginine and lysine, were lower
1494 in DJPI than DJKM although the protein concentration was higher. As both originate
1495 from the same seed and consequently should have similar AA pattern it can be
1496 hypothesised that damage of AA occurred during the production of DJPI.

1497 Diet preparation was done in the certified feed mill facilities of Hohenheim
1498 University, Research Station for Animal Husbandry, Animal Breeding and Small
1499 Animal Breeding in 72800 Eningen, Germany. The total amount of feed needed for the
1500 experiment, except the variable ingredients, was mixed in one lot and subsequently
1501 divided into 5 equal parts. Maize starch and DJKM or DJPI were then supplemented to
1502 achieve the levels as detailed in Table 1, and the diets were mixed again. Diets were
1503 pelleted using a 3-mm die.

Table 1 Composition of the diets (%, as fed)

Treatment[1]	Basal diet	DJKM10	DJKM20	DJPI7.5	DJPI15
Maize			---40.0---		
Soybean meal			---13.0---		
Maize starch	20.0	10.0	-	12.5	5.0
DJKM	-	10.0	20.0	-	-
DJPI	-	-	-	7.5	15.0
Wheat gluten			---10.25---		
Soybean oil			---5.50---		
Amino acids[2]			---3.55---		
Monocalcium phosphate			---4.20---		
Calcium carbonate			---2.10---		
Sodium chloride			---0.10---		
Cholinchloride			---0.20---		
Sodium bicarbonate			---0.30---		
Vitamin and mineral mix[3]			---0.30---		
Titanium dioxide			---0.50---		

[1] DJKM, detoxified *Jatropha* kernel meal; DJPI, detoxified *Jatropha* protein isolate

[2] (% of feed): 1.10 L-lysine monohydrochloride, 0.60 D,L-methionine, 0.4 L-threonine, 0.05 L-tryptophan,
0.55 L-arginine, 0.30 L-isoleucine, 0.25 L-leucine, 0.30 L-valine

[3] Premix contained per kg: Vitamin A 6,000,000 I.U.; Vitamin D_3 1,500,000 I.U.; Vitamin E 15 g; Vitamin B_1 1.5 g; Vitamin B_2 3 g; Vitamin B_6 3 g; Vitamin B_{12} 15 mg; Vitamin K_3 1.2 g; Nicotinic acid 25 g; Pantothenic acid 7 g; Biotin 50 mg; Folic acid 500 mg; Fe 90 g; Mn 120 g; Zn 80 g; Cu 15 g; I 1.7 g; Se 0.5 g; Co 0.6 g

Animals, housing and sampling

The experiment was conducted in the Research Station for Animal Husbandry, Animal Breeding and Small Animal Breeding of the University Hohenheim, 72800 Eningen, Germany. Four hundred turkey hatchlings (B.U.T. Big 6; Gebr. Böcker Putenbrüterei GmbH, Wallhausen, Germany) were allocated to 40 pens of 10 birds (5 male and 5 female), each on a wood shavings bedding. A commercial turkey starter diet was offered in the pre-experimental phase and this starter diet contained a coccidiostat. No other medical treatment was necessary.

Feed was offered for *ad libitum* intake in feeder troughs continuously. Feeders were re-filled with pre-weighed amounts when required. Drinking water was

1523 continuously supplied. Lighting was continuous until day 5, and a regime of 20 h light
1524 to 4 h dark was used from then onward. Temperature was 36°C on days 1 and 2 and
1525 was reduced from day 3 onwards in steps of 0.5 °C per day.

1526 On day 14 of age 8 pens were allocated to each of the 5 experimental diets in a
1527 way that an equal distribution of treatments in the animal house was given.

1528 *Analyses and data evaluation*

1529 Samples of feed and digesta were ground through a sieve with 0.5 mm pore size.
1530 Concentrations of dry matter and crude nutrients were determined according to
1531 VDLUFA standard methods[2]. Concentrations of Ti were determined according to
1532 Boguhn et al. (2009)[3] using an inductively coupled plasma spectrometer (ICP-OES).
1533 Amino acid analysis was conducted as described previously[1]. In brief, after an
1534 oxidation step, samples were hydrolysed in 6 N hydrochloric acid. Norleucine was
1535 used as the external standard. Amino acids were separated and detected with an AA
1536 analyzer (Hitachi L-8900) using different buffer solutions and ninhydrin. Because
1537 histidine and tyrosine are degraded during performic acid oxidation, these AA were not
1538 determined. Tryptophan analysis followed standard procedures[4]. Separation and
1539 detection of tryptophan was conducted using HPLC. The nitrogen concentration was
1540 analysed using a C-N analyser and crude protein was calculated as nitrogen multiplied
1541 by 6.25.

1542 The pc digestibility coefficients (y) of AA and crude protein (CP) were
1543 calculated, on a pen basis, according to the generally accepted equation:

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$$y = 1 - \left(\frac{TiO_2 \text{ in diet (g/kg)}}{TiO_2 \text{ in digesta (g/kg)}} \times \frac{\text{AA or CP in digesta (g/kg)}}{\text{AA or CP in diet (g/kg)}} \right)$$

1547 The quantitative daily intakes of AA and CP were calculated as feed intake (g/d)
1548 multiplied by analysed AA (or CP) concentration in the diet (mg/g). The quantity of

1549 AA pc digested was calculated as AA intake (mg/d) multiplied by the digestibility
1550 coefficient. The digestibility of AA from the two protein feeds was obtained by
1551 calculating the linear regression between quantitative AA intake and the amount of AA
1552 digested up to the terminal ileum.

1553 Performance data were subjected to *glm* procedure using the software package
1554 SAS for Windows 9.2. Significant treatment effects were detected using t-test ($p \leq$
1555 0.05).

1556 Linear regressions were calculated using GraphPad Prism 5.0 (GraphPad
1557 Software Inc., San Diego, USA). Each pen was considered as one unit in the regression
1558 analysis. As parameters for the goodness of fit, r^2 and $s_{y.x}$ will be presented. $s_{y.x}$ is the
1559 standard deviation of residuals, which are the distances between the individual points
1560 from the calculated line.

1561

1562 **Results**

1563 The study could be finished without problems. Body weight gain (BW gain), feed
1564 consumption, and the gain to feed ratio were significantly increased by inclusion of the
1565 test protein feeds (Table 3). BW gain and gain to feed ratio were higher in diets with
1566 DJKM compared to DJPI. The feed consumption was on a high level and highest in the
1567 diet with 20 % DJKM.

1568 **Table 3** Body weight (BW), BW gain, feed consumption and gain to feed ratio of
1569 turkeys in the 7-day experimental period (Means and SD, 8 replicates per treatment)

Treatment	Initial BW	BW gain	Feed consumption	Gain to feed ratio
	g	g/d	g/d	g/g
Control[1]	415^{a}	42^{a}	60^{a}	0.70^{a}
	15	2.0	4.5	0.06
DJKM10	402^{ab}	54^{b}	66^{bcd}	0.81^{b}
	18	2.7	3.7	0.02
DJKM20	397^{b}	57^{c}	70^{d}	0.82^{b}
	10	2.2	3.0	0.01
DJPI7.5	403^{ab}	49^{d}	63^{ac}	0.78^{c}
	18	1.3	2.8	0.03
DJPI15	406^{ab}	50^{d}	65^{c}	0.77^{c}
	16	1.3	2.8	0.02
	0.24	<0.001	<0.001	<0.001

1570 [1] 6-day experimental period
1571 [abcd] Values without a common superscript are significantly different according to t-test ($p \leq$
1572 0.05)

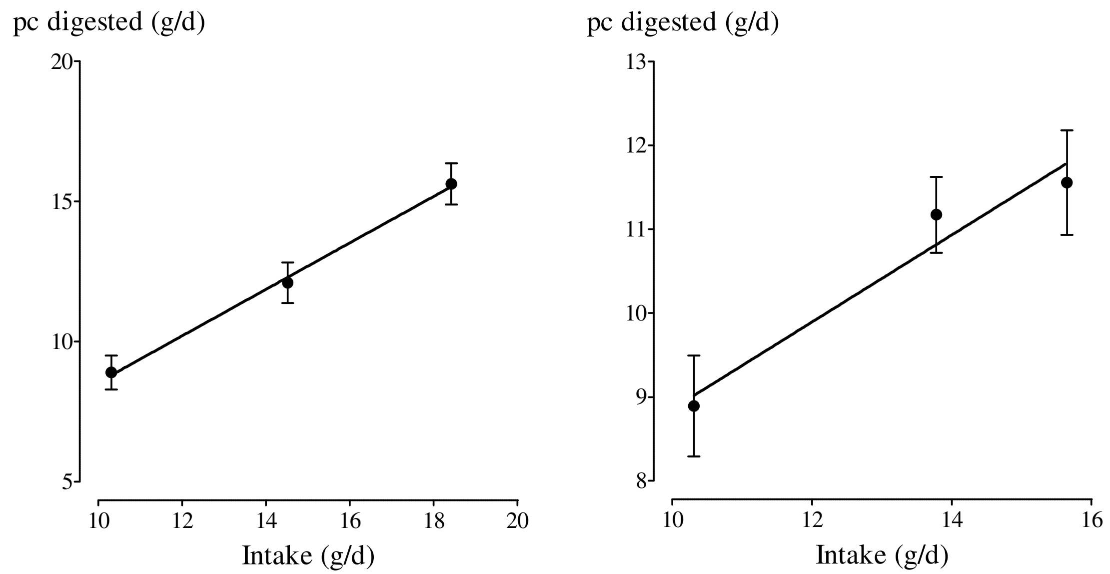
1573

1574 The pc digestibility coefficients of the individual AA from the 5 diets ranged between
1575 0.58 and 0.97, with the lowest and highest values being determined for cystine in diet
1576 DJPI15 and methionine in the control diet, respectively (Table 4).

1577 In regard to the two protein sources the response in digestibility to increased
1578 intake was different between DJKM and DJPI. For DJKM, the amounts of crude
1579 protein and AA digested up to the terminal ileum depended linearly on the intake of
1580 protein and AA (Fig. 1 and Annex Fig. 1). Such linear relationship was found in
1581 several previous studies of our group with different protein sources. In contrast, the
1582 linearity was not as clear for the supplemented DJPI (Fig. 1 and Annex Fig. 2). It
1583 appears that including 15 % instead of 7.5 % DJPI into the diet had a depressing effect
1584 on AA digestibility.

1585 Results of linear regression analysis are shown in Table 5. Estimates for the
1586 slopes represent the digestibility of the respective supplemented protein source.
1587 Estimated intercepts are not relevant in the context of this study. Digestibility of
1588 essential AA ranged from 83% (threonine) to 91% (methionine) and was on an overall
1589 very high level. With the same experimental approach a range in digestibility of
1590 essential AA of soybean meal (80 to 88%) and rapeseed meal (72 to 86%) was found
1591 in turkeys[5]. This comparison indicates that DJKM, based on AA digestibility, is at least
1592 as good as conventional protein sources for poultry.

1593 In contrast, slopes determined for DJPI were low. Among essential AA
1594 digestibility ranged from 46% (tryptophan) to 66% (isoleucine and leucine) (Table 5).
1595 Together with the reduced analysed concentrations of lysine and arginine in DJPI this
1596 indicates that some damage of the protein has occurred that drastically reduced the
1597 value of the protein. Because the depressing effect seemed to be greater with 15%
1598 inclusion than with 7.5% inclusion of DJPI we alternatively calculated the digestibility
1599 with a regression that was based only on the data for the basal diet and the diet that
1600 contained 7.5% DJPI. Calculated digestibilities then ranged between 63% (tryptophan)
1601 and 75% (leucine) (Annex table 2). While this range is much better in comparison to
1602 the calculation based on all data, it still shows that the digestibility was considerably
1603 lower in DJPI than in DJKM. The lower AA digestibility in DJPI could possibly be
1604 due to the more drastic and yet not properly optimised large scale detoxified process.
1605



1606 **Figure 1** Relationship between intake and digestion up to the terminal ileum for
1607 crude protein in turkeys fed different concentrations of DJKM (left) and
1608 DJPI (right) (Means and SD, n = 8 pens per treatment)
1609

Table 4 Precaecal digestibility coefficients of crude protein and amino acids determined for the diets with different concentrations of detoxified *Jatropha* kernel meal (DJKM) and detoxified *Jatropha* protein isolate (DJPI) (Means and SD, n = 8 pens per treatment)

Protein source[1]	Control		DJKM				DJPI			
			100 g/kg		200 g/kg		75 g/kg		150 g/kg	
Crude protein	0.863	± 0.007	0.833	± 0.009	0.848	± 0.007	0.811	± 0.008	0.738	± 0.013
Alanine	0.862	± 0.006	0.840	± 0.010	0.858	± 0.006	0.805	± 0.009	0.730	± 0.011
Arginine	0.926	± 0.003	0.903	± 0.007	0.914	± 0.003	0.860	± 0.007	0.800	± 0.007
Aspartic acid	0.792	± 0.010	0.713	± 0.021	0.740	± 0.009	0.697	± 0.010	0.584	± 0.013
Cystine	0.703	± 0.026	0.626	± 0.032	0.631	± 0.012	0.668	± 0.012	0.580	± 0.022
Glutamic acid	0.936	± 0.003	0.905	± 0.007	0.904	± 0.003	0.886	± 0.004	0.827	± 0.008
Glycine	0.816	± 0.011	0.779	± 0.016	0.799	± 0.007	0.745	± 0.010	0.651	± 0.013
Isoleucine	0.910	± 0.003	0.891	± 0.008	0.898	± 0.004	0.867	± 0.012	0.822	± 0.014
Leucine	0.907	± 0.003	0.886	± 0.007	0.896	± 0.004	0.871	± 0.008	0.826	± 0.009
Lysine	0.931	± 0.003	0.908	± 0.006	0.911	± 0.003	0.906	± 0.007	0.875	± 0.008
Methionine	0.966	± 0.002	0.953	± 0.003	0.950	± 0.002	0.935	± 0.003	0.893	± 0.005
Phenylalanine	0.896	± 0.004	0.878	± 0.009	0.891	± 0.005	0.846	± 0.011	0.785	± 0.011
Proline	0.902	± 0.006	0.872	± 0.013	0.879	± 0.007	0.872	± 0.008	0.819	± 0.009
Serine	0.843	± 0.008	0.792	± 0.014	0.817	± 0.007	0.772	± 0.009	0.677	± 0.014
Threonine	0.862	± 0.008	0.837	± 0.010	0.850	± 0.005	0.820	± 0.010	0.751	± 0.014
Tryptophan	0.842	± 0.008	0.766	± 0.016	0.822	± 0.012	0.779	± 0.008	0.684	± 0.014
Valine	0.890	± 0.006	0.880	± 0.008	0.887	± 0.003	0.847	± 0.016	0.799	± 0.012

Table 5 Amounts of crude protein (g) and amino acids (mg) digested per day up to the terminal ileum (y) depending on the respective daily intake (x), described by a linear regression (parameter estimate and SE of estimate; n = 24 for each regression line)

	Intercept				Slope				r^2		$s_{y.x}$	
Protein source[1]	DJKM		DJPI		DJKM		DJPI		DJK	DJPI	DJKM	DJPIM
Crude protein	0.3	± 0.63	3.7	± 0.76	0.83	± 0.042	0.52	± 0.057	0.95	0.79	0.69	0.61
Alanine	-0.7	± 26.0	155.5	± 29.2	0.85	± 0.037	0.54	± 0.046	0.96	0.86	32.6	27.5
Arginine	11.2	± 48.3	275.4	± 53.8	0.90	± 0.034	0.61	± 0.046	0.97	0.89	69.0	52.0
Aspartic acid	48.9	± 39.8	337.2	± 46.3	0.70	± 0.033	0.34	± 0.044	0.95	0.73	58.0	49.9
Cystine	39.8	± 13.9	124.3	± 34.9	0.48	± 0.057	0.05	± 0.167	0.76	0.004	11.1	11.1
Glutamic acid	235.7	± 213	1129	± 274	0.85	± 0.056	0.55	± 0.079	0.91	0.69	191	167
Glycine	6.7	± 22.1	163.2	± 25.8	0.79	± 0.035	0.43	± 0.046	0.96	0.79	29.3	25.3
Isoleucine	13.1	± 35.7	142.9	± 38.7	0.88	± 0.049	0.66	± 0.055	0.94	0.87	38.6	31.0
Leucine	21.6	± 70.1	262.7	± 79.0	0.86	± 0.049	0.66	± 0.060	0.94	0.85	70.9	59.0
Lysine	51.0	± 75.7	267.2	± 123	0.87	± 0.068	0.63	± 0.124	0.88	0.54	56.8	47.7
Methionine	28.7	± 51.4	196.4	± 68.9	0.91	± 0.083	0.58	± 0.121	0.84	0.62	32.7	27.9
Phenylalanine	1.9	± 32.3	165.4	± 37.9	0.89	± 0.041	0.60	± 0.053	0.96	0.85	37.1	32.4
Proline	64.3	± 87.0	307.7	± 98.5	0.83	± 0.072	0.59	± 0.086	0.86	0.69	63.0	54.9
Serine	19.1	± 32.6	282.1	± 45.9	0.79	± 0.041	0.34	± 0.066	0.95	0.55	38.2	33.5
Threonine	13.6	± 40.1	232.9	± 52.5	0.83	± 0.051	0.48	± 0.073	0.92	0.66	37.7	32.5
Tryptophan	-2.8	± 7.1	47.6	± 8.9	0.83	± 0.042	0.46	± 0.054	0.95	0.77	8.9	8.1
Valine	2.0	± 36.1	155.2	± 37.7	0.88	± 0.041	0.64	± 0.048	0.95	0.89	41.3	31.9

[1]DJKM, detoxified *Jatropha* kernel meal; DJPI, detoxified *Jatropha* protein isolate

Summary and conclusions

The objective of this study was to determine the precaecal digestibility of amino acids in detoxified *Jatropha* kernel meal (DJKM) and a detoxified *Jatropha* protein isolate (DJPI) in turkeys.

Three-week-old turkeys were fed diets based on maize and solvent-extracted soybean meal with 100 and 200 g/kg DJKM or 75 and 150 g/kg DJPI for 7 days. Both protein feeds replaced maize starch in equal proportions so that the changes in the amino acid concentrations of the experimental diets resulted from DJKM or DJPI only. Eight replicated pens of 10 birds were used per treatment. Digesta was collected from a standardised section of the terminal ileum and pooled per pen. The digestibility of amino acids was calculated of each pen and each diet, and the digestibility of the two protein feeds was calculated by linear regression analysis.

The digestibility of amino acids from DJKM was on a very high level. Digestibility of essential amino acids ranged from 83% (threonine) to 91% (methionine) and was not inferior to the range found for conventional protein sources in previous studies with a similar design. Feed intake and growth of turkeys, although only studied for 7 days, did not indicate a negative effect of inclusion of DJKM into the diet. Based on these observations it can be hypothesised that DJKM has great potential as a protein source for poultry. In a next step feeding trials with growing poultry using different inclusion levels of DJKM should be conducted for testing this hypothesis.

The digestibility of amino acids from DJPI was much lower. Among essential AA digestibility it ranged from 46% (tryptophan) to 66% (isoleucine and leucine). The depressing effect on digestibility was greater with 15% than 7.5% inclusion of DJPI in the diet. This product is not yet suitable for being used as a protein feed for poultry. Details of the production process should be checked for possible effects on digestion and be improved.

Annex table 1 Initial body weight (BW) as well as BW gain, feed intake and gain to feed ratio (pen basis) during the experimental phase (7 days)

Pen	Diet	Initial BW (g/bird)	BW gain (g/d)	Feed intake (g/d)	Gain to feed ratio
1	Control[1]	398	40.2	69.7	0.58
2	DJKM10	394	53.0	64.9	0.82
3	DJKM20	406	57.6	69.9	0.82
4	DJPI7.5	412	49.0	64.3	0.76
5	DJPI15	420	50.8	65.5	0.78
6	Control[1]	399	39.1	54.5	0.72
7	DJKM10	376	49.3	59.4	0.83
8	DJKM20	390	56.4	68.7	0.82
9	DJPI7.5	408	50.4	62.1	0.81
10	DJPI15	417	49.7	65.5	0.76
11	Control[1]	414	41.8	58.5	0.71
12	DJKM10	415	57.9	69.2	0.84
13	DJKM20	395	55.7	66.3	0.84
14	DJPI7.5	405	47.2	60.4	0.78
15	DJPI15	403	50.4	66.3	0.76
16	Control[1]	417	45.1	62.2	0.72
17	DJKM10	417	53.7	65.4	0.82
18	DJKM20	381	53.8	65.0	0.83
19	DJPI7.5	415	51.0	63.6	0.80
20	DJPI15	403	49.1	61.3	0.80
21	Control[1]	406	42.3	60.0	0.71
22	DJKM10	420	55.0	68.0	0.81
23	DJKM20	406	58.7	71.1	0.83
24	DJPI7.5	360	47.8	59.5	0.80
25	DJPI15	373	49.4	61.9	0.80
26	Control[1]	418	44.7	57.6	0.77
27	DJKM10	404	56.4	71.8	0.78
28	DJKM20	391	58.1	72.7	0.80
29	DJPI7.5	418	49.6	68.4	0.72
30	DJPI15	419	51.8	67.1	0.77
31	Control[1]	440	42.8	62.3	0.69
32	DJKM10	413	54.0	67.3	0.80
33	DJKM20	409	57.7	69.7	0.83
34	DJPI7.5	404	49.2	63.3	0.78
35	DJPI15	415	52.3	69.5	0.75
36	Control[1]	430	42.9	58.7	0.73
37	DJKM10	377	51.3	64.2	0.80
38	DJKM20	401	61.3	73.6	0.83
39	DJPI7.5	406	50.0	64.7	0.77
40	DJPI15	396	48.5	63.0	0.77

[1] 6-day experimental period

Annex table 2 Results of linear regression analysis calculated only with the data for the basal diet and the diet DJPI7.5 (y is the amounts of crude protein (g) or amino acid (mg) digested per day up to the terminal ileum depending on the respective daily intake (x), parameter estimate and SE of estimate; n = 16 for each regression line)

	Intercept		Slope		r^2	$s_{y.x}$
Crude protein	2.1	± 0.94	0.66	± 0.077	0.84	0.53
Alanine	93.1	± 38.4	0.66	± 0.069	0.87	24.7
Arginine	206.4	± 81.5	0.69	± 0.079	0.84	51.8
Aspartic acid	201.6	± 45.3	0.51	± 0.050	0.88	36.1
Cystine	51.5	± 30.1	0.43	± 0.148	0.38	8.0
Glutamic acid	731.3	± 390	0.68	± 0.120	0.70	169
Glycine	94.1	± 29.3	0.58	± 0.060	0.87	20.2
Isoleucine	101.0	± 60.2	0.73	± 0.094	0.81	31.7
Leucine	167.9	± 120	0.75	± 0.100	0.80	60.1
Lysine	168.9	± 183	0.74	± 0.191	0.52	50.9
Methionine	132.9	± 119	0.70	± 0.219	0.43	30.2
Phenylalanine	102.5	± 52.0	0.71	± 0.081	0.85	31.5
Proline	158.1	± 140	0.74	± 0.130	0.70	55.5
Serine	165.7	± 54.3	0.54	± 0.085	0.74	28.4
Threonine	126.6	± 73.1	0.65	± 0.110	0.71	30.9
Tryptophan	25.4	± 9.4	0.63	± 0.064	0.87	6.5
Valine	106.5	± 61.4	0.71	± 0.088	0.82	33.0

3.1.2 Preparation of protein isolate free of toxin and antinutritional factors and its use in fish and livestock diets ((main responsible institute: Germany 1)

Two methods for preparation and detoxification were developed. In the first method, the protein isolate is prepared which is toxic and then it is detoxified, basically using the same approach as used for the kernel meal. In the second method, 'One-Step Method' the protein isolate preparation and detoxification takes place in one step, making it easier and faster than the first method.

3.1.2.1 Evaluations of the nutritional value of *Jatropha curcas* protein isolate in common carp (*Cyprinus carpio* L.)

Introduction

Fish meal (FM) is the major protein source in compound feeds for intensive fish farming. In an effort to reduce reliance on FM as the primary protein source, most modern nutrient-dense aquaculture diets now use some plant protein ingredients. Several studies have been undertaken to evaluate the nutritional quality of a range of plant protein sources in common carp (*Cyprinus carpio* L.) (Hasan *et al.* 1997; Kumar *et al.* 2008, 2010; Mazurkiewicz 2009; Makkar *et al.* 2009). Detoxified *Jatropha* kernel meal has been reported to have good potential for incorporation in commercial fish diets as a good quality protein-rich feed ingredient (Kumar *et al.* 2008, 2010; Makkar *et al.* 2009).

Modern nutrient-dense diets for aquatic species have little formulation flexibility to accommodate large amounts of indigestible material (neutral detergent fibre). Because of this, many plant protein sources are not viable alternatives for FM, despite having reasonable protein or energy digestibility (Glencross *et al.* 2005). In addition, many plant materials contain antinutritional factors. Modern processing technologies have overcome many of these obstacles, not only by denaturing anti-nutritional factors and solvent extracting much of the unsuitable lipid, but also by removing a large part of the undesirable carbohydrate and other components (Oliva-Teles *et al.* 1994). The resulting protein concentrate or protein isolate products contain high levels of protein, which often have digestibility similar or higher than that of FM protein (Xie & Jokumsen 1998). Techniques for production of protein concentrates and isolates from legumes

are relatively well known. Among these are processes such as dehulling, air classifying, solvent extraction and solubilised extraction (Lasztity *et al.* 2001), all of which have some commercial application. A range of such products produced from soybean already exists in the market and have previously been assessed in common carp, rainbow trout and Atlantic salmon (Kaushik *et al.* 1995; Escaffre *et al.* 1997; Refstie *et al.* 1998).

Jatropha curcas (L.) (physic nut) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. It is a hardy plant, thrives on degraded land and requires limited amounts of nutrients and water. The International *Jatropha* Organization has projected that in 2017 there will be around 32.72 million hectares of land cultivated worldwide with *J. curcas,* producing 160 million tons of seeds and 95% of the total production will be in Asia. *Jatropha* seeds have been extensively investigated as a source of oil. The seed contains about 30-35% oil that can be converted into biodiesel of high quality upon transesterification and used as a substitute for diesel fuel (Makkar & Becker 2009). The seed cake left after mechanical pressing of oil contains 50-60% shells which are indigestible. The protein isolate obtained from the seed cake, using the principle of isoelectric precipitation, is an excellent source of nutrients and contains 80 to 85 % crude protein. However, the presence of high levels of antinutrients such as trypsin inhibitor, lectin and phytate and the major toxic components phorbol esters (PE_S) (Makkar *et al.* 2007) restrict their use in fish feed. Heat labile antinutrients such as protease inhibitors, and lectins are easy to inactivate by moist heating (Makkar & Becker 2009). A method for detoxification of *Jatropha* protein isolate has been developed in our laboratory. It is based on extraction of PEs using organic solvents and inactivation of trypsin inhibitors and lectin by heat treatment. Improved *Jatropha* plant can yield 4 to 5 tons seed per year from one hectare of plantation, which can give approximately 0.3 to 0.4 ton of the protein isolate (Makkar & Becker 2009). This means that there is a possibility of producing enough *Jatropha* protein isolates to meet growing aquaculture industry demand. Our previous studies have shown that detoxified *Jatropha* kernel meal (kernel meal is the product obtained after removal of oil by solvent extraction of the shell-free kernels) is a good protein source for common carp (Kumar *et al.* 2008, 2010; Makkar *et al.* 2009), rainbow trout (*Oncorhynchus mykiss*) (Makkar *et al.* 2009), Nile tilapia (*Oreochromis niloticus*) and white leg shrimp (*Penaeus vannamei*) (unpublished data) diets. Here we report nutritional value of the protein isolate prepared from *Jatropha curcas* seed cake and compare it with soy protein isolate (SPI) and FM in common carp.

Materials and methods

Preparation of the Jatropha protein isolates

Jatropha seed cake obtained using a mechanical screw press (German screw press type Komet D85-1G, Germany) was used for preparation of protein isolate. The chemical composition of the seed cake was: crude protein 23.6 %, oil 9.3 %, and ash 5.8%; all on dry matter basis).

The principle of isoelectric precipitation was used to obtain protein isolate from the seed cake. To 500 g of defatted seed cake (in triplicate) was added 5000 ml of distilled water adjusted to pH 11 using 10 mol L^{-1} NaOH. The mixture was stirred for 1 h at room temperature (20 ^{o}C). Every 15 min the pH was checked and adjusted to 11 using 10 mol L^{-1} NaOH. The contents were centrifuged at 3000 × g for 20 min and the supernatant was collected. The supernatant was brought to pH 4 using 6 mol L^{-1} HCl, stirred for 10 min and kept at 4 ^{o}C for 1 h to precipitate proteins. The contents were centrifuge at 3000 × g for 20 min to obtain the protein isolate. The protein isolate was freeze-dried. Organic solvents were used to detoxify the protein isolate (patent application has been filed for the process of detoxification). After removal of PEs, the protein isolate was autoclaved (121 ^{o}C for 15 min) to inactivate heat labile antinutrients, trypsin inhibitor and lectin.

Diet formulation, experimental system and animals

Fish meal was purchased from Kurt Becker GmbH, Bremen, Germany. Prior to feed formulation, the proximate composition of DJPI, SPI, wheat meal, and FM was determined. Five isonitrogenous diets were formulated. Experimental diets contained crude protein 38%, crude lipid 9%, gross energy 19.5 KJ/g, vitamin premix 2%, mineral premix 2% and titanium oxide (TiO_2) 1%. Lysine monohydrochloride (lysine 80% in this salt) was supplemented at the rate of 2.71% of DJPI inclusion in the diet. Each experimental feed, except the control, contained 500 FTU phytase (NATUPHOS 5000G, BASF, Ludwigshafen, Germany) per kg. The inclusion level of the DJPI and SPI were as follows: Control diet (Control) was prepared with FM and wheat

meal, without DJPI and SPI; J_{50} and J_{75} (50% and 75% of FM protein replaced by DJPI), and S_{50} and S_{75} (50% and 75% of FM protein replaced by SPI). The final mixture of each diet was made into 2 mm diameter moist pellets and then freeze-dried (Table 1).

Table 1 Composition of the experimental diets (g kg^{-1} feed)

Ingredients	Experimental diets[1]				
	Control	J_{50}	J_{75}	S_{50}	S_{75}
Fish meal	484	242	121	242	121
[2]Wheat meal	436	435	435	435	435
Jatropha protein isolate	-	196	295	-	-
Soya protein isolate	-	-	-	172	258
Cellulose	-	23	33	47	70
Sunflower oil	40	64	76	64	76
[3]Vitamin premix	20	20	20	20	20
[4]Mineral premix	20	20	20	20	20
Total	1000	1000	1000	1000	1000
Lysine monohydrochloride	-	5.4	8.0	-	-
Phytase (FTU/kg)	-	500	500	500	500
TiO_2	10	10	10	10	10

[1]Experimental diets
Control: Fish meal and wheat meal, without any *Jatropha* protein isolate and soy protein isolate
J_{50}: 50% of fish meal proteins replaced by *Jatropha* protein isolate
J_{75}: 75% of fish meal protein replaced by *Jatropha* protein isolate
S_{50}: 50% of fish meal protein replaced by soy protein isolate
S_{75}: 75% of fish meal protein replaced by soy protein isolate
[2]Whole wheat meal.
[3]Vitamin premix (g or IU kg-1 premix): retinol palmitate, 500000IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50000IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride,100; biotin, 0.25.
[4]Mineral premix (g kg-1): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67; $CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

Common carp (*Cyprinus carpio* L.) fingerlings obtained from the Institute of Fisheries Ecology and Aquaculture of the Federal Research Center for Fisheries at Ahensburg, Germany were transferred to the University of Hohenheim, Stuttgart, Germany and kept in two 500 l capacity tanks for acclimatisation. After acclimatisation, 45 fish were randomly distributed into

five groups with three replicates; each replicate contained three fish (av. wt. 20.3 ± 0.13 g) in an aquarium (45 l capacity). All the aquaria were supplied with water from a recirculatory system. The system was subjected to a photoperiod of 12 h light : 12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 26.2 – 27.1°C, pH 7.0 – 7.5, dissolved oxygen 6.9 – 7.4 mg l^{-1}, total NH_3 0.1– 0.2 mg l^{-1}, nitrite 0.07 – 0.1 mg l^{-1} and nitrate 1–3 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation above 80%. One day before start of the experiment, the fish were starved and during the experimental period fish were fed at 16 g feed per kg metabolic body mass ($kg^{0.8}$) per day (equal to five times their maintenance requirement) and split into 5 equal rations per day (at 8:00, 10:30, 13:00, 15:30 and 18:00 h). The feeds were dispensed using an automatic feeder. Fish were weighed individually at the beginning of the experiment and at weekly intervals during the experimental period to adjust the feeding level for the subsequent week. The fish were not fed on the weighing day. During last two weeks of the experiment, fish were fed with a diet containing a marker (TiO_2) for digestibility measurement (Mamun *et al.* 2007). During last two weeks of the experiment, faeces were collected daily according to Mamun *et al.* (2007). The collected faeces were centrifuged at 4000 × g for 10 min, the supernatant discarded and the faeces were then stored at −20°C until analysis. At start of the experiment, six fish of the same population were also killed and preserved at −20 °C for analysis of the initial body composition.

The experiment lasted 12 weeks and fish were sampled thereafter. At the end of experiment, one fish per group was anaesthetized with tricaine methanesulfonate (MS222; 250 mg/L). Anaesthetized fish were carefully dissected to isolate intestine and stored in liquid nitrogen for determination of activities of digestive enzymes. One fish per group was killed by a blow to the head with metal rod and then stored at −20 ^{O}C for chemical composition analysis. Prior to determination of the proximate composition, the fish were autoclaved at 121 ^{o}C for 20 min, thoroughly homogenised using an Ultra-Turrax T25, frozen overnight and freeze-dried.

The University of Hohenheim Animal Welfare Committee approved all the experimental procedures involving in keeping, feeding and sampling of common carp.

Proximate analysis and determination of phorbol esters, antinutrients and amino acid

The proximate composition of diet ingredients, diets and whole body of fish was determined using standard methods (AOAC 1990). Phorbol esters (PEs) were determined according to Makkar *et al.* (2007) based on the method of Makkar *et al.* (1997). The results were expressed as equivalents to a standard phorbol-12-myristate 13-acetate (Sigma, Saint Louis, USA). Detection limit of PEs by HPLC was 3 µg/g protein isolates. Trypsin inhibitor activity was determined according to Smith *et al.* (1980) except that the enzyme was added last as suggested by Liu & Markakis (1989). Analysis of the lectin content was conducted by haemagglutination assay (Makkar *et al.* 1997). Phytate content of samples was determined by a spectrophotometric procedure (Vaintraub & Lapteva 1988). Non-starch polysaccharides (NSP) were determined according to Englyst *et al.* (1994). Amino acid composition of FM, DJPI, SPI and wheat meal was determined using an automated amino acid analyser after hydrolysing the samples with 6M HCl at 110 ^{0}C for 24 h (Bassler & Buchholz 1993). The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. Tryptophan content of the above-mentioned samples was determined spectrophotometrically by the method of Pinter-Szakacs & Molnar-Perl (1990).

Growth and nutrient utilization parameters

Growth performance and diet nutrient utilization were assessed in terms of body mass gain (BMG) = [(Final body mass - initial body mass) / Initial body mass] X 100; specific growth rate (SGR) = [(ln final body mass in g) - ln initial body mass in g) / number of trial days] X 100; metabolic growth rate (MGR) = (Body mass gain in g) / [{(initial body mass in g / 1000)$^{\wedge 0.8}$ + (final body mass in g / 1000)$^{\wedge 0.8}$}/2] / number of trial days; feed gain ratio (FGR) = dry feed fed (g)/body mass gain (g); protein efficiency ratio (PER) = body mass gain (g)/crude protein fed (g); protein productive value (PPV) = [(final fish body protein in g - initial fish body protein in g) / total protein consumed in g] X 100; lipid productive value (LPV) = [(final fish body lipid in g - initial fish body lipid in g) / total crude lipid consumed in g] X 100 and energy productive value (EPV) = [(final fish body energy - initial fish body energy)/(gross energy intake)] X 100.

Digestibility measurement and efficiency of digestible nutrients and gross energy

Titanium dioxide in the feed and faeces was determined according to the method described by Richter et al. (2003). The percentage of apparent dry matter digestibility of diets was calculated according to Maynard *et al.* (1981). Apparent dry matter digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces)}] X 100

Apparent digestibility coefficients (ADCs) of nutrients and energy of the experimental diets were calculated according to Maynard & Loosli (1969).

The nutrient and energy digestibility were obtained using the following formula:

Apparent nutrient digestibility (%) = [1 – {(% TiO_2 in feed) / (% TiO_2 in faeces) X (% Nutrient or energy in faeces) / (% Nutrient or energy in feed)}] X 100

ADCs of the test ingredients were calculated based on the digestibility of the reference diet and the test diets using an equation used by Bureau *et al.* (1999):

$ADC_I = ADC_T + ((1\text{-}s)\ D_R/sD_I)\ (ADC_T - ADC_R)$

where: ADC_I = Apparent digestibility coefficient of test ingredient; ADC_T = Apparent digestibility coefficient of test diet; ADC_R = Apparent digestibility coefficient of the reference diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = % nutrient (or kJ/g gross energy) of the test ingredient; s = Proportion of test ingredient in test diet mash.

Digestible nutrients and gross energy retained (%) = (Nutrient and energy retained in the whole body/Digestible nutrient and digestible energy) X100

Digestible nutrients and energy = Total offered of nutrients and gross energy through feed X digestibility coefficient.

Hepatosomatic index (HSI), intestinal somatic index (ISI) and digestive enzymes assay

Hepatosomatic index, and ISI are calculated as indicated below:

HSI = Liver mass (g) X 100 / body mass (g) and ISI = Intestinal mass (g) X 100 / body mass (g).

Amylase activity was estimated using dinitro-salicylic-acid (DNS) method (Rick & Stegbauer 1974). Amylase activity was expressed as mmol of maltose released from starch per min at 37 ^oC. Protease activity was determined by the casein digestion method of Drapeau (1974), and one unit of enzyme activity was defined as the amount of enzyme needed to release

acid soluble fragments equivalent to $\Delta 0.001A_{280}$ per minute at 37 ^{O}C and pH 7.8. Lipase activity was assayed by the method of Cherry & Crandell (1932), and one unit of enzyme was the amount of enzyme that hydrolyses 1.0 micro equivalent of fatty acid from a triglyceride in 24 h at pH 7.7 at 37 ^{O}C.

Statistical analysis

All data were subjected to a one-way analysis of variance ANOVA and the significance of the differences between means was tested using Duncan's multiple range test ($P<0.05$). The software used was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). Values are expressed as means ± standard deviation.

Results and discussion

The use of plant protein products in aquaculture diets is generally limited by their low levels of digestible protein and/or energy. The seed cakes of soybeans, lupins, pea and *Jatropha* represent some of those plant products that contain considerable amount of proteins and efforts have been made to further enhance their protein levels by processing technologies (Glencross *et al.* 2005; Makkar & Becker 2009; Makkar *et al.* 2009; Øverland *et al.* 2009). While protein isolate is commercially available from soybean-derived sources, similar such products from other plant products are still in a dormant phase. Notable in the products evaluated thus far is an increasing protein content, usually at the expense of the lignin and carbohydrate content of the seed cake. Based on the reports from other studies, it could be reasoned that an increase in the protein content of these product would be usually concomitant with a decline in the levels of antinutritional factors (Makkar & Becker 2009). Kaushik *et al.* (1995) evaluated nutritional value of a wide range of soybean products in rainbow trout and showed that they have high protein digestibility. Clearly these observations are consistent with those observed in the present study for *Jatropha* protein isolate.

Phorbol esters and antinutrients content in protein isolate

Phorbol esters content in untreated protein isolate was 1.2 mg/g. However, these were not detected in DJPI. Trypsin inhibitor and lectins were also not detected in DJPI and SPI; whereas phyate levels in DJPI and SPI were 2.95% and 0.94%, and NSP levels 10.5% and 1.04% respectively (Table 2).

Table 2 Proximate composition, antinutrients content and amino acid contents of feed ingredients

	Fish meal	*Jatropha* protein isolate	Soy protein isolate	Wheat meal
Proximate composition (g kg $^{-1}$)				
Dry matter	940	945	957	941
Crude protein	65.5	808	922	145
Crude lipid	88	9.7	10	16.3
Crude ash	142	93	37.9	14
Gross energy (KJ/g)	21.1	19.3	22.0	18.7
Antinutrients				
Trypsin inhibitor (mg trypsin inhibited per g sample)	-	ND	ND	-
Lectin[a]	-	ND	ND	-
Phytate (% dry matter)	-	2.95	0.95	-
Non-starch polysaccharides (g kg $^{-1}$)	-	105	10.4	-
Essential amino acids composition (g kg $^{-1}$)				
Arginine	35.3	86.0	67.9	5.4
Histidine	17.7	24.0	24.4	3.4
Iso leucine	22.8	33.8	36.5	4.2
Leucine	41.6	55.8	68.1	9.1
Lysine	40.9	18.9	52.1	3.3
Phenylalanine	21.8	38.6	43.2	6.5

Methionine	16	11.8	12.1	2.0
Threonine	23	26.5	31.1	3.7
Tryptophan	4.9	8.9	10.4	1.4
Valine	29.3	58.6	37.4	5.1
Non-essential amino acids composition (g kg $^{-1}$)				
Alanine	43.3	32.8	40.9	4.6
Asparginine	60.5	76.5	122.8	7.2
Cystine	4.3	1.4	9.8	2.9
Serine	25.5	35.1	46.0	6.3
Glutamine	79.4	119.4	174.9	44.9
Glycine	59.8	36.4	37.2	5.6
Tyrosine	14.8	20.0	31.0	3.3
Proline	36.9	41.0	50.2	14.5

ND: Not detected

[a:]Minimum amount of material (mg mL^{-1} assay medium) that produced agglutination.

Proximate and amino acid composition of feed ingredient and experimental diets

Proximate composition and amino acid profiles of feed ingredients and diets are shown in Tables 2 and 3. Diets contained about 38% crude protein and were isonitrogenous. Crude lipid and ash were in the range of 8.8–9.7% and 9.2–10.9% respectively. All experimental diets had almost similar amino acid composition (Table 3). In all the diets, contents of essential amino acids were essentially as per the requirement of the common carp (NRC 1993). Calculated digestible protein/digestible energy (DP/DE) ratios of the experimental diets were in the range from 19.1 to 20.7 g MJ^{-1} (Table 3). Optimum dietary DP/DE ratio studied in various commercially important protein resources ranged from 17 – 26 (g MJ^{-1}) (Page & Andrew 1973; Garling & Wilson 1976; Takeuchi *et al.* 1979; Anderson 1996; Einen & Roem 1997). A diet containing DP/DE in the range of 18 – 24 g MJ^{-1} was reported to promote optimum growth of common carp (Takeuchi *et al.* 1979). Our value falls within the range of the optimum for growth and feed utilization.

Table 3 Proximate, amino acid composition (g kg^{-1}) and digestible protein to digestible energy ratio (DP/DE, g MJ^{-1}) of the experimental diets

Treatment*	Control	J_{50}	J_{75}	S_{50}	S_{75}
Proximate (g kg^{-1})					
Dry matter	952	951	955	961	962
Crude protein	384	382	381	380	385
Crude lipid	92	94	97	88	91
Crude ash	109	105	103	96	92
Gross energy (KJ/g)	19.1	19.8	20.4	19.4	20.1
Essential amino acids (g kg^{-1})					
Arginine	24.53	27.75	31.99	22.57	24.14
Histidine	12.30	10.47	10.70	9.96	9.92
Iso leucine	15.85	13.97	14.56	13.62	14.00
Leucine	28.91	24.96	25.45	25.74	26.56
Lysine	28.43	20.44	19.96	20.29	19.83
Phenylalanine	15.15	15.67	16.85	15.53	16.61
Methionine	11.12	7.05	6.29	6.82	5.93
Threonine	15.99	12.37	12.21	12.52	12.42
Tryptophan	3.41	3.54	3.83	3.58	3.89
Valine	20.36	20.79	23.05	15.74	15.41
Non-essential amino acids (g kg^{-1})					
Alanine	30.09	18.91	16.92	19.51	17.79
Asparginine	42.05	32.77	33.02	38.89	42.13
Cystine	3.34	2.58	2.19	3.99	4.31
Glycine	17.72	15.79	16.18	16.82	17.69
Glutamine	55.18	62.15	64.36	68.83	74.26
Proline	41.56	24.04	20.41	23.31	19.27
Serine	10.29	8.94	9.13	10.35	11.22
Tyrosine	25.65	23.27	22.87	23.87	23.72
DP/DE (g MJ^{-1})	19.6	19.7	19.1	20.5	20.7

Amino acid compositions of the experimental diets were calculated from amino acid profile of individual feed ingredients.
*See footnotes to Table 1.

Fish behaviour, feed intake and growth

Sometimes the use of plant protein products in aquaculture diets is limited by the effects of the ingredients on the palatability of the diets to the fish (Burel *et al.* 1998). Based on the visual observation during feeding time, palatability or acceptability of feed was good and the behaviour of fish was normal. No left feed was observed in the aquaria. These findings are in contrast with those reported by others who observed a decline in feed intake of soy and lupin protein isolates fed at higher than 40% inclusion levels to common carp and rainbow trout (Escaffre *et al.* 1997; Glencross *et al.* 2004).

Weekly body mass gains of fish are given in Figure 1. These indicate that from third week onwards there was differential growth among the groups and lower body mass development was observed in J_{75} and control groups than other groups. This trend was maintained till the end of the experiment. Growth performance and nutrient utilization parameters are presented in Table 4. Highest BMG, SGR, MGR, PER, LPV were observed for S_{75} group, which were not statistically different from those for J_{50} and S_{50} groups and significantly higher ($P < 0.05$) than those for all other groups. Lowest PPV was observed in control, which was not statistically different from that for J_{75} group and significantly lower ($P > 0.05$) than those for all other groups. Lowest feed gain ratio was observed for S_{75} group, and this value significantly ($P < 0.05$) differed from those for all other groups. Significantly higher ($P < 0.05$) LPV was observed in plant protein fed groups than control group. Since overall growth performance and protein or energy utilisation of this group was similar to the FM fed group, the current study demonstrates that a high replacement level (up to 75%) of FM by a single plant-protein source such as DJPI is possible in common carp. Escaffre *et al.* (1997) have reported reduced growth in carp fed a diet containing SPI at levels $\geq$ 60%. On the other hand, many researchers (Glencross *et al.* 2005; Kaushik *et al.* 2004; Overland *et al.* 2009) have shown that soy, lupin and pea protein isolates can replace 50-75% of FM protein in trout and Atlantic salmon diets without impairing the growth performance and nutrient utilization. Despite the existence of some variability between fish species in the utilisation of plant products, results of most studies confirm that high dietary levels (>40% of total protein) of plant derived proteins depress growth and feed efficiency in carp (Hasan *et al.* 1997; Mazurkiewicz 2009). This poor growth performance commonly found in fish fed plant protein-rich diets, in most cases, was related to reduction in the voluntary feed intake (consequently a lower intake of essential

nutrients and digestible energy) (Gomes *et al.* 1995). However, in some studies conducted with rainbow trout, the total replacement of FM by soybean products (with or without supplemented L-methionine) was successfully achieved (Wilson 1992; Kaushik *et al.* 1995).

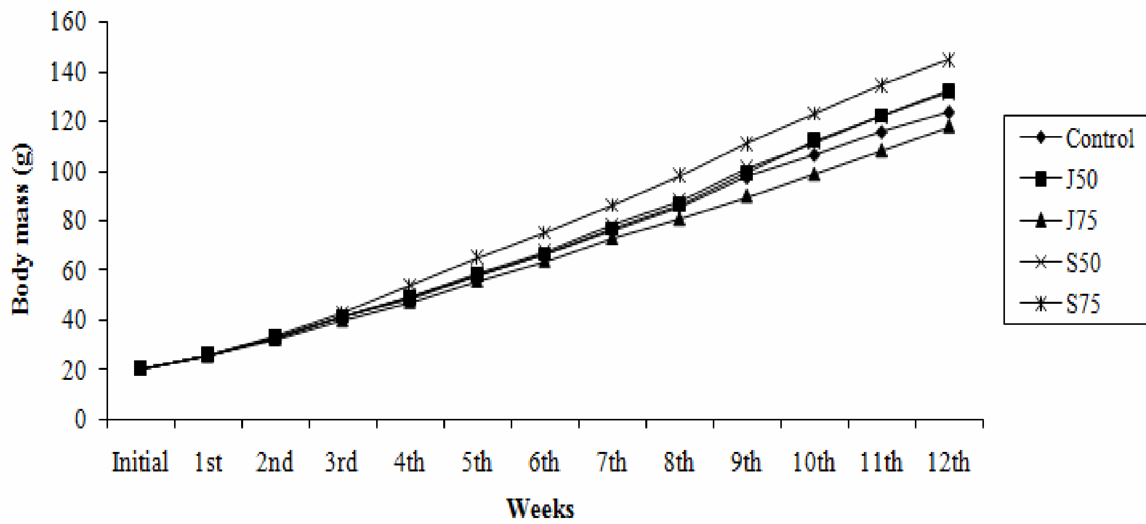


Figure 1 Body mass gain of common carp (*Cyprinus carpio* L.) fed experimental diets for 12 weeks

Table 4 Growth performance and nutrient utilisation of common carp (*Cyprinus carpio* L.) fed the experimental diets for 12 weeks

Treatment*	IW (g)	FW (g)	BMG	SGR	MGR	FGR	PER	PPV	LPV	EPV
Control	20.3 ± 0.12	124^{b} ± 9.0	510^{b} ± 42	2.15^{b} ± 0.08	13.2^{b} ± 0.50	1.36^{a} ± 0.06	1.91^{b} ± 0.11	30.4^{c} ± 2.84	35.3^{c} ± 7.78	22.1^{b} ± 1.06
J_{50}	20.3 ± 0.11	128^{ab} ± 5.7	533^{ab} ± 25	2.20^{ab} ± 0.05	13.5^{ab} ± 0.29	1.31^{a} ± 0.03	2.01^{ab} ± 0.05	34.2^{b} ± 1.37	48.0^{b} ± 8.15	23.8^{ab} ± 5.95
J_{75}	20.2 ± 0.08	118^{b} ± 13.5	481^{b} ± 65	2.09^{b} ± 0.14	12.8^{b} ± 0.83	1.39^{a} ± 0.10	1.86^{b} ± 0.14	33.7^{bc} ± 2.83	56.0^{a} ± 10.50	24.1^{ab} ± 2.19
S_{50}	20.4 ± 0.14	131^{ab} ± 7.0	546^{ab} ± 38	2.22^{ab} ± 0.07	13.6^{ab} ± 0.40	1.29^{a} ± 0.05	2.02^{ab} ± 0.05	36.4^{ab} ± 1.14	53.7^{ab} ± 4.21	27.1^{a} ± 1.65
S_{75}	20.4 ± 0.12	145^{a} ± 2.4	610^{a} ± 9	2.33^{a} ± 0.01	14.3^{a} ± 0.10	1.19^{b} ± 0.03	2.17^{a} ± 0.07	38.7^{a} ± 2.21	67.2^{a} ± 8.27	28.9^{ab} ± 1.91
SEM	0.04	3.04	14.47	0.03	0.18	0.02	0.03	1.01	3.42	0.94

* See footnotes to Table 1.
Values are mean (n = 3) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).
IW- Initial weight, FW - Final weight, BMG (%) - Body mass gain, SGR (%) – Specific growth rate and FGR – Feed gain ratio; MGR ($gkg^{0.8}$ day^{-1}) - Metabolic growth rate, PER - Protein efficiency ratio, PPV (%)- Protein productive value, LPV (%) - Lipid production value and EPV (%) - Energy production value.

Chemical composition of whole body of fish

Whole body chemical composition of fish was significantly affected by dietary treatments (Table 5). Moisture content exhibited inverse relationship with lipid and protein contents. Similar trend has been reported in a study by Hasan *et al.* (1997), wherein FM protein was replaced by plant protein such as mustard, sesame, linseed, copra and groundnut oil cakes. Significantly higher crude lipid deposition was observed in all plant protein fed groups compared to control group. As plant protein increased in the common carp diet, lipid retention in the whole body also increased. Higher lipid content in fish of plant protein fed groups led to higher value of LPV and EPV in these groups (Table 4). Similarly, Hasan *et al.* (1997) and Mazurkiewicz (2009) observed that FM protein replaced by plant protein in common carp diet exhibited higher lipid deposition. Makkar *et al.* (2009) observed higher LPV in common carp fed detoxified *Jatropha* kernel meal and soybean meal based diets compared to FM based diet. There is evidence that replacement of FM by plant protein sources such as corn gluten meal and soy protein concentrates increases hepatic lipogenic enzyme activities in seabass that leads to higher whole body lipid (Dias 1999; Kaushik *et al.* 2004). In the present study, higher value of HSI in plant protein fed groups suggests higher lipid deposition in liver. Hepatosomatic index values of about 1, as observed here, are common in common carp (Yılmaz & Genc 2006; Makkar *et al.* 2009).

Crude protein of whole body was higher in plant protein fed groups than control group (Table 5), which concurs with the higher value of PPV in plant protein groups (Table 4). Efficient protein synthesis requires sufficient availability of all essential amino acids. Unbalanced amino acid concentrations in a diet or different solubility of individual amino acids results in increased protein degradation, and thereby increased protein turnover. Cheng *et al.* (2003) reported that the plant protein (soy protein) based diets decrease nitrogen retention in salmon and trout because these diets have less digestible energy and an amino acid profile that is sub optimal for muscle growth. Interestingly in our study crude protein content in whole body was higher in DJPI and SPI fed groups. In accordance with this, other researchers (Hasan *et al.* 1997; Mazurkiewicz 2009) also found that the body protein content increased significantly when plant protein replaced FM in common carp diet. Almost similar amino acid contents in DJPI, SPI and FM (Table 3) along with supplementation of lysine in the diets (J_{50} and J_{75} diets) might have resulted in the increased protein accretion in test groups (J_{50}, $J_{75,}$ S_{50} and S_{75}) compared to control group. Jahan

et al. (2003) have shown that proper combination of FM and plant proteins increases protein retention in carp (Jahan *et al.* 2003). These observations suggest that DJPI and SPI containing diets contained optimum digestible energy and a balanced amino acid profile for optimum growth of common carp.

Table 5 Chemical composition of whole body of common carp (*Cyprinus carpio* L.) of different experimental groups at the start and at the end of the experiment (% wet basis ± SD)

Treatment*	Moisture	Crude protein	Crude lipid	Ash	Gross energy (kJ/g)
Initial fish	78.3 ± 1.54	14.1 ± 0.11	4.2 ± 0.22	3.3 ± 0.13	4.3 ± 0.14
Control	76.4^{a} ± 1.21	15.6^{b} ± 0.68	5.3^{b} ± 1.15	2.7^{a} ± 0.31	5.4^{b} ± 0.26
J_{50}	74.7^{b} ± 1.15	16.5^{ab} ± 0.5 1	6.6^{ab} ± 1.50	2.8^{a} ± 0.35	5.9^{a} ± 0.29
J_{75}	73.7^{b} ± 0.58	17.0^{a} ± 0.31	7.1^{a} ± 0.96	2.7^{a} ± 0.36	6.4^{a} ± 0.45
S_{50}	74.7^{b} ± 0.58	16.6^{a} ± 0.41	6.7^{ab} ± 0.54	2.7^{a} ± 0.21	6.4^{a} ± 0.21
S_{75}	74.3^{b} ± 0.71	17.0^{a} ± 0.71	7.3^{a} ± 0.96	2.5^{a} ± 0.26	6.6^{a} ± 0.47
SEM	0.42	0.21	0.34	0.07	0.17

* See footnotes to Table 1

Values are mean (n = 3) ± standard deviation.

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Nutrient digestibility and digestible nutrients and energy retained

Highest ADCs of protein and energy were observed for the S_{75} group, which were not statistically different to those for J_{50} group and significantly higher ($P < 0.05$) than for all other groups; whereas, ADCs of dry matter and lipid did not differ significantly among the five groups (Table 6). Apparent digestibility coefficients of oil seed meal proteins and plant protein isolates are 80-95% and 90-98% respectively for tilapia, trout, Atlantic salmon and common carp (Jauncey & Ross 1982; Glencross *et al.* 2005; Makkar *et al.* 2009). Common carp is reported to be able to digest the plant proteins well, generally slightly better than carnivorous fish species (NRC 1983). The protein digestibility coefficient is a key factor in the evaluation of the quality of a diet and in particular in determination of its potential for the synthesis of new tissues. Detoxified *Jatropha* protein isolate and SPI, in combination with FM protein, showed excellent dry matter, crude

protein, lipid and energy digestibilities in the present study (Table 6), indicating excellent utilization of feed ingredients. Makkar *et al.* (2009) reported that apparent protein digestibility values ranged between 80–92% for carp fed plant protein (detoxified *Jatropha* kernel meal and soybean meal) based diets. In the present study, when compared to FM proteins, *Jatropha* and soy protein isolates have higher apparent protein digestibility (at 50% replacement of FM protein), which could be attributed to the absence of trypsin inhibitor and lectin, presence of low levels of NSPs, and addition of phytase to mitigate the effects of phytate, if any. In another study Kaushik *et al.* (1995), have used a SPI (containing protease inhibitors <3 mg/g) as a FM replacer in trout diet and found ADCs of protein of SPI fed groups was similar to FM fed group. However, digestibility of protein from corn gluten meal was significantly lower than that from FM in carp and rainbow trout (Escaffre *et al.* 1997; Gomes *et al.* 1995). Energy digestibility of DJPI and SPI protein based diets was considerably lower than protein digestibility. Our results are in concurrence with those obtained in studies by Gouveia *et al.* (1993) and Makkar *et al.* (2009) in trout and common carp respectively wherein they observed considerably lower energy digestibility of plant protein based diets than protein digestibility.

Digestible protein, lipid and energy retained were in the range of 34-41%, 45-84% and 25-31% respectively (Table 6). Digestible energy retained of diets did not differ significantly among the five groups. Plant protein fed groups exhibited higher retention of digestible protein and lipid than control group, indicating that common carp has utilized DJPI and SPI better than FM and retained more efficient than control group.

Table 6 Apparent digestibility coefficient of the dry matter, nutrients and energy of diets, and efficiency of digestible nutrients and energy of diets in common carp (*Cyprinus carpio* L.)

Treatment*	Dry matter digestibility	Protein digestibility	Lipid digestibility	Energy digestibility	Digestible protein retained (%)	Digestible lipid retained (%)	Digestible energy retained (%)
Control	$82^{b} \pm 1.53$	$90^{b} \pm 1.03$	$94^{a} \pm 1.58$	$88^{b} \pm 1.28$	$33.6^{b} \pm 3.09$	$44.8^{b} \pm 11.97$	$25.1^{a} \pm 1.45$
J_{50}	$86^{a} \pm 2.54$	$93^{a} \pm 1.57$	$95^{a} \pm 1.67$	$91^{a} \pm 1.64$	$36.7^{ab} \pm 1.64$	$61.5^{ab} \pm 16.35$	$26.2^{a} \pm 6.74$
J_{75}	$83^{b} \pm 3.21$	$89^{b} \pm 2.01$	$94^{a} \pm 2.53$	$89^{b} \pm 2.08$	$37.2^{ab} \pm 3.58$	$75.8^{a} \pm 15.78$	$27.1^{a} \pm 2.32$
S_{50}	$83^{b} \pm 3.21$	$91^{b} \pm 1.94$	$94^{a} \pm 2.52$	$88^{b} \pm 2.08$	$39.6^{a} \pm 1.21$	$68.2^{ab} \pm 7.19$	$31.0^{a} \pm 2.17$
S_{75}	$88^{a} \pm 2.08$	$94^{a} \pm 1.59$	$96^{a} \pm 1.15$	$92^{a} \pm 2.67$	$41.0^{a} \pm 2.54$	$83.7^{a} \pm 11.01$	$31.4^{a} \pm 1.90$
SEM	0.82	0.53	0.43	0.58	0.89	4.54	1.03

* See footnotes to Table 1.

Values are mean (n = 3) ± standard deviation.

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Principal in defining the nutritional value of a particular ingredient is the examination of its influence on the digestive and absorptive processes of the animal and it can be addressed through digestibility studies (Cho & Slinger 1979). Lower apparent digestibility coefficient of protein was observed for DJPI than for SPI (at 75% replacement level); whereas, apparent digestibility coefficient of protein was similar to that for SPI (at 50% replacement level) in common carp (Table 7).

Table 7 Fractional protein digestibility of feed ingredients

Ingredients	Replacement of fish meal protein (%)	Protein digestibility (%)
Jatropha protein isolate	50%	$98^{a} \pm 2.04$
	75%	$88^{b} \pm 1.01$
Soy protein isolate	50%	$93^{ab} \pm 6.95$
	75%	$98^{a} \pm 2.38$
SEM		1.59

Values are mean (n = 3) ± standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Digestive enzyme activities and intestinal somatic index

Dietary inclusion of DJPI and SPI did not alter the digestive enzymes (amylase, protease and lipase) activities (Table 8). Phytate and trypsin inhibitor inhibit activities of digestive enzymes such as trypsin, pepsin and alpha-amylase (Alarcon *et al.* 1999), and phytate forms complexes with minerals (Sugiura *et al.* 1999) and proteins (Moyano *et al.* 1999); thereby modifying digestion processes and thus impairing intestinal absorption of nutrients. Heat labile antinutrients (trypsin inhibitors and lectins) were not detected in the DJPI and SPI; whereas, heat stable enzyme inhibitor (phytate) was present in DJPI and SPI. Phytate content in DJPI and SPI were 2.9% and 0.95% respectively. For plant based feeds, we used 500 FTU phytase per kg feed, which might be sufficient to hydrolyse the phytate content in DJPI and SPI. No difference in activities of digestive enzymes could be due to the absence of trypsin inhibitors and lectins and addition of phytase in the plant

protein based diets. Our results are in contrast with those of Escaffre *et al.* (1997), who observed that increasing levels of dietary soy protein concentrate induced a significant decline in intestinal trypsin activity in common carp. In our previous study, common carp fed with detoxified *Jatropha* kernel meal and soybean meal exhibiting lower protease, amylase and lipase activities (Makkar *et al.* 2009). In that study detoxified *Jatropha* kernel meal and soybean meal contained 9.3% and 2.5% phytate respectively, which is almost 4 to 5 times higher than in DJPI and SPI. High content of phytate could inhibit the digestive enzyme activities by forming complexes with minerals and proteins during digestion processes.

It is known that carnivorous and omnivorous fish require longer time to digest plant protein based diets (Buddington *et al.* 1997; Makkar *et al.* 2009). Direct relationship between the amount of dietary plant protein and ISI has been reported earlier in fish (Makkar *et al.* 2009). In carp, higher intestinal somatic index was observed in fish fed DJPI and SPI based diets compared with FM fed diet (Table 8). Similarly, intestinal somatic index was higher in the plant protein fed groups than in control group in the common carp and trout (Santigoga *et al.* 2008; Makkar *et al.* 2009). From a physiological view point, a longer intestinal somatic index would facilitate an increase in digestibility and retention time by enhancing contact time of the digestive enzymes and the feed components, resulting in increase in their digestion and absorption. Omnivorous fish like common carp appear to possess compensation mechanism, such as an increase in intestinal index and as a result increase in digestive activity, to achieve a digestive balance and growth rates similar to those observed for FM fed group.

Table 8 Activities of digestive enzymes (U/mg protein), hepato somatic index (HSI) and intestinal somatic index (ISI) of fish and digestible protein to digestible energy (g digestible protein/MJ digestible energy) ratio of different experimental groups

Treatment*	Amylase	Protease	Lipase	HSI	ISI
Control	$20.1^{a} \pm 3.36$	$40.0^{a} \pm 2.82$	$8.5^{a} \pm 0.85$	$0.87^{b} \pm 0.15$	$1.48^{b} \pm 0.18$
J_{50}	$18.6^{a} \pm 5.81$	$37.1^{a} \pm 3.91$	$8.4^{a} \pm 0.46$	$0.99^{a} \pm 0.17$	$1.66^{a} \pm 0.24$
J_{75}	$18.4^{a} \pm 4.40$	$32.7^{a} \pm 3.04$	$7.8^{a} \pm 1.39$	$1.12^{a} \pm 0.20$	$1.63^{a} \pm 0.10$
S_{50}	$19.0^{a} \pm 4.59$	$37.2^{a} \pm 5.56$	$7.9^{a} \pm 0.93$	$1.04^{a} \pm 0.04$	$1.58^{a} \pm 0.11$
S_{75}	$18.1^{a} \pm 5.44$	$38.1^{a} \pm 3.39$	$7.7^{a} \pm 1.37$	$1.04^{a} \pm 0.05$	$1.62^{a} \pm 0.15$
SEM	1.15	1.02	0.25	0.04	0.06

* See footnotes to Table 1

Values are mean (n = 3) ± standard deviation.

Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Conclusions

The results of the current study showed that the detoxified *Jatropha curcas* protein isolate (DJPI) fed groups exhibited good growth performance (almost seven times increase in fish body mass after 12 weeks). Detoxified *Jatropha curcas* protein isolate show potential for use in aquaculture diets. It can be used as one of the promising fish meal replacers in the diets of common carp. It can replace up to 75% of fish meal protein without sacrificing fish yield.

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3.1.2.2. Comparative nutritional evaluation of *Jatropha curcas* protein isolate and soy protein isolate in common carp (*cyprinus carpio* l.) fingerlings (in this study protein isolate prepared by 'One-Step Method' was used)

Introduction

Aquaculture is growing rapidly at an average rate of 8.9% per year, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems (FAO 2007). This rapid growth is because of the intensification of the culture systems. This activity requires feeds with high levels of protein, which taxes finite global sources of fish meal (FM) supplies. Therefore, alternative feed ingredients are required to provide the essential nutrients for the growth and quality of aquaculture production. *Jatropha curcas* (L.) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. Its seeds are rich in oil and protein. *Jatropha* seed cake (JSC), obtained after oil extraction using mechanical presses, is an excellent source of protein. However, presence of shells containing approximately 45% lignin, toxic and antinutritional constituents restricts its use in fish feed. *Jatropha* protein isolate (JPI) was prepared from JSC and was detoxified by a novel method which isolates and detoxifies it in one step (patent application filed).

Materials and methods

Using *Cyprinus carpio* L. fingerlings an eight week experiment was conducted to evaluate the nutritional quality of the detoxified *Jatropha* protein isolate (DJPI) and to compare it with that of soya protein isolate (SPI). The protein contents of DJPI and SPI were 85% and 92% respectively. Fingerlings (75; av. wt. 11.4 ± 0.25g) were randomly distributed in five groups with three replicates and fed iso-nitrogenous diets (crude protein 38%): C_{ontrol} (fish meal (FM) based protein), J_{50} and J_{75} (50% and 75% of FM protein replaced by DJPI), and S_{50} and S_{75} (50% and 75% of FM protein replaced by SPI).

Results and discussion

Highest body mass gain (BMG, %), specific growth rate (SGR, %.day^{-1}) and metabolic growth rate (MGR, g.kg$^{0.8}$.day^{-1}) were observed for the S_{50} group which were not statistically different from that for S_{75} and J_{50} groups but were significantly ($P < 0.05$) higher than for all other groups (Table 1). Lowest feed conversion ratio (FCR) was observed in S_{50} group which was not statistically different from that for S_{75} group but was significantly lower ($P > 0.05$) than for all other groups; whereas, protein efficiency ratio (PER) exhibited opposite trend (Table 1).

Table 1 Growth performance and nutrient utilization

Treatment	BMG (%)	SGR (%.day^{-1})	MGR (g.kg$^{0.8}$.day^{-1})	FCR	PER
C_{ontrol}	$370^{bc} \pm 27$	$2.76^{bc} \pm 0.10$	$12.2^{bc} \pm 0.47$	$1.18^{ab} \pm 0.04$	$2.16^{b} \pm 0.07$
J_{50}	$401^{ab} \pm 25$	$2.86^{ab} \pm 0.09$	$12.6^{ab} \pm 0.37$	$1.18^{ab} \pm 0.10$	$2.18^{b} \pm 0.18$
J_{75}	$338^{c} \pm 31$	$2.64^{c} \pm 0.12$	$11.5^{c} \pm 0.50$	$1.27^{a} \pm 0.02$	$2.00^{b} \pm 0.04$
S_{50}	$451^{a} \pm 45$	$3.04^{a} \pm 0.15$	$13.4^{a} \pm 0.64$	$1.07^{c} \pm 0.02$	$2.46^{a} \pm 0.04$
S_{75}	$421^{ab} \pm 24$	$2.95^{ab} \pm 0.08$	$12.9^{ab} \pm 0.30$	$1.12^{bc} \pm 0.04$	$2.36^{a} \pm 0.09$
SEM	12.52	0.05	0.20	0.02	0.05

Values are mean ± standard deviation (n = 3).
Mean values in the same column with different superscripts differ significantly ($P < 0.05$).

Overall growth performance and nutrient utilizations of DJPI fed groups was similar to the FM fed group which implies that DJPI in combination with FM protein induces excellent nutrient utilization and DJPI can be used as protein source in common carp. Escaffre et al. (1997) have reported reduced growth in carp fed a diet containing SPI at levels $\geq$ 60%. On the other hand, Glencross et al. (2005) observed that lupin protein isolate can replace 50-75% of FM protein in trout and Atlantic salmon diets without impairing the growth performance and nutrient utilization.

White blood cells (1.29-1.31x10^{5}cells.mm^{-3}), hemoglobin (Hb, 7.5-9.4g.dl^{-1}), mean cell volume (390-438fL), mean corpuscular hemoglobin (56-73pg); and alkaline phosphatase

(ALP) and alanine transaminase (ALT) activities and glucose level in blood did not differ significantly among the five groups (Table 2). ALP and ALT are released into blood during organ damage (Racicot et al., 1975). Similar levels of ALP and ALT in all the groups indicate normal organ function on feeding of DJPI. Lowest red blood cell (RBC), hematocrit (Hct) level (44-59%), concentration of potassium (1.2-2.0mmol/l) ion in blood, relative intestinal length (1.18-1.55), hepatosomatic index (0.86-1.23) and intestinal somatic index (2.30-3.02) were observed in C_{ontrol} group and these were statistically lower ($P > 0.05$) than those in all other groups; whereas, creatinine (0.2-0.5 mg/dl), total bilirubin (0.2-0.3mg.dl^{-1}) and urea nitrogen (2.7-4.7mg.dl^{-1}) concentration in blood exhibited opposite trend. Highest globulin concentration (0.6-1.0 mg/dl) in blood was observed in S_{75} group which is statistically not different from that in J_{75} group but was higher than those in other groups. Lowest albumin concentration (1.8-2.5g/dl) was observed in S_{75} group which was similar to J_{75} group but was lower ($P > 0.05$) than those in other groups. Blood ions such as sodium (131-134mmol.l^{-1}), calcium (10.6-11.5mg.dl^{-1}) and phosphorus (14.3-16.5mg.dl^{-1}) concentrations in blood did not differ significantly among the five groups. Hematological and physiological parameters were within the normal ranges, suggesting no clinical toxicity on feeding DJPI.

Table 2 Blood chemistry and metabolic enzymes

Treatment	RBC (10^6 cells.ml^{-1})	Hb (g.dl^{-1})	Hct (%)	ALP (U.l^{-1})	ALT (U.l^{-1})	Glucose (mg.dl^{-1})
C_{ontrol}	$1.1^e \pm 0.02$	$8.1^a \pm 0.7$	$44^b \pm 4.2$	$65.7^a \pm 13$	$74^a \pm 17$	$67^a \pm 8$
J_{50}	$1.3^c \pm 0.03$	$9.4^a \pm 0.5$	$52^a \pm 6.1$	$62.7^a \pm 4$	$79^a \pm 12$	$61^a \pm 12$
J_{75}	$1.4^a \pm 0.01$	$8.4^a \pm 3.0$	$59^a \pm 3.0$	$68.3^a \pm 7$	$68^a \pm 10$	$87^a \pm 25$
S_{50}	$1.2^d \pm 0.02$	$8.5^a \pm 0.5$	$53^a \pm 2.1$	$59.3^a \pm 10$	$70^a \pm 11$	$61^a \pm 7$
S_{75}	$1.4^b \pm 0.03$	$7.5^a \pm 0.9$	$59^a \pm 7.2$	$67.3^a \pm 16$	$65^a \pm 13$	$68^a \pm 11$
SEM	0.03	0.36	2.2	2.52	3.11	3.99

Values are mean ± standard deviation (n = 3).
Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Conclusions

Common carp can efficiently use detoxified *Jatropha* protein isolate (DJPI) as a source of protein. DJPI could replace 75% FM protein in common carp diets, without sacrificing growth and nutrient utilization; and without affecting physiological and haematological parameters. The results of this study enlarge the portfolio of plant protein sources that can be used in fish diets, and open a new market opportunity for use of a new feed resource in the feed industry.

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3.1.2.3 Detoxification of protein concentrate using adsorbents (main responsible institute: Germany 1)

Introduction

In almost all countries, oil is produced from the whole seeds using a mechanical press. The seed cake left as a by-product after extracting oil using a mechanical press could contain 50 to 60 % shells which are largely indigestible. The presence of high levels of shells in the seed cake is one of the constraints in its utilization in animal diets. Therefore, at present it is used as a fertilizer. Other constraints in the utilization of seed cake or its fractions as livestock feed are the presence of toxic phorbol esters and antinutritional factors such as trypsin inhibitor, lectin and phytate.
In our previous study [see section or publication: Makkar et al. (2008)], protein isolate was prepared from the seed cake by dissolving proteins present in the cake at pH 11 and separating the shells by filtration or centrifugation; and then precipitating the solubilised proteins at iso-electric pH. The recovery of the protein isolate on dry matter basis was 17% and its protein content 80%. However, this preparation contained high amounts of phorbol esters, trypsin nhibitor, lectin and phytate. The hypothesis of this study was that the treatment of the dissolved proteins at alkaline conditions with various adsorbents before precipitation of protein at iso-electric pH would make the protein isolate free of phorbol esters.

Materials and Methods

Materials

Various adsorbents used (listed in Table 1) were procured from SuedChemie. Other chemicals were the same as reported in Makkar et al. (2008).

Preparation of solubilized protein solutions

Solubilization of proteins from the seed cake was done at pH 11, as reported in section or in Makkar et al. (2008). The solubilized proteins fractions were divided into two fractions: one fraction at pH 11 was kept as it is; and the pH of the second fraction was

brought down to pH 8 (at this pH the proteins were still in the solution and did not precipitate down).

Treatment with adsorbents

The solublized protein fractions at pH 11 and pH 8 were separately treated with the adsorbents. The levels of adsorbents used were 1 % and 3% (both w/v basis) and the contact time was 30 min. Thereafter, the adsorbents were removed by centrifugation (7000 *g* for 10 min). The remaining solution containing solubilized proteins were brought down to pH 4, kept in cold for overnight and then centrifuges to obtain the protein isolates. The crude protein and phorbol esters (PEs) were determined as described in Makkar et al. (2008).

Results and Discussion

Table 1 presents PE levels in the protein isolate and their reduction in protein isolates prepared on using 3% adsorbents. Initially the experiments were conducted using 1% adsorbents and since no decrease in PE levels in the protein isolates was obtained (results not show), we increased the level to 3 %.

As is evident from these results, only the adsorbents EX M 1694 and EX 0096 were most effective, but they could reduce the PE levels by only 11% at pH 11 treatment. The treatment of adsorbents at pH to 8 did not bring any additional benefit. Infact, at pH 8, the adsorbents were less effective. It may be noted that a substantial amount of protein was taken away by the adsorbents (Table 2).

Table 1 Phorbol esters (PE) levels in the protein isolate and percent reduction of PEs in adsorbents on using adsorbents

3 % Adsorbent	mg PE/g protein isolate		Reduction at pH 11 (%)	Reduction at pH 8 (%)
	pH 11	pH 8		
No adsorbent	5.24, 5.09, 5.24, 5.12 **(5.17)**	5.02, 5.16, 5.40, 4.84 **(5.10)**		
EX M 1694	4.25, 4.77, 4.57, 4.75 **(4.59)**	6.96, 5.83, 6.44 **(6.41)**	**11.3**	**-25.7**
EX M 1833	5.50, 5.88, 5.37, 5.21 **(5.49)**	4.57, 4.61, 4.68 **(4.62)**	**-6.2**	**9.4**
Tonsil EX 1221 (I)	5.26, 5.37, 5.05, 5.14 **(5.21)**	6.43, 6.06, 6.14 **(6.21)**	**-0.7**	**-21.7**
No adsorbent	5.19, 5.41, 5.34, 5.12 **(5.26)**	5.24, 4.99, 5.35, 4.88 **(5.12)**		
EX 0096	4.73, 4.52, 4.63, 4.86 **(4.69)**	5.06, 5.24 **(5.15)**	**11.0**	**-0.7**
Kieselgel 60	4.83, 4.84, 4.80 **(4.82)**	4.97, 4.78, 5.09, 4.94 **(4.94)**	**8.4**	**3.4**
Aluminiumoxid neu	5.01, 4.97, 4.94**(4.97)**	5.04, 5.33 **(5.19)**	**5.5**	**-1.4**

Table 2 Distribution of protein in different fractions obtained during protein isolate preparation

3 % Adsorbent	pH 11			pH 8		
	CP in pellet	CP in supernatant	CP in adsorbent	CP in pellet	CP in supernatant	CP in adsorbent
No adsorbent	83.0, 83.6 **(83.3)**	16.4, 16.1 **(16.2)**		83.7, 83.6 **(83.7)**	16.7, 16.4 **(16.6)**	
EX M 1694	69.4, 70.8 **(70.1)**	9.1, 9.3 **(9.2)**	**20.2**	54.7, 56.3 **(55.5)**	6.9, 6.8 **(6.8)**	**38.0**
EX M 1833	56.4, 60.8 **(58.6)**	11.6, 11.2 **(11.4)**	**29.5**	63.5, 63.3 **(63.4)**	5.5, 5.4 **(5.4)**	**31.5**
Tonsil EX 1221 (I)	68.7, 68.2 **(68.4)**	12.8, 12.5 **(12.7)**	**18.4**	57.2, 56.0 **(56.6)**	10.3, 9.6 **(9.9)**	**33.7**
No adsorbens	83.5, 84.5 **(84.0)**	17.8, 18.5 **(18.1)**		79.7, 80.6 **(80.1)**	18.3, 18.0 **(18.1)**	
EX 0096	73.8, 73.7 **(73.8)**	11.8, 12.4 **(12.1)**	**16.2**	70.7, 71.6 **(71.2)**	8.1, 7.9 **(8.0)**	**19.1**
Kieselgel 60	80.7, 80.8 **(80.7)**	13.9, 14.1 **(14.0)**	**7.4**	75.9, 77.1 **(75.9)**	11.4, 12.6 **(11.4)**	**10.9**
Aluminium oxid Neu	80.2, 81.3 **(80.8)**	16.8, 17.1 **(17.0)**	**4.4**	80.3, 80.1 **(80.2)**	15.0, 15.9 **(15.5)**	**2.7**

Conclusions

None of the adsorbents could make the isolates free of PEs, an essential requirement for using the protein isolates for feeding purposes. It was concluded that the use of adsorbents do not have any potential in making protein isolates free of the main toxin, i.e., phorbol esters.

3.1.3 Challenges and opportunities for using byproducts from the production of biodiesel from *Jatropha* oil as livestock feed (main responsible institute: Germany 1)

Introduction

The genus *Jatropha* is extremely old and may have already existed 70 million years ago on the ancient continent "Gondwanaland" before it split up to form the individual continents. It is considered to be the most primitive member of the large genus Euphorbiaceae. The genus *Jatropha* consists of between 165–175 species. *Jatropha curcas* is a shrub or a small to medium sized tree. It is native to almost all tropical regions. There are two genotypes of *J. curcas*, toxic and non-toxic. Non-toxic genotype is available only in Mexico.

Jatropha curcas is used to prevent or control erosion, and even reclaim eroded lands in many countries. Ambitious plans have been drawn up in many countries in south east Asia, Africa and Latin America to plant *J. curcas* since it can survive under severe conditions of drought and severe dry seasons. In addition, it is a multipurpose plant and seeds yield high quantity (30 to 38% oil in seeds) and quality oil that is easily converted into biodiesel by the conventional, proven processes. The oil of the toxic *J. curcas* is not edible because of its toxin phorbol ester. Large plantations of *J. curcas* have already taken place in India, Myanmar, Indonesia and China with the prime aim to produce biodiesel from the seed oil. This has resulted in increasing quantity of byproducts that could be used as livestock feed. This paper presents challenges and opportunities for using these byproducts in rations for all farm animals including fish.

Challenges

There are five main byprodcts from the *Jatropha* biodiesel production chain that could be used as livestock feed: **kernel meal (defatted shell-free kernel) or seed cake (screw-pressed whole seeds)**, **acid gum** and **fatty acid distillate** produced during the degumming and deodorisation processes respectively before the transesterification process and **glycerol** produced during the transesterification process in the diesel production.

Antinutritional factors in kernel meal

Trypsin inhibitor and lectin activities are high in the kernel meal and these activities are similar to that in raw soya bean meal (Makkar *et al.,* 1998). Trypsin inhibitor could decrease protein digestibility and lectins could cause toxicity. However, lectin activity of both the toxic and non-toxic meals, as determined by haemagglutintation assay, was almost similar. Curcin is considered to be a lectin and the similar haemagglutination of toxic and non-toxic genotypes suggest that curcin is not the principle toxin present in *Jatropha* seeds. Phytate content is also very high (ca 9%). Phytate is known to decrease absorption of minerals, particularly calcium, zinc and iron. It may also be noted that the levels of trypsin inhibitor and phyate in the kernel meals from both the toxic and non-toxic genotypes *J. curcas* are almost similar.

Tannins, cyanogens, glucosinolates and amylase inhibitors have not been detected in the *Jatropha* meals (Makkar and Becker, 1998). Similar levels of saponins were observed in kernel meals from both toxic and non-toxic genotypes (2.6 to 3.4%), and these saponins did not possess haemolytic activity. The level of non-starch polysaccharides in *Jatropha* meal is similar to that in soya bean meal and lower than other conventional protein rich feed resources. They do not appear to elicit adverse effects. In our studies on common carp (*Cyprinus carpio*) and Nile tilapia at 75% replacement of fishmeal protein in the diet by heat treated meal from the non-toxic genotype of *Jatropha*, the growth of both these species of fish was as good as for the fish fed 100% fishmeal protein (our unpublished observations).

To mitigate adverse effects of phytate, the addition of phytase enzyme should be considered for feeds containing kernel meal from the non-toxic *Jatropha* genotype. This would also spare the supplement of phosphorus to the diets and decrease phosphorus release into the water channel, thereby decreasing environmental pollution.

Main toxic principle in kernel meal

Phorbol esters are present in high concentration in the kernel meal from the toxic genotype (2.8 mg/g) but absent in kernel meal from the non-toxic genotype. Phorbol esters, diterpenes of phorbol type cause severe toxic symptoms in livestock. At least six phorbol esters are present in *Jatropha* seeds (Haas *et al.*, 2002). The phorbol esters are

reported to mimic the action of diacyl glycerol, activator of protein kinase C, which regulates different signal transduction pathways. Interference with the activity of protein kinase C affects a number of processes including, phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression. They are also co-carcinogens and have purgative and skin-irritant activities. In humans accidental poisoning by *Jatropha* seeds has been reported to elicit giddiness, vomiting and diarrhea. Mortality has also been reported in a number of animal species viz. mice, chicks and goats (Makkar and Becker, 1998; Goel *et al*., 2007) when force fed to these species.

From the above results, it is evident that the main toxic principle present in *Jatropha* seed meal is phorbol esters. Trypsin inhibitor, lectin and phytate might aggravate adverse effects, but are not responsible for acute toxicity. We have isolated pure phorbol esters from *Jatropha* oil and mixed them in standard fish diet and reproduced toxic symptoms observed on feeding *Jatropha* kernel meal (Makkar and Becker, 1998).

Seed shell as an indigestible material in seed cake

In small scale oil extraction units, the oil is produced from whole seeds using a screw press. The seed cake left as a by-product after oil extraction by screw press can contain as much as 500 g/kg of shells as the indigestible material. Therefore there is a need to separate high quality protein from the shells.

Phorbol esters in gums, fatty acid distillate and glycerol produced during biodiesel production

By-products of the vegetable oil pre-treatment and biodiesel production process, such as gums, fatty acid distillate, and glycerine have several applications in food and feed industry and the presence of phorbol esters could render them unfit for edible purposes. In one of our studies we follow the flow of phorbol esters during various stages of pre-treatment and biodiesel production from *Jatropha* oil (Makkar *et al*., 2008b). During the degumming step a fraction called acid gums is produced. Phorbol esters were present in the acid gums. This implies that the use of these acid gums in animal feed is not possible. On the other hand phorbol esters were not detected in the fatty acid distillate

produced during the stripping or deodorisation process. However, the presence of possibly toxic phorbol ester degradation products in this fraction could not be ruled out (Makkar *et al.*, 2008). At present no information is available on the nature or toxicity of the possible degradation products.

Phorbol esters were not detected in the glycerine samples in our study ((Makkar *et al.*, 2008b), however phorbol esters were detected in glycerine samples obtained from other industrial plants (Company 1: 0.67–0.97 mg/g; Company 2: 0.13 mg/g). These results suggest that different oil pre-treatment conditions could affect the presence of phorbol esters in glycerine produced from toxic *Jatropha* oil. The presence of phorbol esters in these fractions could render them unsuitable as a feed ingredient.

Opportunities

Kernel meal

One ton of toxic seeds yields approximately 615 kg of kernels with a potential oil recovery of approximately 351 kg using solvent extraction. The crude protein content in the kernel meal is approximately 60%. The levels of all essential amino acids except lysine are comparable with the FAO reference protein for a growing child of 2 to 5 years of age. A comparison between the amino acid composition of *Jatropha* meal and soya beans (Vasconceles *et al.*, 1997) revealed an almost similar pattern for all essential amino acids, except lysine and sulphur amino acids; lysine is lower and and sulphur amino acids higher in the *Jatropha* meals. The levels of essential amino acids, in the *Jatropha* meals are higher than or similar to those of castor bean meal. The non-protein nitrogen in *Jatropha* meal formed only 9.0% of the total nitrogen in the *Jatropha* meals suggesting the presence of high levels (91%) of true protein (Makkar *et al.*, 1998).

Digestibility and metabolizable energy of heat treated (121° C, 66% moisture, 30 min) kernel meal, using the *in vitro* gas method (Menke *et al.*, 1979) were lower compared to those for soya bean meal (78 vs 88%) and (11 vs 13 MJ/kg) respectively. Digestibility of the *Jatropha* kernel meal protein determined by treatment with pepsin followed by trypsin was similar to that of toasted soya bean meal (90%), whereas *in vitro* rumen digestibility of nitrogen was lower by approximately 50% (Makkar *et al.*, 1998), suggesting that *Jatropha* kernel meal has a high level of rumen undegradable protein which is available postruminally.

For effective utilization of kernel meal from the non-toxic *J. curcas*, it is imperative to heat treat the meal to inactivate trypsin inhibitor and lectins and to add phytase in the diet to mitigate the adverse effects of phytate in monogastric animals. The high protein efficiency in rats and the rapid growth observed in fish fed heat treated non-toxic *Jatropha* meal (Makkar and Becker, 1999) suggested that the protein quality of *Jatropha* kernel meal is very high. Since the levels of crude protein, essential amino acids and of other constituents and the in vitro parameters that give information on the extent of nutrient availability given in the above are almost similar for the kernel meals obtained from the toxic and non-toxic *J. curcas*, it is reasonable to conclude that the *Jatropha* kernel meal from the toxic genotype would be an excellent protein sources once it has been detoxified.

Recently we have developed a process for detoxification of the kernel meal from the toxic *J. curcas* and the feeding results with carp have been conducted. No histopathological lesions in the organs, and no changes in enzyme activities and normal blood parameters were observed. The performance of group in which 75% of fish meal protein was replaced by detoxified *Jatropha* kernel meal was comparable to the group in which 75% of fish meal protein was replaced by soya bean meal group but was lower than the fishmeal group; whereas, performance of the group in which 50% fish meal protein was replaced by the detoxified *Jatropha* kernel meal was better than that of 50% and 75% soya bean meal fed groups and similar to that of the fishmeal group (Kumar *et al.*, 2008).

Seed cake

The seed cake left as a by-product after oil extraction by screw press has approximately 22% crude protein and a high content of indigestible shells (up to 50%). Using the principle of iso-electric precipitation, the protein concentrate prepared from the screw pressed cake obtained from the toxic genotype contained a substantial amount of phorbol esters (0.86–1.48 mg/g), trypsin inhibitor, lectins and phytate The amino acid composition of the protein concentrate mirrored that of the kernel meal and the available lysine was unaffected by the treatment of producing the protein concentrate (Makkar *et al.*, 2008a). As for the kernel meal, *in vitro* rumen protein digestibility of the protein

concentrate was low and protein digestibility using pepsin and pancreatin was high (Selje-Assmann et al., 2007), suggesting high value of protein concentrate for high yielding animals. To make the protein concentrates suitable for use as an ingredient in livestock feed, phorbol esters must be removed, and trypsin inhibior and lectins inactivated by heat treatment. The adverse effects of phytate could be mitigated by addition of phytase in the diet. This material after detoxification would form an excellent protein supplement for farm animal species.

Acid gums, fatty acid distillate and glycerol

Amongst these byproducts, glycerol is recovered in substantial amounts in the biodiesel production (10%). In order to enable safe use of these byproducts, a process is needed for isolation of phorbol esters in the toxic oil before the oil goes for biodiesel production. The phorbol esters isolated could be used for various agricultural and pharmaceutical applications since they have strong molluscicidal and pesticidal activities. On the other hand these fractions obtained during the biodiesel production from the oil from the non-toxic *J. curcas* would be safe for inclusion in livestock diets.

Our study has shown that the stripping or deodorisation process as performed in our study leads to destruction of phorbol esters. At present no information is available on the nature of the degraded products and their possible toxicity. There is a need for further research to evaluate the innocuous nature of fatty acid distillate and glycerol so produced.

Conclusions

Increasing amounts of byproducts would be available from the increasing biofuel production. Most of these byproducts are rich in protein (kernel meal, seed cake, extrusion cake, distillers dried grain, corn gluten) and energy (fatty acid distillate, glycerol), and have tremendous potential to be fed to livestock and fish species. Before these are incorporated into the diets, there is a need to evaluate the presence of phorbol esters and their degraded products in the byproducts obtained from the biodiesel production from the toxic *Jatropha* oil; and to monitor the presence of mycotoxins, pesticides and pesticide residues, and phyto-harmones in the by-products from the biofuel industry based on edible seed meals and cereals.

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3.1.4 Separation of shells from screw pressed cake using sieving (responsible group: Germany 1)

Introduction

Jatropha curcas is a hardy plant and can grow on degraded land with little resources. It belongs to Euphorbiaceae family and its seeds contain about 30–35% oil, which can be used as fuel directly or can be converted to biodiesel by transesterification. Large scale plantations of this plant have taken place in many countries in Asia, Africa and Latin America with the aim to use oil as bio-diesel [1, 2]. In these countries, oil is produced from the whole seeds using a mechanical press. The seed has an outer black-coloured hard shell and inside a white-coloured kernel. The proportion of the shell to kernel is approximately 0.35 to 0.65. The seed cake left as a by-product after extracting oil using a mechanical press could contain 50 to 60 % shells which are largely indigestible. The presence of high levels of shells in the seed cake is one of the constraints in its utilization in animal diets. Therefore, at present it is used as a fertilizer [3]. Other constraints in the utilization of seed cake or its fractions as livestock feed are the presence of toxic phorbol esters and antinutritional factors such as trypsin inhibitor, lectin and phytate [2].

The objective of this study was to optimize the conditions for removal of shells from the seed cake by using a physical method i.e. grinding of the seed cake following by sieving, to obtain protein rich fractions which could be used as livestock feed after detoxification. The protein quality of the meal obtained after de-oiling (using hexane or pertroleum benzene) of *Jatropha* kernels obtained after removal of shells is high. The levels of essential amino acids except lysine are higher in this meal than in the FAO reference protein for a growing child of 2–5 years [2]. In our laboratory, conditions have been standardised for detoxification of the protein-rich kernel meal (obtained from de-shelled seeds) and protein isolate (obtained from the seed cake using the principle of iso-electric precipitation) [4-6], and the same detoxification process can be used for protein rich fraction(s) obtained through the sieving process. In addition, the protein rich

fractions obtained, without detoxification, could also be a starting material for non-edible applications of proteins, for example preparation of films and coatings [7].

Materials and Methods

Seeds

Jatropha curcas seeds, collected from the wild trees, were procured from Jaipur, Rajasthan, India.

Seed cake

It was prepared using a mechanical screw press (German screw press type - Komet D85-1G), maximal capacity of material input was 25 kg/h and the screw press was powered by a 3.0 kW electrical motor. The pressing conditions were: screw speed, 290 rpm, worm shaft R 8 mm and worm diameter 56 mm, cylinder P1 and nozzle N12. Eight kg of the seeds gave 5.02 kg press cake.

Grinding of seed cake

The seed cake was ground to pass through a 1 mm sieve using a laboratory mill.

Sieving of the ground cake

The ground cake was passed through sieves of different sizes using Retsch Type 3D sieving machine configured with 3 sieves of mesh size 350 µm, 250 µm and 160 µm (each 200 mm in diameter and 50 mm height) and sieving aids (rubber beads). The sieves were fitted on one another: 350 µm sieve on the top, 250 µm sieve in the middle and 160 µm sieve in the bottom. The ground cake was loaded on the top sieve and passed through top to the bottom sieve. The sieving motion of this machine produces three-dimensional vibrations. The machine was run at intensity 80 for a total run time of 25 min with 5 breaks (5 x 5 min). During the sieving process, clusters formed on the sieves, due to the sieving motion, were frequently dispersed on the sieves using a soft rubber spade. After 25 min, four fractions were obtained: > 350 µm, left on the top sieve; > 250 µm but < 350 µm, left on the middle sieve; > 160 µm but < 250 µm, left on the bottom sieve; and < 160

µm which passed through the bottom sieve. The sieving process was repeated four times, giving four replicates.

Determination of chemical composition

The moisture content of the samples was determined by oven-drying to a constant weight at 105 ^{o}C. Crude protein (CP) as nitrogen x 6.25, lipid and ash contents were determined in accordance with the standard methods of AOAC [8].

Extraction and estimation of phorbolesters by HPLC

The samples were extracted in methanol and phorbol esters were determined on a reverse phase C18 (LiChrospher 100, endcapped 5 µm) 250 x 4 mm I.D. column protected with a guard column containing the same material as the main column according to the procedure outlined in [9, 10]. The four phorbol ester compound peaks that appeared between 26 and 31 min were identified and integrated at 280 nm. The results are expressed as equivalent to a standard, phorbol-12-myristate 13-acetate, which appeared between 34 and 36 min.

Determination of shell content

Shell content in various fractions was determined by using acid detergent lignin (ADL) as the marker since it was present only in shells and not in kernels. The factor for converting ADL to shell content was 2.52 (ADL content of shells was 39.7%).

Statistical evaluation

The data were subjected to ANOVA and statistical comparisons between the feeding groups were made using the Tukey Honest Significant Difference (HSD) test using Statistica for Windows (release 5.1 H, '97 edition). The significance of observed differences was tested at $P<0.05$. The values are reported as Means ± SD.

Results and Discussion

In our previous study [11], another approach was used for separating shells from the seed cake. In this approach, protein isolate was prepared from the seed cake by dissolving

proteins present in the cake at pH 11 and separating the shells by filtration or centrifugation; and then precipitating the solubilised proteins at iso-electric pH. The recovery of the protein isolate on dry matter basis was 17% and its protein content 80%. This process of isolating a protein rich material from the seed cake, being a chemical one, requires skilled manpower, a laboratory equipped with basic facilities, and environment unfriendly chemicals. In addition, these resources required are not available near the sites of the seed cake production in most developing countries; and thus requires transport of the bulky material to a central facility for preparation of the protein isolate.

The aim of this study was to obtain a protein rich fraction with minimum possible shells. In this regard only the fraction < 160 µm (the fraction that passed through all the sieves) met this requirement. It had 37.1% crude protein and 18.9% shells. The recovery of this fraction, on dry matter basis, was 24.1%. The other two fractions: > 160 µm but < 250 µm, and > 250 µm but < 350 µm also had reasonably high content of crude protein, 31.7 and 29.3% respectively and shell content of 25.5 and 32.6% respectively. The recoveries of these fractions, on dry matter, were 11.2 and 8.9% (Table 1).

If the three fractions (< 160 µm, > 160 µm but < 250 µm, and > 250 µm but < 350 µm) are pooled, the dry matter recovery is 43.4%, and it contains 34.3% crude protein and 23.1% shells. Infact the pooled sample has only 3 percentage units lower crude protein and 4 percentage units higher shells, but the recovery of the dry matter is almost two-fold compared to the best fraction (< 160 µm) in terms of protein content. The use of this pooled sample would make the sieving process simple since the ground seed cake (initially passed through 1 mm sieve) would need to be passed through just one sieve of mesh size 315 µm. The material that passed through this sieve would infact be the pooled fraction.

The fraction remaining on the 350 µm sieve contain 2.2% N and hence could be used as a N-fertilizer (Figure 1). In addition, this fraction contains 82% shells, which makes it a good material for improvement of soil structure and soil organic matter. Furthermore, this fraction could also be used for isolating lignin for various industrial, cosmetic, food and pharmaceutical applications [12].

Since the main purpose of producing the seed cake is to extract oil for biofuel production, it would be advisable to recover the oil from the pooled fraction (calculated

oil content of the pooled fraction is 14%) using organic solvent. If this is done, the crude protein content of the pooled fraction after de-oiling would be 50.6% and the shell content 34.1%. The NDF and ADL contents of this de-oiled fraction would be 49.6% and 13.5%, implying that for ruminants, only 13.5% (ADL) would potentially be unavailable; while for the monogastric animals a larger amount (49.6%, NDF) would be unavailable. The pooled fraction, after detoxification, would be a good feed for ruminants (Figure 1). Its crude protein content (approximate 51%) would be higher than that of soybean meal (approximate 45%). Although in comparison to soybean meal, it has almost twice the NDF (NDF content of soybean meal 17.2%; [13]). It may be noted that NDF content of other conventional oil seed meals is: rapeseed meal 26%, sunflower meal 19%, cottonseed meal 29% and safflower meal 60% [14].

For monogastric animals, the best fraction (< 160 μm) after de-oiling would also not be a fraction of choice because of 31.5% NDF. The high NDF values of the best fraction or the pooled de-oiled fraction preclude their use in fish, shrimp or poultry diets. For these animal species, the protein isolate prepared using the chemical process based on iso-electric pH [11] after detoxification would be ideal. Alternatively, the protein isolate from the pooled fraction or the best fraction obtained from the sieving process could also be prepared (Figure 1). The advantage of this would be the removal of the bulky and less useful material (for the isolate preparation) containing shells (the fraction left on the 350 μm sieve; containing 2.2% N and 82% shell) at the site of the seed cake production, for use locally as a fertilizer. This will also enable transport of only the protein rich material to the central and more specialized facilities (which generally would be farther away) for protein isolate preparation, decreasing cost of the transportation.

Phorbol esters, the main toxic agents are present in all the fractions obtained using the sieving process (Table 1) and hence the fractions must be detoxified, before feeding to livestock. Both *Jatropha* kernel meal and protein isolates have been detoxified [4-6]. Furthermore, these protein rich plant sources have been shown to be of high biological value and can be safely incorporated into fish diets. The fractions obtained after sieving can also be subjected to the same process of detoxification.

The physical process based on sieving has advantages and disadvantages. A disadvantage, as stated earlier, is that the fractions can not be used in fish, shrimp or

poultry diets. The advantages are that the sieving method is simple, can be conducted by a semi- or un-skilled person at the site of the cake production, and requires little resources. In addition, it would take lesser time and is expected to cost lesser compared to the chemical process.

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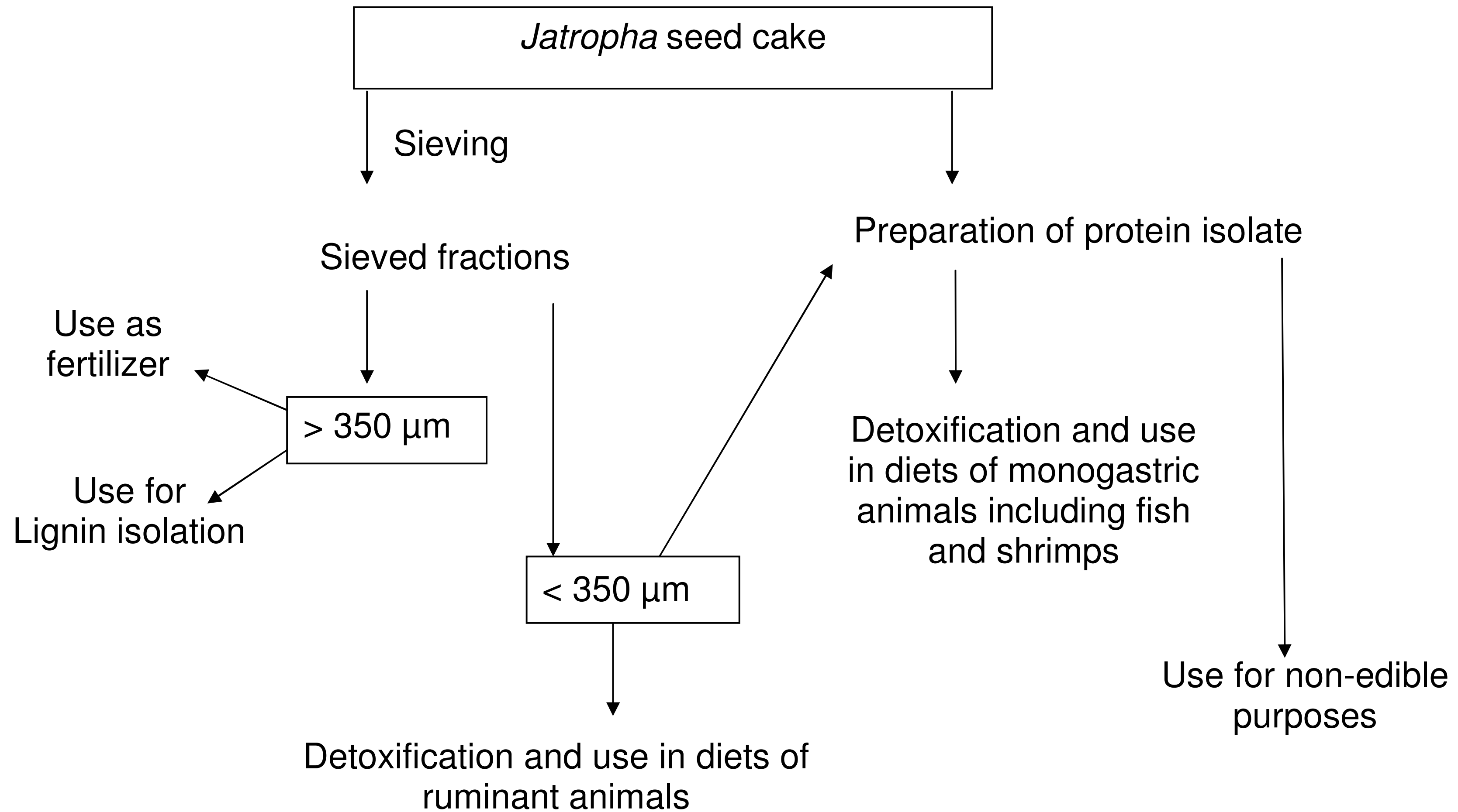
1 **Table 1** Recovery of sieved fractions, proximate composition (% in dry matter), estimated shell content, and
2 phorbol ester content (mg/g) of the original seed cake and sieved fractions
3

	Fraction (% dry matter)	Crude protein	Lipid	Ash	NDF	ADF	ADL	Shell (%)	Phorbol esters
Original seed cake	100.0	23.1	9.5	5.8	57.0	48.2	24.0	60.5	1.00
Fraction, > 315 µm	56.6[a] ± 0.88	13.8[a] ± 0.41	5.6[a] ± 0.19	4.2[a] ± 0.05	71.2[a] ± 0.94	59.1[a] ± 1.79	32.5[a] ± 0.82	82.0[a] ± 2.06	0.83[c] ± 0.07
Fraction, > 250 µm but < 315 µm	8.1[b] ± 0.65	29.3[b] ± 0.21	11.4[b] ± 0.19	7.7[b] ± 0.18	42.1[b] ± 1.13	31.1[b] ± 1.71	12.9[b] ± 0.53	32.6[b] ± 1.53	1.68[b] ± 0.05
Fraction, > 160 µm but < 250 µm	11.2[c] ± 1.12	31.7[c] ± 0.18	12.7[c] ± 0.26	8.4[c] ± 0.17	37.0[c] ± 0.53	26.7[c] ± 1.06	10.1[c] ± 0.31	25.5[c] ± 0.79	1.88[a] ± 0.09
Fraction, <160 µm	24.1[d] ± 0.78	37.1[d] ± 0.07	15.5[d] ± 0.26	9.1[d] ± 0.49	29.1[d] ± 0.60	18.8[d] ± 0.73	7.5[d] ± 0.33	18.9[d] ± 0.83	1.88[a] ± 0.09
SEM	4.97	0.49	2.24	0.93	4.11	3.93	2.55	6.44	0.11

4 NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin
5 Values are mean (n = 4) ± standard deviation. SEM, standard error of mean
6 For the fractions, mean values in the same column with different superscripts differ significantly ($P < 0.05$)
7
8

Figure 1 Possible approaches for using *Jatropha* seed cake



3.1.5 Development of a method for determination of shells in screw pressed cake using NIRS (responsible group: Germany 1)

Sample Preparation

For the sample preparation, defined amounts of seeds, kernel and shells mixed, to get a high range of shell content within the samples (Table 1).

Table 1. Mixing of different quantities of whole seeds, kernels and shells

Total g	Whole seeds g	Kernel g	Shell g	Calculated shell content %
500	490	10	0	34.30
500	475	25	0	33.25
500	425	75	0	29.75
500	415	85	0	29 5
500	390	110	0	27.30
500	370	130	0	25.90
500	330	170	0	23.10
500	315	185	0	22.05
500	290	210	0	20.30
500	270	230	0	18.90
500	230	270	0	16.10
500	215	285	0.0	15.05
500	185	315	0	12.95

500	170	330	0	11.90
500	130	370	0	9.10
500	115	385	0	8.05
500	500	0	0	35.0
500	450	50	0	31.50
500	400	100	0	28.0
500	350	150	0	24.50
500	300	200	0	21.0
500	250	250	0	17.50
500	200	300	0	14.0
500	150	350	0	10.50
500	100	400	0	7.0
500	475	535	25	38.50
500	450	550	50	41.50
500	425	565	75	44.80
500	400	580	100	48.0
500	375	595	125	51.30
500	350	610	150	54.50
500	325	625	175	57.80
500	300	640	200	61.0
500	275	655	225	64.30

Assuming that the shell content of whole seeds is 35 %

The artificial prepared samples were used to produce press cake by pressing through a screw press.

Thereafter the samples were ground to 1mm. The ground samples were taken for various wet analyses: Fibre fractions (neutral detergent fibre, NDF; acid detergent fibre, ADF; acid detergent lignin, lignin) using Van Soest's detergent method, lipid using Soxhlet method and protein using a C-/ N- Analyser

Measuring with NIRS

The samples were lightly pressed in a Petri dish.

Table 2 shows the calibration protocols of the different nutrients

Table 2. Calibration protocol

User	Administrator
Date/Time	27/04/2010 13:01
Software	NIRCal V5.2 (Build 3000)
Project File Name	Protein_23.04.10
Project Comment	
Project GUID	{915A5A74-C738-41AA-B272-22D2735C0944}
Calibration Name	Protein, 23.04.10
Calibration Comment	
Calibration GUID	{1166A963-B616-4CC2-9236-B6DC85816A74}
Calibration Version	0
Properties in Project	Moisture, Protein, NDF, ADF, ADL, Lipid. (total 6/6)
Properties in Calibration Set	Protein. (total 1/6)
Spectra in Project	165
Spectra in Calibration Set	117

Spectra in Validation Set	45
Spectra in Calibration Set	1-6, 10-15, 19-24, 28-33, 37-42, 46-48, 55-60, 64-69, 73-78, 82-96, 100-105, 109-114, 118-123, 127-132, 136-159, 163-165. (total 117/165)
Spectra in Validation Set	7-9, 25-27, 34-36, 43-45, 49-54, 61-63, 70-72, 79-81, 97-99, 106-108, 115-117, 124-126, 133-135, 160-162. (total 45/165)
Spectra unused (U-Set)	16-18. (total 3/165)
Validation Method	Validation Set
C-Set Spectra	
Instrument type / serial	NIRFlex N500 / 1000035027
y-Unit / Measurements / Scans	Reflectance / 1 / 32
V-Set Spectra	
Instrument type / serial	NIRFlex N500 / 1000035027
y-Unit / Measurements / Scans	Reflectance / 1 / 32
Spectra Resolution	4 1/cm
Spectra y-Unit	Reflectance

Wavelengths Project Set	4000-10000. (total 1501/1501)

Wavelengths Calibration Set	5000-7144, 7404-10000. (total 1187/1501)
Number of Data Pretreatments	2
Data Pretreatment Sequence (short form)	n01,db1
Data Pretreatment Sequence (detailed)	1. Normalization between 0 to 1*, 5000-7144, 7404-10000. (total 1187/1501) 2. First Derivative BCAP
Method	PLS
Max Iterations	3000
Mean Centering	Yes
Number of Primary PCs	9
Secondary/Calibration PCs	1-5. (total 5/9)
Blow Up Parameter	
Residual Blow Up	2
Score Blow Up	1.05
Max C-Set Spectra Residual	0.000160496
Max Allowed Residual for Calibration	0.000320992

Q-Value V5	0.888325
Validation Method	Validation Set
C-Set Residual too big	0
V-Set Residual too big	0
Num Properties	1

Rel. Consistency	0.00203619
Weighted BIAS	0.00278519
Validity	0.00271911
Comparability	0.000341493
Precision	0.0180399
Weighted Accuracy	0.0949706

Property Statistics	Protein [%]
C-Set BIAS	0
V-Set BIAS	-0.0770661
C-Set SEE (SEC)	0.496118
V-Set SEE (SEP)	0.499165
Consistency	99.3897
C-Set Regression Coefficient	0.997622
V-Set Regression Coefficient	0.997281
C-Set Regression Intercept	0.11999
V-Set Regression Intercept	0.218253
C-Set Regression Slope	0.99525
V-Set Regression Slope	0.994079
C-Set Orig. min	13.71
V-Set Orig. min	14.63
C-Set Orig. max	41.38
V-Set Orig. max	39.97
C-Set Orig. mean	25.2633
V-Set Orig. mean	23.844
C-Set Orig. sdev	7.19876
V-Set Orig. sdev	6.77332

C-Set Pred. min	13.8129
V-Set Pred. min	14.3007
C-Set Pred. max	40.9284
V-Set Pred. max	40.0968
C-Set Pred. mean	25.2633
V-Set Pred. mean	23.9211
C-Set Pred. sdev	7.18165
V-Set Pred. sdev	6.75157
C-Set RSS	28.5515
V-Set RSS	11.2305
C-Set Durbin-Watson	0.989315
C-Set Durbin-Watson in range 1.5 to 2.5	No
V-Set Durbin-Watson	0.751798
V-Set Durbin-Watson in range 1.5 to 2.5	No
C-Set Resid. min	-1.57637
V-Set Resid. min	-1.09658
C-Set Resid. max	1.23535
V-Set Resid. max	1.22844
V-Set t-value	1.03568
V-Set t-Test(n-1,2-tail) Confidence [%]	69.399
C-Set n	117
V-Set n	45

Table 3 shows values chemical constituents by wet method (Original) and those obtained by NIRS.

Table 3 Means (with deviation) of the samples used for the calibration, using wet and NIRS methods

a) NDF

	Average Predicted	Original	Abs. Dev.	Rel. Dev.
Spectra Name	NDF [%]	NDF [%]	[%]	[%]
7869	27.30	26.61	-0.69	-2.6
7870	25.96	26.97	1.01	3.7
7773	28.45	27.25	-1.20	-4.4
7743	27.93	27.74	-0.19	-0.7
7772	32.04	31.37	-0.67	-2.1
7868	30.91	31.38	0.47	1.5
7867	32.77	34.04	1.27	3.7
7771	35.77	34.58	-1.19	-3.4
7740 Repeat	33.82	34.81	0.99	2.8
7866	36.37	34.87	-1.50	-4.3
7741	35.50	34.97	-0.53	-1.5
7742	33.00	35.92	2.92	8.1
7865	37.07	37.76	0.69	1.8
7864	38.16	38.19	0.03	0.1
7739	40.65	38.35	-2.30	-6.0
7862	40.04	40.03	-0.01	0.0

7863	44.13	42.25	-1.88	-4.5
7738	43.59	43.32	-0.27	-0.6
7737	45.91	45.19	-0.72	-1.6
7860	45.61	45.75	0.14	0.3
7856	46.08	45.98	-0.10	-0.2
7854	48.06	46.39	-1.67	-3.6
7857	46.74	46.42	-0.32	-0.7
7858	46.52	46.58	0.06	0.1
7861	45.66	47.88	2.22	4.6
7859	48.10	47.99	-0.11	-0.2
7855	48.75	49.47	0.72	1.5
7736	50.21	49.82	-0.39	-0.8
7852	50.29	51.33	1.04	2.0
7850	50.96	51.86	0.90	1.7
7853	51.31	52.23	0.92	1.8
7848	53.11	52.56	-0.55	-1.0
7735	52.25	53.41	1.16	2.2
7774	52.52	54.45	1.93	3.6
7851	52.56	53.51	0.95	1.8
7744	55.29	53.92	-1.37	-2.5
7849	55.01	54.82	-0.19	-0.4
7847	57.51	56.53	-0.98	-1.7
7745	56.64	56.68	0.04	0.1
7845	58.98	57.90	-1.08	-1.9
7746	58.08	57.94	-0.14	-0.2
7775	58.24	58.22	-0.02	0.0
7747	59.61	59.73	0.12	0.2
7776	61.40	61.88	0.48	0.8
7846	63.58	62.25	-1.33	-2.1
7748	62.31	62.68	0.37	0.6
7749	63.72	62.88	-0.84	-1.3

7750	64.27	63.72	-0.55	-0.9
7779	64.86	64.06	-0.80	-1.3
7777	63.58	64.84	1.26	1.9
7778	66.80	66.84	0.04	0.1
7751 Repeat	67.55	66.90	-0.65	-1.0
7752	67.40	67.43	0.03	0.0
7780	67.08	68.80	1.72	2.5
7781	70.58	71.49	0.91	1.3

b) Crude protein

	Average Predicted	Original	Abs. Dev.	Rel. Dev.
Spectra Name	Protein [%]	Protein [%]	[%]	[%]
7781	13.96	13.71	-0.25	-1.8
7751 Repeat	13.91	14.06	0.15	1.1
7752	14.47	14.63	0.16	1.1
7780	15.62	15.61	-0.01	-0.1
7778	16.14	15.75	-0.39	-2.5
7846	15.78	15.85	0.07	0.4
7779	16.89	15.98	-0.91	-5.7
7750	16.10	15.99	-0.11	-0.7
7749	17.06	16.92	-0.14	-0.8
7777	17.38	17.50	0.12	0.7
7748	17.73	18.21	0.48	2.6
7776	18.78	18.73	-0.05	-0.3
7845	18.38	18.82	0.44	2.4
7747	18.81	19.25	0.44	2.3
7775	19.86	19.28	-0.58	-3.0
7746	20.05	19.69	-0.36	-1.8
7745	20.64	20.68	0.04	0.2

7847	20.08	20.92	0.84	4.0
7744	21.67	21.18	-0.49	-2.3
7774	22.78	22.17	-0.61	-2.7
7848	22.83	22.23	-0.60	-2.7
7735	22.42	22.36	-0.06	-0.3
7849	22.36	22.74	0.38	1.7
7855	23.37	22.93	-0.44	-1.9
7851	23.11	23.18	0.07	0.3
7853	24.07	23.43	-0.64	-2.7
7736	23.38	23.77	0.39	1.7
7850	24.61	24.04	-0.57	-2.4
7856	24.30	24.19	-0.11	-0.4
7857	23.87	24.54	0.67	2.7
7852	24.14	24.57	0.43	1.8
7737	25.77	25.52	-0.25	-1.0
7854	24.91	25.81	0.90	3.5
7858	26.53	26.35	-0.18	-0.7
7738	26.66	27.18	0.52	1.9
7739	27.15	27.28	0.13	0.5
7859	27.53	27.36	-0.17	-0.6
7740 Repeat	28.54	28.16	-0.38	-1.3
7860	29.17	28.83	-0.34	-1.2
7861	29.36	29.32	-0.04	-0.1
7863	30.02	30.21	0.19	0.6
7771	29.94	30.47	0.53	1.8
7862	32.22	32.16	-0.06	-0.2
7864	32.21	32.17	-0.04	-0.1
7742	33.32	32.63	-0.69	-2.1
7865	32.69	32.71	0.02	0.1
7773	34.06	33.66	-0.40	-1.2
7741	30.99	33.69	2.70	8.0

7772	32.67	33.73	1.06	3.1
7743	35.33	34.87	-0.46	-1.3
7867	37.45	36.40	-1.05	-2.9
7866	35.79	36.85	1.06	2.9
7868	37.54	37.00	-0.54	-1.4
7869	39.83	39.97	0.14	0.4
7870	40.84	41.38	0.54	1.3

c) ADL

	Average Predicted	Original	Abs. Dev.	Rel. Dev.
Spectra Name	ADL [%]	ADL [%]	[%]	[%]
7743	6.54	6.68	0.14	2.1
7870	6.85	6.74	-0.11	-1.7
7869	7.39	7.09	-0.30	-4.2
7773	8.39	7.90	-0.49	-6.2
7868	9.44	8.85	-0.59	-6.7
7741	10.78	9.35	-1.43	-15.3
7866	11.49	10.39	-1.10	-10.6
7772	10.93	10.68	-0.25	-2.3
7867	9.76	10.70	0.94	8.8
7740 Repeat	11.51	11.31	-0.20	-1.8
7742	9.36	11.62	2.26	19.5
7864	12.45	12.23	-0.22	-1.8
7865	12.20	12.23	0.03	0.3
7771	12.87	12.59	-0.28	-2.2
7862	13.75	13.87	0.12	0.9
7739	15.53	14.48	-1.05	-7.2
7863	16.03	14.98	-1.05	-7.0
7857	17.66	17.30	-0.36	-2.1
7738	16.97	17.54	0.57	3.3

7856	17.78	17.60	-0.18	-1.0
7737	18.10	17.85	-0.25	-1.4
7861	17.48	18.40	0.92	5.0
7854	19.08	18.54	-0.54	-2.9
7858	18.11	18.68	0.57	3.1
7859	18.71	18.99	0.28	1.5
7860	17.52	19.10	1.58	8.3
7855	19.20	19.95	0.75	3.7
7852	20.36	20.54	0.18	0.9
7736	21.03	21.02	-0.01	-0.1
7848	21.60	21.13	-0.47	-2.2
7850	20.18	21.14	0.96	4.6
7853	20.49	21.36	0.87	4.1
7735	22.36	21.75	-0.61	-2.8
7847	22.15	21.81	-0.34	-1.6
7851	21.34	21.84	0.50	2.3
7849	22.05	21.87	-0.18	-0.8
7746	23.03	22.34	-0.69	-3.1
7744	22.96	22.39	-0.57	-2.5
7745	23.00	22.64	-0.36	-1.6
7774	22.42	22.68	0.26	1.2
7775	24.77	23.98	-0.79	-3.3
7845	24.41	24.11	-0.30	-1.2
7747	23.66	24.67	1.01	4.1
7749	25.00	24.83	-0.17	-0.7
7748	24.80	25.48	0.68	2.7
7776	26.08	25.65	-0.43	-1.7
7846	26.04	26.13	0.09	0.3
7750	25.62	26.35	0.73	2.8
7752	26.06	26.40	0.34	1.3
7751 Repeat	26.72	26.89	0.17	0.6

7777	27.30	27.18	-0.12	-0.4
7779	27.51	27.19	-0.32	-1.2
7780	27.62	27.55	-0.07	-0.3
7778	28.20	27.72	-0.48	-1.7
7781	28.79	28.86	0.07	0.2

Results for ADF and lipid are not shown. The results are similar to those for NDF and protein respectively.

Table 4 gives results of the samples which were not used for the calibration, they have also differnet particle sizes or they are more than one year old.

Table 4 Means (with deviation) of the samples used for the calibration, using wet (original) and NIRS methods

a) NDF

	Average Predicted	Original	Abs. Dev.	Rel. Dev.
Spectra Name	NDF [%]	NDF [%]	[%]	[%]
5402	47.36	49.33	1.97	4.0
5403	50.15	51.26	1.11	2.2
5405	36.63	32.33	-4.30	-13.3
5406	52.14	53.80	1.66	3.1
5407	33.19	25.61	-7.58	-29.6
5920	21.11	46.35	25.24	54.5
5921	47.47	48.53	1.06	2.2
5922	45.26	50.76	5.50	10.8
5923	46.48	48.37	1.89	3.9
5924	48.27	51.61	3.34	6.5
5925	45.23	49.74	4.51	9.1

5926	46.61	47.01	0.40	0.9
5927	45.51	46.63	1.12	2.4
5928	47.45	49.15	1.70	3.5
6051	50.17	51.22	1.05	2.0
6055	49.37	52.74	3.37	6.4
6059	49.29	53.91	4.62	8.6
6420	48.81	51.77	2.96	5.7
6734	53.95	67.43	13.48	20.0
6735	48.88	40.52	-8.36	-20.6
6736	48.22	33.09	-15.13	-45.7
6737	43.71	26.26	-17.45	-66.5
6738	54.80	65.43	10.63	16.2
6739	47.96	36.73	-11.23	-30.6
6740	47.19	34.52	-12.67	-36.7
6741	43.47	27.61	-15.86	-57.4
6742	54.32	65.90	11.58	17.6
6743	48.18	38.36	-9.82	-25.6
6744	47.48	33.83	-13.65	-40.3
6745	43.16	26.57	-16.59	-62.4
6746	53.56	65.73	12.17	18.5
6747	47.55	38.23	-9.32	-24.4
6748	47.40	33.62	-13.78	-41.0
6749	43.34	27.12	-16.22	-59.8
7100	46.19	46.18	-0.01	0.0
7101	45.97	44.22	-1.75	-3.9
7102	46.67	41.44	-5.23	-12.6
7103	47.48	44.00	-3.48	-7.9
Pure Shells	66.24	80.24	14.00	17.4
Mix 6735 + Shells_1	51.13	44.10	-7.03	-16.0
Mix 6735 + Shells_2	52.91	49.90	-3.02	-6.0
Mix 6735 + Shells_3	53.67	52.11	-1.56	-3.0

Mix 6735 + Shells_4	55.08	54.21	-0.87	-1.6
Mix 6735 + Shells_5	55.86	56.54	0.68	1.2
Mix 6735 + Shells_6	57.02	58.32	1.30	2.2
Mix 6735 + Shells_7	58.16	59.53	1.37	2.3
Mix 6735 + Shells_8	57.11	60.92	3.81	6.3
Mix 6735 + Shells_9	59.55	62.44	2.89	4.6
Mix 6735 + Shells_10	58.51	63.61	5.10	8.0
Mix 6735 + Shells_11	59.18	64.80	5.61	8.7
Mix 6735 + Shells_12	60.20	65.43	5.23	8.0
Mix 6735 + Shells_13	61.76	66.19	4.43	6.7
Mix 6735 + Shells_14	61.31	67.27	5.96	8.9
Mix 6735 + Shells_15	60.76	67.90	7.15	10.5
Mix 6735 + Shells_16	60.30	69.63	9.33	13.4

b) Crude protein

Spectra Name	Average Predicted CP [%]	Original CP [%]	Abs. Dev. [%]	Rel. Dev. [%]
6051	21.06	20.58	-0.48	-2.3
6055	21.20	20.84	-0.36	-1.7
6059	21.00	20.80	-0.20	-1.0
6420	22.07	21.30	-0.77	-3.6
6734	17.84	12.60	-5.24	-41.5
6735	23.09	26.65	3.56	13.4
6736	24.31	28.81	4.50	15.6
6737	28.51	34.13	5.62	16.5
6738	17.62	13.43	-4.19	-31.2
6739	23.37	26.70	3.33	12.5
6740	24.47	29.10	4.63	15.9
6741	28.29	34.20	5.91	17.3

6742	17.96	12.71	-5.25	-41.3
6743	23.31	27.20	3.89	14.3
6744	24.33	29.30	4.97	17.0
6745	28.46	34.30	5.84	17.0
6746	18.03	12.50	-5.53	-44.2
6747	23.47	27.00	3.53	13.1
6748	24.22	29.20	4.98	17.0
6749	28.47	24.50	-3.97	-16.2
Shells	11.54	5.00	-6.54	-130.9
Mix 6735 + Shells_1	21.45	24.70	3.25	13.2
Mix 6735 + Shells_2	20.23	21.54	1.30	6.1
Mix 6735 + Shells_3	19.89	20.33	0.44	2.2
Mix 6735 + Shells_4	18.70	19.19	0.49	2.5
Mix 6735 + Shells_5	17.67	17.92	0.24	1.4
Mix 6735 + Shells_6	17.80	16.95	-0.85	-5.0
Mix 6735 + Shells_7	16.53	16.29	-0.25	-1.5
Mix 6735 + Shells_8	17.60	15.53	-2.07	-13.3
Mix 6735 + Shells_9	15.96	14.70	-1.26	-8.6
Mix 6735 + Shells_10	17.07	14.06	-3.01	-21.4
Mix 6735 + Shells_11	15.88	13.42	-2.46	-18.4
Mix 6735 + Shells_12	15.60	13.07	-2.53	-19.3
Mix 6735 + Shells_13	14.42	12.66	-1.76	-13.9
Mix 6735 + Shells_14	14.75	12.07	-2.68	-22.2
Mix 6735 + Shells_15	14.59	11.72	-2.87	-24.5
Mix 6735 + Shells_16	15.81	10.78	-5.03	-46.6
Mix 6735 + Shells_17	13.59	9.39	-4.20	-44.7
Mix 6735 + Shells_18	13.33	8.35	-4.98	-59.7
Mix 6735 + Shells_19	12.84	7.68	-5.16	-67.3
Mix 6737 + Shells_1	26.00	31.22	5.22	16.7
Mix 6737 + Shells_2	26.15	29.02	2.87	9.9
Mix 6737 + Shells_3	26.35	26.83	0.48	1.8

Mix 6737 + Shells_4	25.60	25.21	-0.39	-1.6
Mix 6737 + Shells_5	24.31	23.51	-0.80	-3.4
Mix 6737 + Shells_6	24.17	21.93	-2.24	-10.2
Mix 6737 + Shells_7	22.42	20.82	-1.59	-7.7
Mix 6737 + Shells_8	19.17	19.41	0.25	1.3
Mix 6737 + Shells_9	22.82	18.25	-4.57	-25.0
Mix 6737 + Shells_10	20.62	17.43	-3.19	-18.3
Mix 6737 + Shells_11	21.29	16.74	-4.55	-27.2
Mix 6737 + Shells_12	19.24	15.98	-3.25	-20.4
Mix 6737 + Shells_13	18.97	15.43	-3.54	-22.9
Mix 6737 + Shells_14	16.81	14.63	-2.18	-14.9
Mix 6737 + Shells_15	17.99	13.85	-4.14	-29.9
Mix 6737 + Shells_16	16.59	12.55	-4.03	-32.1
Mix 6737 + Shells_17	16.45	11.40	-5.05	-44.3
Mix 6737 + Shells_18	17.22	10.20	-7.02	-68.8
Mix 6737 + Shells_19	15.19	9.31	-5.89	-63.2
Mix 6736 + Shells_1	23.85	26.68	2.84	10.6
Mix 6736 + Shells_2	20.05	19.46	-0.60	-3.1
Mix 6736 + Shells_3	19.45	18.56	-0.89	-4.8
Mix 6736 + Shells_4	19.10	16.69	-2.40	-14.4
Mix 6736 + Shells_5	18.66	15.91	-2.75	-17.3
Mix 6736 + Shells_6	18.75	15.16	-3.59	-23.7
Mix 6736 + Shells_7	18.18	14.70	-3.47	-23.6
Mix 6736 + Shells_8	17.69	14.15	-3.53	-25.0
Mix 6736 + Shells_9	18.46	13.69	-4.77	-34.8
Mix 6736 + Shells_10	18.63	13.37	-5.26	-39.3
Mix 6736 + Shells_11	18.48	13.02	-5.46	-41.9
Mix 6736 + Shells_12	17.88	12.83	-5.04	-39.3
Mix 6736 + Shells_13	17.39	12.51	-4.88	-39.0
Mix 6736 + Shells_14	16.65	12.17	-4.48	-36.8

Mix 6736 + Shells_15	15.05	11.93	-3.12	-26.2
Mix 6736 + Shells_16	15.80	10.33	-5.46	-52.9
Mix 6736 + Shells_17	14.03	9.05	-4.98	-55.0
Mix 6736 + Shells_18	13.14	8.40	-4.74	-56.5
Mix 6736 + Shells_19	13.14	7.75	-5.39	-69.4

c) ADL

Spectra Name	Average Predicted ADL [%]	Original ADL [%]	Abs. Dev. [%]	Rel. Dev. [%]
5402	21.75	17.74	-4.01	-22.6
5403	23.04	19.74	-3.30	-16.7
5405	14.75	10.86	-3.89	-35.8
5406	22.98	20.85	-2.13	-10.2
5407	13.38	7.44	-5.94	-79.8
5920	16.08	20.25	4.17	20.6
5921	22.90	19.14	-3.76	-19.7
5922	21.33	20.47	-0.86	-4.2
5923	22.73	20.03	-2.70	-13.5
5924	24.33	20.85	-3.48	-16.7
5925	20.95	17.88	-3.07	-17.2
5926	21.42	18.80	-2.62	-13.9
5927	20.62	20.35	-0.27	-1.4
5928	22.07	21.16	-0.91	-4.3
6051	23.10	21.94	-1.16	-5.3
6055	23.35	22.87	-0.48	-2.1
6059	23.66	22.62	-1.04	-4.6
6420	22.65	21.11	-1.54	-7.3
6734	27.78	31.17	3.39	10.9
6735	20.68	12.33	-8.35	-67.7

6736	19.47	8.79	-10.68	-121.5
6737	15.31	6.45	-8.86	-137.3
6738	28.18	29.13	0.95	3.3
6739	20.35	11.05	-9.30	-84.2
6740	18.85	9.36	-9.49	-101.4
6741	15.10	7.02	-8.08	-115.0
6742	27.39	30.27	2.88	9.5
6743	20.36	11.75	-8.61	-73.3
6744	19.05	9.34	-9.71	-104.0
6745	15.04	6.85	-8.19	-119.5
6746	27.45	30.43	2.98	9.8
6747	20.18	12.18	-8.00	-65.6
6748	18.99	9.65	-9.34	-96.8
6749	14.57	7.35	-7.22	-98.3
Mix 6735 + Shells_1	22.38	14.34	-8.04	-56.1
Mix 6735 + Shells_10	26.97	25.28	-1.69	-6.7
Mix 6735 + Shells_11	29.02	25.95	-3.07	-11.8
Mix 6735 + Shells_12	29.11	26.30	-2.81	-10.7
Mix 6735 + Shells_13	30.12	26.73	-3.39	-12.7
Mix 6735 + Shells_14	29.69	27.33	-2.36	-8.6
Mix 6735 + Shells_15	29.92	27.69	-2.23	-8.1
Mix 6735 + Shells_16	28.77	28.66	-0.11	-0.4
Mix 6735 + Shells_17	31.69	30.09	-1.60	-5.3
Mix 6735 + Shells_18	32.04	31.16	-0.88	-2.8
Mix 6735 + Shells_19	32.47	31.86	-0.61	-1.9
Mix 6735 + Shells_2	23.81	17.59	-6.22	-35.4
Mix 6735 + Shells_3	24.27	18.83	-5.44	-28.9
Mix 6735 + Shells_4	25.49	20.01	-5.48	-27.4
Mix 6735 + Shells_5	26.46	21.32	-5.14	-24.1
Mix 6735 + Shells_6	27.08	22.31	-4.77	-21.4
Mix 6735 + Shells_7	28.42	22.99	-5.43	-23.6

Mix 6735 + Shells_8	26.56	23.77	-2.79	-11.7
Mix 6735 + Shells_9	28.76	24.62	-4.14	-16.8
Mix 6736 + Shells_1	19.29	11.09	-8.20	-73.9
Mix 6736 + Shells_10	25.86	25.53	-0.33	-1.3
Mix 6736 + Shells_11	25.77	25.91	0.14	0.5
Mix 6736 + Shells_12	26.60	26.11	-0.49	-1.9
Mix 6736 + Shells_13	27.41	26.46	-0.95	-3.6
Mix 6736 + Shells_14	28.29	26.84	-1.45	-5.4
Mix 6736 + Shells_15	29.77	27.10	-2.67	-9.9
Mix 6736 + Shells_16	29.75	28.83	-0.92	-3.2
Mix 6736 + Shells_17	31.00	30.21	-0.79	-2.6
Mix 6736 + Shells_18	32.40	30.92	-1.48	-4.8
Mix 6736 + Shells_19	32.00	31.62	-0.38	-1.2
Mix 6736 + Shells_2	24.19	18.93	-5.26	-27.8
Mix 6736 + Shells_3	24.78	19.91	-4.87	-24.5
Mix 6736 + Shells_4	25.13	21.93	-3.20	-14.6
Mix 6736 + Shells_5	25.64	22.78	-2.86	-12.6
Mix 6736 + Shells_6	25.28	23.59	-1.69	-7.2
Mix 6736 + Shells_7	26.25	24.09	-2.16	-9.0
Mix 6736 + Shells_8	26.83	24.68	-2.15	-8.7
Mix 6736 + Shells_9	26.05	25.18	-0.87	-3.4
Mix 6737 + Shells_1	18.22	8.99	-9.23	-102.7
Mix 6737 + Shells_10	24.47	22.82	-1.65	-7.2
Mix 6737 + Shells_11	23.48	23.66	0.18	0.7
Mix 6737 + Shells_12	25.48	24.11	-1.37	-5.7
Mix 6737 + Shells_13	26.02	24.65	-1.37	-5.6
Mix 6737 + Shells_14	28.70	25.41	-3.29	-12.9
Mix 6737 + Shells_15	27.50	25.86	-1.64	-6.3
Mix 6737 + Shells_16	28.74	27.09	-1.65	-6.1
Mix 6737 + Shells_17	28.88	28.90	0.02	0.1
Mix 6737 + Shells_18	27.74	30.26	2.52	8.3

Mix 6737 + Shells_19	30.26	31.13	0.87	2.8
Mix 6737 + Shells_2	17.87	13.10	-4.77	-36.4
Mix 6737 + Shells_3	17.43	14.67	-2.76	-18.8
Mix 6737 + Shells_4	18.03	16.16	-1.87	-11.5
Mix 6737 + Shells_5	19.88	17.81	-2.07	-11.6
Mix 6737 + Shells_6	19.96	19.07	-0.89	-4.7
Mix 6737 + Shells_7	21.59	19.93	-1.66	-8.4
Mix 6737 + Shells_8	25.87	20.91	-4.96	-23.7
Mix 6737 + Shells_9	21.36	21.99	0.63	2.9
Pure Shells	33.94	34.61	0.67	1.9

Results for ADF and lipid are not shown. The results were similar to those for NDF and protein respectively.

Summary

The samples which were prepared for the calibration and afterwards measured as samples showed lower deviations as compared to those for the samples with different particle sizes or for the samples that were older than one year. The prediction of crude protein, neutral detergent fibre and oil was better than that of lignin.

Conclusions

The calibration equations are unsatisfactory and need to be improved using a large set of sample. Further work is required.

3.1.6 Additional related side work that emanated from the project (only title and summary of the work are presented here. Full papers of the work listed here are available at: http://Jatropha.uni-hohenheim.de)

3.1.6.1 *Jatropha platyphylla*, a new non-toxic *Jatropha* species: Physical properties and chemical constituents including toxic and antinutritional factors of seeds

Abstract

Local communities in Mexico consume *Jatropha platyphylla* seeds after roasting. The kernels of *J. platyphylla* contained *ca* 60% oil and were free of phorbol esters. The kernel meal of this *Jatropha* species contained trypsin inhibitor, lectins and phytate. However, trypsin inhibitor and lectins are heat labile so this explains why the local people can eat roasted seeds without ill effects. Heat treated *J. platyphylla* kernel meal (JPKM) was free of trypsin inhibitor and lectin activities. Crude protein content of JPKM was 75%. Heated JPKM and soybean meal were included in a standard diet (crude protein 36%) for Nile tilapia (*Oreochromis niloticus)* to replace 50% of the fish meal protein. The growth of fish in all the three groups was statistically similar and the blood biochemical parameters that serve as biomarkers for toxicity were within the normal ranges. This is the first study that confirms the non-toxic nature of *J. platyphylla*.

3.1.6.2 Dietary inclusion of *Jatropha platyphilla* kernel meal in the diet of Nile tilapia *(Oreochromis niloticus L.):* growth, metabolic, nutritional and haematological responses

Abstract

Jatropha platyphylla is a multipurpose and drought-resistant shrub, available in Mexico, locally known as "sangregrado" and belonging to the family *Euphorbiaceae*. Its seeds are rich in oil and protein. *Jatropha platyphylla* kernel meal (JPKM) obtained after oil extraction contained 70-75% crude-protein. The kernel meal was free of phorbol-esters and lectins, the main toxins present in other *Jatropha* species: however, it contained

phytate and trypsin-inhibitor. The levels of essential amino acids (except lysine) were higher in JPKM than in soybean meal (SBM). Using Nile tilapia (*Oreochromis niloticus)* fingerlings a 12-week experiment was conducted to evaluate the nutritional quality of the JPKM and compare with that of SBM and fishmeal. Two experiments were conducted simultaneously under two different experimental conditions. The first experiment was in a recirculatory-system to evaluate the nutritional and haematological responses and the second in a respirometric-system to evaluate metabolic response. Fingerlings 15 fish; average weight 13.7±0.21 g for recirculatory-system and another 15 fish 13.9±0.17 g for respirometer-system were randomly distributed in three treatment-groups with five-replicates for each system. Fish were fed three iso-nitrogenous diets (crude-protein 36%), control diet containing fishmeal based protein and two other diets replacing 62.5% fismeal protein with JPKM (*Jatropha* group) and SBM (Soybean group). The growth performance, feed-conversion-ratio, protein-efficiency-ratio and energy-retention did not differ significantly among the three groups in both experimental (recirculatory and respirometer) set ups. Higher protein productive value was observed in plant protein fed group than control group, whereas apparent lipid conversion exhibited reverse trend in both experimental set ups. RBC count, hematocrit and blood-glucose contents were higher in plant-protein fed groups than control group, while other hematological-parameters (WBC count, haemoglobulin, mean-cell-volume; calcium and sodium ions, total-bilirubin and urea-nitrogen in the blood) and metabolic enzymes (alkaline-phosphatase and alanine-transaminase) activities in blood did not differ significantly among the three groups. Average metabolic rate, energy expenditure per g protein fed and retained in the body did not differ significantly among the three groups. The results from the present study established that JPKM is a promising and good quality protein source for Nile tilapia feed and it can replace 62.5% of fishmeal protein in the diet.

3.1.6.3 Are *Jatropha curcas* phorbol esters degraded by rumenmicrobes?

Abstract

Jatropha curcas, a non-edible oil plant, is being promoted as a biofuel plant in a number of countries in tropical and subtropical regions. The kernel meal left after extraction of the oil is a potentially protein-rich feed ingredient. However, the presence of highly toxic phorbol esters limits its use. Degradation of *J. curcas* phorbol esters by rumen microbes, using an in vitro rumen fermentation system, has been investigated in this study.

The difference between phorbol ester contents in the residues obtained with and without substrates at 0, 24, 48 or 72 h of the incubations was statistically similar. Phorbol esters did not affect either the gas or short chain production in the in vitro rumen fermentation system.

Rumenmicrobes can not degrade phorbol esters. In addition, the phorbol esters do not adversely affect rumen fermentation. Ruminants are expected to be as prone as monogastric animals to the toxicity of *Jatropha* seeds.

3.1.6.4 Nutritional, Biochemical, and Pharmaceutical Potential of Proteins and Peptides from *Jatropha*: Review

Abstract

Increased bioenergy consciousness and high demand for animal products have propelled the search for alternative resources that could meet the dual demands. *Jatropha* seeds have potential to fit these roles in view of their multipurpose uses, broad climatic adaptability features, and high oil and protein contents. During the past five years many large-scale cultivation projects have been undertaken to produce *Jatropha* seed oil as a feedstock for the biodiesel industry. The present review aims at providing biological significance of *Jatropha* proteins and peptides along with their nutritional and therapeutic applications. The nutritional qualities of the kernel meal and protein concentrates or isolates prepared from seed cake are presented, enabling their efficient use in animal nutrition. In addition, (a) biologically active proteins involved in plant protection, for

example, aquaporin and betaine aldehyde dehydrogenase, which have roles in drought resistance, and β-glucanase, which has antifungal activity, as well as those having pharmaceutical properties, and (b) cyclic peptides with various biological activities such as antiproliferative, immunomodulatory, antifungal, and antimalarial activity are discussed. It is expected that the information collated will open avenues for new applications of proteins present in *Jatropha* plant, thereby contributing to enhance the financial viability and sustainability of a *Jatropha*-based biodiesel industry.

3.1.6.5 Optimization of conditions for the extraction of phorbol esters from *Jatropha* oil

Abstract

The production of *Jatropha curcas* seeds as a biodiesel feedstock is expected to reach 160 Mt by 2017. The present study aims at extracting phorbol esters (PEs) as a co-product from *Jatropha* oil before processing it to biodiesel. The conditions were optimized for extraction of PEs in organic solvents by using a magnetic stirrer and an Ultra turrax. The extent of reduction in PEs was >99.4% in methanol using any of the stirring tools. However, the extraction using Ultra turrax affected considerably the colour of the remaining oil. Therefore, further solvent:oil ratio, time and temperature were optimized using a magnetic stirrer to get PE rich fraction-I (48.4 mg PEs g^{-1}) and virtually PE-free oil. PEs were 14 fold higher in this fraction than the control oil. PEs, extracted in methanol from the untreated *Jatropha* oil, at 1 mg L^{-1} produced 100% mortality in snails (Physa fontinalis). The methanol extract from virtually PE-free oil when concentrated 20 and 25 time the untreated *Jatropha* oil (equivalent of 20 mg L^{-1} and 25 mg L^{-1} PEs in the control oil) was nontoxic to snails. PE rich fraction-I, obtained as a co-product, can be used in agricultural, medicinal and pharmaceutical applications and the remaining oil can be used for biodiesel preparation. The remaining oil will be friendly to the environment and workers.

3.1.6.6 Quality of Biodiesel Prepared from Phorbol Ester Extracted *Jatropha curcas* Oil

Abstract

Jatropha curcas seeds are rich in oil (28–32%), which can be converted to high quality biodiesel. The oil is non-edible due to the presence of toxic compounds, namely, phorbol esters (PEs). PEs have a number of agricultural/medicinal/pharmaceutical applications and hence their recovery generates a value added co-product towards the biodiesel production chain. This study aims to assess the effects of PE extraction on quality of both the residual oil and the biodiesel production from it. Two Approaches (1, use of an Ultra-turrax; and 2, use of a magnetic stirrer) were used with an effective treatment time of 2 and 5 min, resulting in 80 and 78% extraction of PEs, respectively. The phosphorus content was reduced by 70.2 and 75.8%, free fatty acids by 55.3 and 55.6%, and the fatty acid composition did not change in the residual oils. The peroxide value increased from 2.69 (untreated oil) to 3.01 and 3.49 mequiv O2/kg in the residual oils following Approach 1 and Approach 2, respectively. The biodiesel prepared from both residual oils met European (EN 14214:2008) and American biodiesel standard (ASTM D6751-09) specifications. Oxidative stability indices for both the biodiesels were well within the permitted limit. It is concluded that PEs could be isolated in active forms for various applications by either of the two methods with a high yield and the residual oil can be processed to produce high quality biodiesel.

3.1.6.7 Toxicity of *Jatropha curcas* phorbol esters in mice

Abstract

Phorbol esters are the main toxins in *Jatropha curcas* seed and oil. The aim of this study was to assess the acute toxicity of phorbol esters given by intragastric administration and to determine the LD50 for Swiss Hauschka mice. The LD50 and 95% confidence limits

for male mice were 27.34 mg/kg body mass and 24.90–29.89 mg/kg body mass; and the LD5 and LD95 were 18.87 and 39.62 mg/kg body mass, respectively. The regression equations between the probits of mortalities (Y) and the log of doses (D) was

Y = −9.67 + 10.21 log (D). Histopathological studies on the organs from the dead mice showed: (1) no significant abnormal changes in the organs at the lowest dose (21.26 mg/kg body mass) studied, (2) prominent lesions mainly found in lung and kidney, with diffused haemorrhages in lung, and glomerular sclerosis and atrophy in kidney at doses P32.40 mg/kg body mass, and (3) multiple abruption of cardiac muscle fibres and anachromasis of cortical neurons at the highest dose of 36.00 mg/kg body mass. The results obtained would aid in developing safety measures for the *Jatropha* based biofuel industry and in exploiting the pharmaceutical and agricultural applications of phorbol esters.

3.1.6.8 Fate of *Jatropha Curcas* phorbol esters in soil

Abstract

Jatropha curcas seeds contain 30-40% oil, which can be converted to biodiesel of high quality. The nitrogen rich seedcake is currently used as a fertilizer. Phorbol esters (PE) are the main toxins present in the oil and seedcake. These are co-carcinogens and exhibit antibacterial, antiviral, and insecticidal activities and are toxic to animals. This study reports the fate of PE in soil and to determine its bioactivity during the process of degradation. Two approaches for incorporation of PE in soil were used. In the first approach silica bound to PE and in the second approach seedcake were used. The degradation studies were conducted at temperatures: room temperature, RT, 22-23°C; 32°C and 42°C, and moistures: 13% and 23%. At zero day, concentration of PE in soil was 2.6 and 0.37 mg/g for approach 1 and 2 respectively. When silica bound to PE was used, 100% degradation was observed after 19, 12, 12 days at RT, 32°C and 42°C at 13% moisture, and after 17, 9 and 9 days at 23% moisture. Similarly when the cake was incorporated in soil, 100% degradation was found after 21, 17 and 17 days at 13%

moisture, and after 23, 17, and 15 days at 23% moisture level at RT, 32°C and 42°C. The rate of degradation (%/day) increased with increase in temperature and moisture content (Approach 1: 2.91, 21.77 and 22.42, and 4.13, 23.00 and 23.25 at 13% and 23% moisture, Approach 2: 2.58, 7.07 and 8.67, and 7.94, 10.44 and 14.17 at 13% and 23% moisture; at RT, 32°C and 42°C). Snails (*Physa fontinalis*) and brine shrimp (*Artemeia salina*) assays were used to determine activity of PE in soil. With decrease in PE concentration in soil, the mortality decreased. In conclusion, PE are biodegradable in soil and their degraded products appear to be innocuous.

3.2 University of Hohenheim, Stuttgart (Engineering Group: Inst 440e; Germany 2)

1 Objective 1: Systematical analyzes of engineering properties...
1.1 Introduction...
1.2 Materials and Methods...
1.3 Results and discussion ....
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2 Objective 2: Optimization of oil-pressing with respect to oil and press cake.....................
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3 Objective 2.1: Oil clarification system..
3.1 Introduction...
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4 Objective 3: Preparation for *Jatropha* oil for direct use in plant oil stoves........................
4.1 Introduction...
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5 Objective 3.1: Gaseous emissions of a plant oil stove when fuelled with oil from *Jatropha curcas* . ...
5.1 Introduction...
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6 Objective 4: Analysis of *Jatropha* seed shells as an energy source...................................
6.1 Introduction...
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Objective 1: Systematic analyzes of engineering properties

Introduction

Jatropha curcas L. is a drought – resistant shrub/tree belonging to the Euphorbiaceae family [1, 2]. Cultivated in Central and South America, *J.curcas* was distributed by Portuguese seafarers in Southeast Asia, India and Africa [3]. The plant and its seeds are non edible by animals and humans and the toxicity of the seeds is mainly due to the presence of diterpine [2, 4]. The existing distribution of *J.curcas* shows that its introduction has been the most successful in drier regions of the tropics. It grows on well-drained soils with good aeration and is well adapted to marginal soils of low nutrient content [2].

The potential use of extracted oil from *J.curcas* as transesterified oil (biodiesel) or as a blend with diesel has been studied [1, 5-7]. The calorific value and cetane number of *J.curcas* oil are comparable to diesel, but the density and viscosity are much higher [8]. Since the density of oil is high, the engine performance, emissions and combustion parameters can be reached by optimizing the injector opening pressure, injection time, injection rate and enhancing the swirl level of the operating engine [1, 6]. High viscosity of the *Jatropha curcas* oil is not advantageous for the compression ignition engine. Pramanik (2003) has studied the performance of an engine using blends and *Jatropha* oil in a single cylinder (compression ignition) engine and compared the performance obtained with a diesel engine. Adequate thermal efficiencies of the engine were obtained with blends containing up to 50 % volume of *Jatropha* oil. 40–50 % of *J.curcas* oil can be replaced without any engine modification and preheating of the blends [1, 6].

In the oil industry, different processes are undertaken before oil extraction occurs. When *J.curcas* fruits arrive for oil extraction different processes are conducted before: (a) dehulling, separating hull from nut, (b) deshelling, separating shell from kernel, (c) drying and then (d) oil extraction. Physical, mechanical and chemical properties of seed and kernel are needed for the design of equipment to handle, transport, process, store and assess the product quality [1, 9, 10]. The aim of this study was to investigate the physical, mechanical and chemical properties of *Jatropha curcas* L. seeds, as part of an optimization program for deshelling and oil extraction of *J.curcas* for direct use in plant

oil stoves. The essential parameters were bulk/solid density, volume, porosity, surface area, specific surface area, coefficient of friction, static angle of repose, rupture force, deformation at rupture point, deformation ratio at rupture point, hardness, energy used for rupture, moisture content, crude lipids, oil density, kinematic viscosity, gross calorific value, iodine value, water content of oil, C – impurities and acid value.

Materials and Methods

Material used and separation

For this study, dried *J.curcas* seeds (mc. 9 % db.) were imported from India. The place of the seeds' origin can be characterized by an annual precipitation of 1000-1200 mm and a temperature range between 15-35°C. The *J.curcas* plantation was 11-12 years old with yields as high as 7500 kg/ha/a. Seeds were harvested manually during November-December 2007 (harvest season) and stored in jute bags in a warehouse facility at a temperature range of 14-30°C.

J. curcas seeds were inspected and manually cleaned to avoid foreign matter. Seeds were separated by a pneumatic conveyor and labeled into four groups according to their average weight Fig. 1-1.

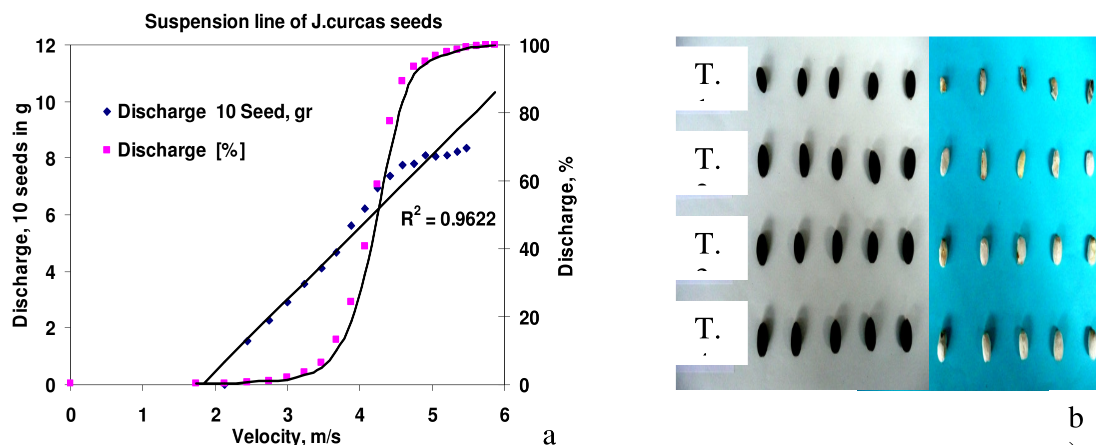

Fig. 1-1 Suspension line of *J. curcas* seeds (a) and selected groups of seeds and kernels based on their average weight (b) via a pneumatic conveyor

Kernel groups were obtained by breaking down the seeds and were classified according to their respective seed groups. Seed and kernel groups were used to determine the

physical, mechanical and chemical properties of *J.curcas*.

Measurements of physical, mechanical and chemical properties were performed at the Institute of Agricultural Engineering in the Tropics and Subtropics, University of Hohenheim.

Physical Properties

One hundred seeds and kernels of each group were randomly selected and weighed individually (five replicates) with a precision electronic balance, with an accuracy of ± 0.001 g. The thousand unit mass was calculated by multiplying the hundred unit mass by ten [1, 11-14]. One hundred measurements of the axial dimensions x, y and z namely length (x), breath (y) and height (z) from *J. curcas* seed and kernels (Fig. 1-2) were recorded with digital vernier callipers (Mitutoyo, model Absolute Digimatic, Japan) with an accuracy of ± 0.01mm.

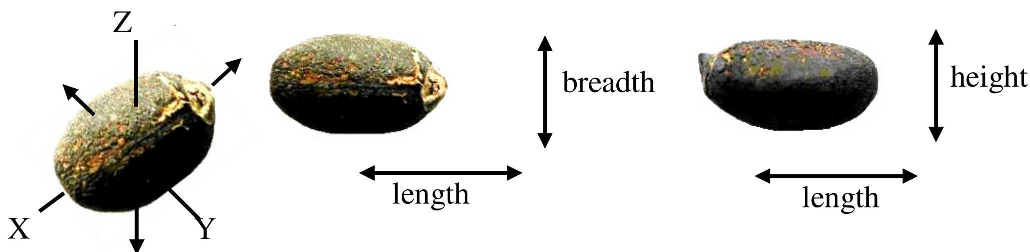

Fig. 1-2 Three major dimensions of *J. curcas* seeds where axis x is length; axis y is breadth and axis z is height

The geometric mean diameter (d_g) and the arithmetic mean diameter (d_a) were calculated as follows [12, 15]:

$$d_a = \frac{(x + y + z)}{3}; \qquad d_g = (x \cdot y \cdot z)^{1/3} \qquad \text{Eq. 1}$$

Sphericity (ϕ) was calculated based on the isoperimetric property of a sphere [15], the higher the sphericity value for the seed the closer its shape is to a sphere is [1].

$$\phi = \frac{(x \cdot y \cdot z)^{1/3}}{a} = \frac{d_g}{a} \qquad \text{Eq. 2}$$

Bulk density, solid density, volume and porosity

The bulk density (ρb) is the ratio of the mass sample to its total volume [16]. It was calculated by putting the bulk material into a container (17cm x 22cm) with known weight and volume and weighed [1]. A constant feeding rate and impaction were applied

for better distribution and settlement of the sample [17]. Bulk density was calculated with the following formula:

$$\rho_b = \frac{w}{v} \qquad \text{Eq. 3}$$

where: *w* is the weight of the sample (g), *v* is the total volume of the sample (ml)
The solid density (ρs) or true density is defined as the ratio of mass of the sample to its true volume, without considering any air spaces among samples [18]. True volume (v) was determined based on the assumption that *J. curcas* seeds are similar to a scalene ellipsoid where a>b>c (Fig. 2-2) and was calculated for one hundred samples. The formula was derived from the volume of a prolate ellipsoid as follows [15],

$$v = \frac{4\pi(x \cdot y \cdot z)}{3} \qquad \text{Eq. 4}$$

Porosity (ε) indicates the amount of pores in the bulk material [15]. It was calculated for one hundred samples as follows,

$$\varepsilon = \left[1 - \frac{\rho_b}{\rho_s}\right]100 \qquad \text{Eq. 5}$$

Surface area and specific surface area

Approximate surface area (S) and specific surface area (S_s) were determined following the calculations based on prolate ellipsoids [15]. By definition, the specific surface area is the surface area of one sample multiplied by the number of samples in a given mass and divided by the bulk volume. This last one was found by multiplying the mass of one sample by the number of samples in the given mass and by dividing it by the bulk density [1, 19].

Surface area in mm^2 and specific surface area in mm^2/cm^3 for seeds and kernels were calculated for one hundred samples (five replications were made for each group) as follows;

$$S = 2\pi\left[\frac{y}{2}\right]^2 + 2\pi\left[\frac{x \cdot y}{4e}\right]\sin^{-1} e \qquad \text{where: } e = \left[1 - \left[\frac{y}{x}\right]^2\right]^{1/2} \qquad \text{Eq. 6}$$

$$S_s = \frac{S\rho_b}{m} \qquad \text{Eq. 7}$$

Coefficient of static friction

The static coefficient of friction (μ) of *J. curcas* seeds and kernels against different

structural materials, namely plywood, aluminium, stainless steel and rubber was determined (Fig.1-3). A wooden (container) frame (10cmx10cmx6cm) placed on different flat surfaces was filled with samples. The filled container was pulled along the flat surface and the force required to cause motion was recorded by a fixed spring scale Model Kern HDB 5k5.

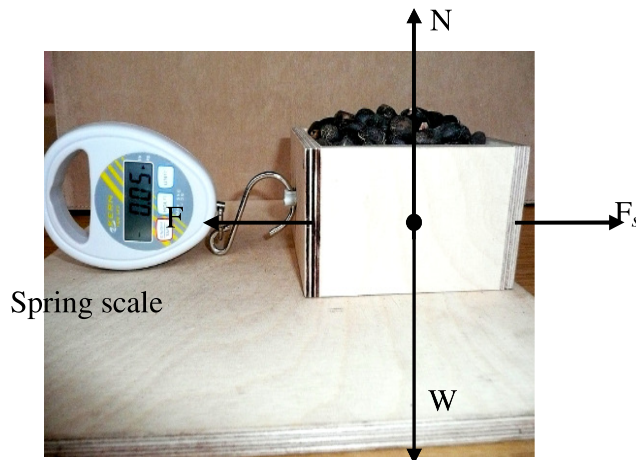

Fig. 1-3 A filled container and spring scale for determination of the coefficient of friction of seeds on a plywood surface

The static coefficient of friction is the ratio of force required to slide the sample over a surface divided by the normal force pressing the sample against a surface [12] was calculated as follows:

$$\mu = \frac{f}{w} \qquad \text{Eq. 8}$$

where: f (N) is the force required to just cause motion of the weight w (g) of the object

Static and dynamic angle of repose

The angle of repose (θ), indicates the cohesion among the individual units of any material. The higher the cohesion, the higher is the angle of repose [1]. The angle of repose for *J. curcas* seeds and kernels was measured using the filling method to determine the static angle of repose or the emptying method to determine the dynamic angle of repose.

For the filling method, a Hele-Shaw Cell [20] was built (Fig. 1-4). The sample was poured into the cell through a hopper fixed onto the right side in order to ensure a constant feed rate to create a natural slope. The slope angle namely the static angle of

repose was read with a protractor.

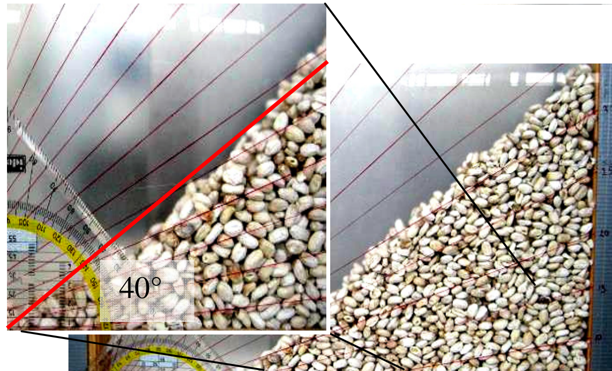

Fig. 1-4 Hele-Shaw Cell for determination of the angle of repose of seeds and kernels

For the emptying method, a bottomless cylinder was used (Fig. 1-5) (10cmx5cm) [14, 21]. The cylinder was placed over a flat surface and sample material was filled into it. The cylinder was raised slowly allowing the sample to flow down to assume a natural pile and slope. The dynamic angle of repose was calculated from the measurements of the vertical depth and the diameter of spread of the sample material as follows:

$$\theta = \tan^{-1}\frac{2h}{d} \quad \text{Eq. 9}$$

where h is the height of the pile (cm), d the diameter of the sample (cm)

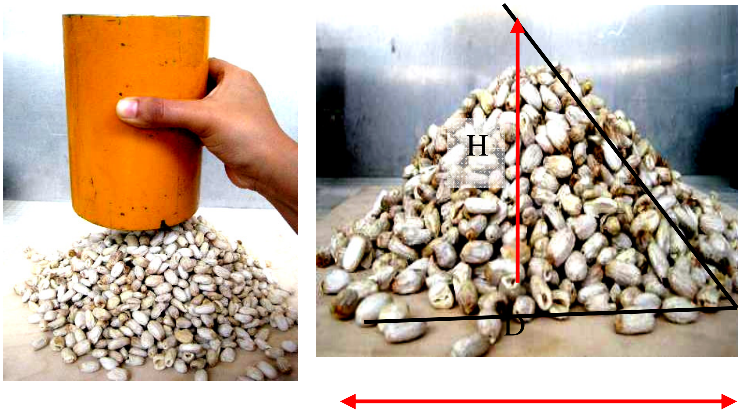

Fig. 1-5 Determination of the dynamic angle of repose in kernels

Mechanical properties: rupture force, deformation at rupture point, deformation ratio at rupture point, hardness and energy for rupture

Laboratory compression tests were carried out by using a compression tester (Instron – 4300 Series, and data was acquired and processed using the software BlueHill2. Twenty

units of *J. curcas* seeds or kernels from each of the four fractions were tested in horizontal (x), transversal (y) and vertical (z) loading directions (see Fig. 3). The rupture force, deformation at rupture point, deformation ratio at rupture point, hardness and energy used for rupture were all measured at a velocity of 1.25 mm/min, which is suitable for highly oil-bearing materials [22].

The rupture force, FR (N) is the minimum force required to break the sample. The deformation at rupture point, RDP (mm) is the deformation along the loading direction. The deformation ratio at rupture point, RDR is the axial strain at rupture point of the sample. It was calculated as a ratio of deformation at the rupture point to the length of the sample in the loading direction:

$$R_{DR} = \frac{R_{DP}}{d} \qquad \text{Eq. 10}$$

where d is the x,y or z dimension of seeds or kernels (mm).

Hardness, H (N/mm) is the ratio of rupture force and deformation at rupture point. It was calculated as:

$$H = \frac{F_R}{R_{DP}} \qquad \text{Eq. 11}$$

Energy for rupture, ER (N mm) is the energy needed to rupture the sample Fig. 1-6, which could be determined from the area under the curve between the initial point and the rupture point.

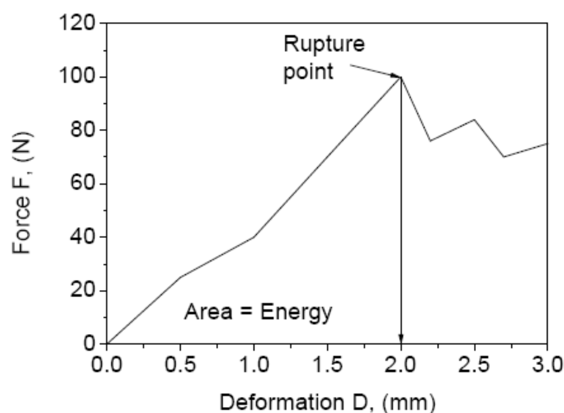

Fig. 1-6 Typical force-deformation curve for compressed J.curcas nut

The area was measured using a polarplanimeter with an accuracy of ± 0.2 % (model:

OTT-Kompensations-Polarplanimeter).

Chemical properties

Chemical properties were analyzed at the Oil Laboratory of the institute of Agricultural Engineering in the Tropics and Subtropics at Hohenheim University. The samples were prepared following the guidelines laid down in the Deutsches Institut für Normung (DIN standards) for fat and oil derivatives.

Table 1-1 demonstrates the chemical parameters analyzed in this study and the methods used according to the literature.

Table 1-1 Method used for analyzing chemical properties of J.curcas oil

Chemical properties		
Moisture content (seeds & kernels	% db	[23]
Oil content (seeds & kernels)	% db	[24]
Density	kg/m^3	[25]
Kinematic viscosity	mm^2/s	[26]
Gross calorific value	MJ/kg	[27]
Iodine value	g/100g	[28]
Acid number	KOH/g	[29]
Impurities. C	% mass/max	[30]
Water content in oil	mg/kg	[31]

The results obtained were analyzed according to the “Pre-standard DIN V 51605 for rapeseed oil as fuel”. This pre-norm was created to standardize the quality of the oil produced for satisfactory engine operation [32]. Despite the fact, that this standard was created for rapeseed oil, the limiting values can be compared for other oils that are used in engines [33]. The *J. curcas* oil meets the quality standards laid down for rapeseed as a fuel [34].

Results and discussion

Seed dimensions

Thousand unit mass and unit mass of seeds and kernels increased with an increase of the sample group. Differences between the mean values were significant at $p \leq 0.01$ (Table 1-2, Table 1-3). The coefficients of correlation showed that the length and unit mass ratio in seeds xs/ms were highly significant ($R^2 = 0.866$) when compared to the length and unit mass ratio in kernels xk/mk ($R^2 = 0.667$). This result indicated that the unit mass of the kernel was closely related to its length, while the unit mass of the seed showed less influence on length with its length. Moreover, there was a close correlation between the unit mass of seeds and the unit mass of kernels ms/mk ($R^2 = 0.884$).

Table 1-2 Some physical properties of *J. curcas* seeds at 9% mc.db.

	Group			
Properties	T.1 0.2g - 0.35g	T.2 0.36g - 0.5g	T.3 0.51g - 0.7g	T.4 0.71g - 0.9g
1000 unit mass, g	270.44±13.48a	432.08±3.58b	606.58±8.90c	771.10±6.73d
Unit mass, g	0.27±0.05a	0.43±0.04b	0.61±0.06c	0.77±0.05d
Length, mm	16.20±1.27a	16.97±1.02b	17.74±0.85c	18.46±0.71d
Breadth, mm	10.27±0.74a	10.72±0.59b	11.04±0.45c	11.24±0.37d
Height, mm	7.75±0.59a	8.29±0.59b	8.67±0.44c	9.04±0.45d
Arithmetic mean	11.41±0.69a	12.00±0.58b	12.48±0.42c	12.91±0.33d
Geometric mean	10.87±0.65a	11.46±0.56b	11.93±0.39c	12.33±0.31d

Mean±SD, n=500. Different letters in columns indicate significant differences between

Seed length in the four groups ranged from 16.20 mm to 18.46 mm, while seed breadth ranged from 10.27 mm to 11.24 mm and seed height ranged from 7.75 mm to 9.04 mm. Kernel length in the four groups ranged from 12.82 mm to 15.14 mm, while breadth mean values ranged from 6.09 mm to 8.85 mm, and height mean values ranged from 4.44 mm to 7.09 mm. Seeds and kernels of the T.4 group were longer, thicker and higher than the seeds of the groups T.3, T.2 and T.1.

Table 1-3 Some physical properties of *J. curcas* kernels at 9% mc.db.

	Group			
Properties	t.1 0.2g - 0.35g	t.2 0.36g - 0.5g	t.3 0.51g -	t.4 0.71g - 0.9g
1000 unit mass, g	119.60±5.30a	216.96±21.96b	354.72±12.71c	491.48±0.48d
Unit mass, g	0.12±0.05a	0.22±0.06b	0.35±0.07c	0.49±0.05d
Length, mm	12.82±1.10a	13.60±0.99b	14.55±0.70c	15.14±0.52d
Breadth, mm	6.09±0.90a	7.03±0.64b	8.19±0.63c	8.85±0.36d
Height, mm	4.44±0.83a	5.35±0.78b	6.30±0.72c	7.09±0.57d
Arithmetic mean	7.78±0.69a	8.66±0.48b	9.68±0.42c	10.36±0.27d
Geometric mean	6.99±0.75a	7.97±0.52b	9.07±0.49c	9.82±0.31d

Mean±SD, n=500. Different letters in columns indicate significant differences between

These observations indicated that the seed dimensions increased proportionally with an increase of the sample group, and the Tukey's Test confirmed that the seed and kernel

dimensions were significant at $p \leq 0.01$. Linear equations of the length, breadth and height for seeds and kernels can be expressed as follows:

$$x_s = 0.7551 \cdot m_s + 15.454 \qquad (R^2 = 0.9997) \qquad (1)$$

$$y_s = 0.3247 \cdot m_s + 10.006 \qquad (R^2 = 0.9689) \qquad (2)$$

$$z_s = 0.4247 \cdot m_s + 7.3792 \qquad (R^2 = 0.9910) \qquad (3)$$

$$x_k = 0.7919 \cdot m_k + 12.049 \qquad (R^2 = 0.9932) \qquad (4)$$

$$y_k = 0.9456 \cdot m_k + 5.1763 \qquad (R^2 = 0.9904) \qquad (5)$$

$$z_k = 0.889 \cdot m_k + 3.5744 \quad (R^2 = 0.9986) \qquad (6)$$

The coefficients of correlation showed that the length to breadth ratio for seeds xs/ys (R^2 =0.557) was more significant than its length to height ratio xs/ys (R^2 =0.278). Contrary to this, the length to height ratio in kernels xk/zk (R^2 =0.574) was more significant than its length to breadth ratio xk/yk (R^2 =0.429), and the seed to kernel length ratio xs/xk, seed to kernel breadth ratio ys/yk, and seed to kernel breadth ratio zs/zk showed low significant correlations. These results indicated that the breadth of seeds was more closely related to its length than to its height; while the breadth of kernels showed less influence on length. The following general expression can be used to describe the relationship of seed dimensions:

$$x_s = 1.60 \cdot y_s$$

Likewise, the following general expression can be used to describe the relationship of kernel dimensions:

$$x_k = 2.91 y_k = 4.56 z_k$$

Geometric and arithmetic mean diameter of seeds and kernels increased with an increase of the sample group. Differences between the mean values were significant at $p \leq 0.01$ (Table 1-2, Table 1-3). Linear equations of the arithmetic and geometric diameters in seeds and kernels can be expressed as follows:

$$d_a = 0.5549 \cdot m_s + 10.679 \qquad (R^2 = 0.9074) \qquad (7)$$

$$d_g = 0.4835 \cdot m_s + 10.438 \qquad (R^2 = 0.9923) \qquad (8)$$

$$d_a = 0.8757 \cdot m_k + 6.9324 \qquad (R^2 = 0.9948) \qquad (9)$$

$$d_g = 0.9595 \cdot m_k + 6.063 \qquad (R^2 = 0.9950) \qquad (10)$$

The coefficients of correlation showed that the length to arithmetic diameter ratio of seeds xs/dxs (R^2 =0.919), and the length to arithmetic diameter ratio of kernels xk/dxk (R^2 =0.848) were highly significant when compared with the seed to kernel arithmetic diameter ratio dxs/dxk (R^2 =0.630). These results indicated that arithmetic diameter values for seeds and kernels were closely related to length, while the arithmetic diameter of seeds showed less influence on the arithmetic diameter of kernels. Likewise, the length to geometric diameter ratio in seeds xs/dgs (R^2 =0.800), and the length to geometric diameter ratio of kernels xk/dgk (R^2 =0.749) were highly significant when compared with the seed to kernel geometric diameter ratio dgs/dgk (R^2 =0.556).

Sphericity, surface area and specific surface area

Table 1-4 shows sphericity, surface area and specific surface area of seeds and kernels of all groups. It was calculated that seeds have mean values of sphericity of 0.67, while kernels have mean values of 0.60.

Table 1-4 Sphericity, surface area and specific surface area of *J. curcas* seeds and kernels

Particle	Sphericity	Surface area (mm2)	Specific surface area (mm2/cm3)
Seed			
T.1	0.67±0.04a	434.91±53.57a	4.19±1.02a
T.2	0.68±0.03ac	474.59±45.89b	3.21±0.43ab
T.3	0.67±0.02a	507.45±36.00c	3.12±0.35b
T.4	0.67±0.02ab	532.89±30.09d	3.05±0.20c
Kernel			
t.1	0.55±0.05a	186.83±40.52a	5.31±1.87a
t.2	0.59±0.05b	233.27±30.64b	4.01±1.17bc
t.3	0.62±0.04c	298.40±32.97c	3.86±0.79c
t.4	0.65±0.03d	339.59±20.65d	3.35±0.33d

Mean±SD, n = 500. Different letters in columns indicate significant

During deshelling, low sphericity values (i.e. flattened shapes) may be present for loosely bound shells which could create disadvantages when rolling or conveying the material as observed in sunflower seeds [35].

Garnayak et al, 2008 and Sirisomboon et al, 2007, have reported sphericity mean values of 0.64 in seeds, and from 0.66 to 0.68 in kernels, which are contrary to the results obtained in this investigation. This may be due to the sampling procedure, since the above mentioned authors studied longer, thicker and heavier seeds. Statistical analysis indicated that the differences among the sphericity mean values in seed groups were almost none significant, while differences of mean sphericity values in kernels were significant at $p \leq 0.01$. In other words, the average sphericity values found in seeds were almost the same (Table 1-4). The following equations show the best fit for seed and kernel sphericity values respectively,

$$\phi = -0.002 \cdot m_s^2 + 0.0082 \cdot m_s + 0.6672 \quad (R^2 = 0.9558) \qquad (11)$$

$$\phi = 0.0344 \cdot m_k + 0.5161 \qquad (R^2 = 0.9876) \qquad (12)$$

Surface area mean values in seeds and kernels were larger in the group T.4 than those in smaller groups, while specific surface area values in T.4 seeds and kernels were the lowest among groups. The coefficients of correlation showed that the length to surface area ratio of seeds xs/Ss(R^2 = 0.825), the length to surface area ratio of kernels xk/Sk (R^2 =0.783), and the length to specific surface area ratio of seeds xs/Ss (R^2 =-0.707) were highly significant, while the length and specific surface area ratio of seeds xs/Sss, the specific surface area to surface area ratio of kernels Ss/Sk and the seed to kernel specific surface area ratio Sss/Ssk showed low correlation. These results indicated that the surface area of seeds and kernels were closely correlated to length, thus T.4 seeds require higher energy transfer ratios for deshelling than the smaller ones due to its higher exposed area. The following linear equations show the best fit for the seed and kernel surface area and specific surface area respectively,

$$S_s = 32.682 \cdot m_s + 405.76 \quad (R^2 = 0.9906) \qquad (13)$$

$$Ss_s = -0.3527 \cdot m_s + 4.2763 \qquad (R^2 = 0.7187) \qquad (14)$$

$$S_k = 52.343 \cdot m_k + 133.66 \quad (R^2 = 0.9929) \qquad (15)$$

$$Ss_k = -0.6043 \cdot m_k + 5.6416 \qquad (R^2 = 0.8710) \qquad (16)$$

During pneumatic sample classification (i.e. grouping), both surface area and specific surface area values are needed to calculate the resistance of the sample against the pneumatic airflow of the conveyor, that later may influence the deshelling efficiency. Further, both surface area and shape characteristics of the sample may have a considerable effect on the formation of different angles of repose [36].

Bulk density, solid density, volume, and porosity

Bulk and solid density values in seeds and kernels increased with an increase in the sample group (Table 1-5). Seed density values were lower than those found in the kernel, which might be due to the presence of shell fragments in the seeds, which may reduce the mass per unit volume ratio. A linear increase in seed density owing to a mass increase is presented in the following linear equations,

$$\rho_{bs} = 0.065 \cdot m_s + 0.18 \qquad (R^2 = 0.9929) \qquad (17)$$

$$\rho_{ss} = 0.1555 \cdot m_s + 0.3656 \qquad (R^2 = 0.9960) \qquad (18)$$

Likewise, the following linear equations show the linear equations for kernel densities,

$$\rho_{bk} = 0.063 \cdot m_k + 0.235 \qquad (R^2 = 0.9788) \tag{19}$$

$$\rho_{sk} = 0.1389 \cdot m_k + 0.7145 \qquad (R^2 = 0.9588) \tag{20}$$

Differences between average density values were significant at $p \leq 0.01$. In comparison with sunflower seeds, the solid density values in *J. curcas* seeds and kernels were similar, while the bulk density values in *J. curcas* seeds and kernels were lower than those in sunflower [37]. Bulk and solid density are used in determining the size of storage bins and containers.

Table 1-5 Density, porosity and volume of *J. curcas* seed and kernels

Particle	Bulk density	Solid density	Volume (m3)	Porosity (%)
Seed				
T.1	0.25±0.01a	0.51±0.13a	0.54±0.09a	49.87±12.86a
T.2	0.30±0.01b	0.69±0.13b	0.63±0.09b	57.07±6.90b
T.3	0.38±0.00c	0.83±0.08c	0.71±0.07c	54.87±4.51cd
T.4	0.44±0.01d	0.98±0.06d	0.79±0.06d	55.11±2.84d
Kernel				
t.1	0.30±0.01a	0.82±0.27a	0.15±0.04a	59.41±12.75ad
t.2	0.35±0.00b	1.04±0.31b	0.21±0.04b	63.12±11.7b
t.3	0.44±0.01c	1.15±0.27c	0.31±0.05c	59.26±9.67ac
t.4	0.48±0.01d	1.24±0.16d	0.40±0.04d	60.77±4.92dc

Mean±SD, n=5. Different letters in rows indicate significant differences

The volume of seeds and kernels increased with an increase of the sample group. Differences among volume mean values in seeds and kernels were significant at $p \leq 0.01$. The coefficients of correlation showed that the length to volume ratio in seeds xs/vs (R^2 =0.852) and, the length to volume ratio in kernels xk/vk (R^2 =0.628) were highly significant when compared to the seed to kernel volume ratio vs/vk (R^2 =0.552). These results indicated that volumetric values in seeds and kernels were closely related to length, while seed volume showed a lower correlation with the kernel volume. The following equations show the best fit for the seed and kernel volumetric values respectively,

$$v_s = 0.0804 \cdot m_s + 0.4681 \qquad (R^2 = 0.9975) \tag{21}$$

$$v_k = 0.0851 \cdot m_k + 0.056 \qquad (R^2 = 0.9948) \tag{22}$$

Porosity values of the bulk of seeds in the four groups were lower than those in the bulk of kernels. These results indicate that aeration of the kernel bulk may be easier than that of seed bulk as observed previously by Sirisomboon, 2007. Porosity values varied from 49-57% for seeds and, from 59-63% for kernels. The following polynomial equation was found to describe the variation of porosity in seeds,

$$\varepsilon_s = -1.7418 \cdot m_s^2 + 10.06 \cdot m_s + 42.142 \qquad (R^2 = 0.7521) \qquad (23)$$

Nevertheless, differences between the porosity mean values for kernels were quite noticeable but for seeds were almost insignificant.

Static coefficient of friction

On all the surfaces studied (plywood, aluminium, stainless steel and rubber) the static coefficient of friction of kernels was higher than for seeds (Fig. 1-7, Fig. 1-8). This was mainly due to the viscous surface of kernels as observed by Sirisomboon, 2007. The static coefficient of friction for plywood was the highest for kernels (Fig. 7). For transportation reasons, containers made of plywood could be used. Contact between kernels and plywood surfaces can be reduced in order to prevent material losses that might have an influence on kernel meal yields.

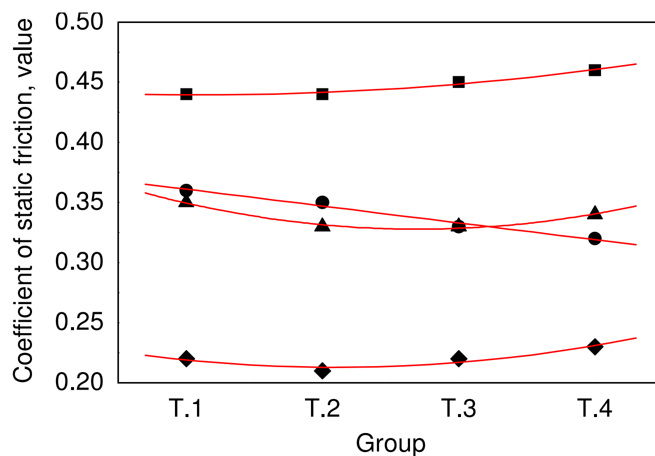

Fig. 1-7 Coefficient of static friction of *J. curcas* seeds on various surfaces: plywood (▲), aluminium (●), stainless steel (♦), rubber (■)

The static coefficient of friction of seeds on aluminium showed a decreasing tendency to drop with an increase of the sample groups, while the contrary was observed for kernels. The first aspect was due to the high sphericity values found in seeds (0.67, see Table 1-4) that allowed seeds to slip over the aluminium sheet more easily, thereby creating less friction between the surfaces.

The oily and viscous surfaces present in kernels may have an influence on the increase in frictional forces existing with respect to the kernel grouping.

The coefficient of static friction of seeds and kernels for stainless steel remained almost constant with respect to the four sample groups. This result is probably due to the smoother surfaces inherent in stainless steel sheeting allowing the seeds and kernels to slide over the surface more easily. During the deshelling and pressing operations it was recommendable

to use aluminium and stainless steel components in order to offset the negative impact of process efficiency and product quality when using other materials.

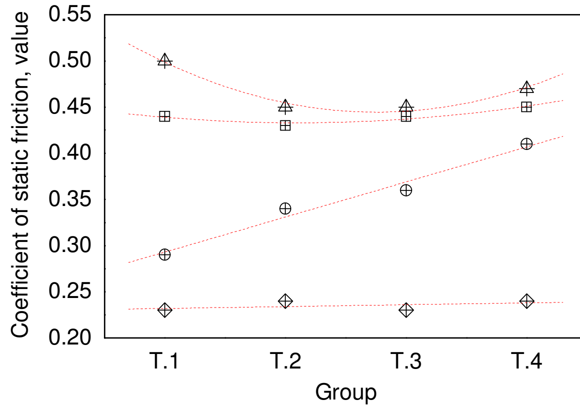

Fig. 1-8 Coefficient of static friction of *J. curcas* kernels on various surfaces: plywood (▲), aluminium (●), stainless steel (♦), rubber (■)

Additionally, static coefficient of friction of seeds on rubber was higher compared to that on other surfaces. Similar results were found for the coefficient of static friction of kernels on rubber surfaces. During deshelling, rubber conveyors can be used; hence the frictional forces present in this material, which might have a negative influence in yield and quality (protein content) of the kernel meal. Therefore, further studies of possible effects of the frictional forces compared to the kernel meal and quality should be evaluated. The following linear and polynomial equations show the best fit for the coefficients of static friction in seeds,

$$\mu_{ps} = 0.0055 \cdot m_s^2 - 0.0313 \cdot m_s + 0.3735 \qquad (R^2 = 0.9908) \qquad (24)$$

$$\mu_{as} = 0.016 \cdot m_s + 0.381 \qquad (R^2 = 0.9726) \qquad (25)$$

$$\mu_{ss} = 0.004 \cdot m_s^2 - 0.0156 \cdot m_s + 0.228 \qquad (R^2 = 0.7585) \qquad (26)$$

$$\mu_{rs} = 0.0052 \cdot m_s + 0.434 \qquad (R^2 = 0.9657) \qquad (27)$$

Likewise, the following linear and polynomial equations show the best fit for the coefficients of static friction in kernels,

$$\mu_{pk} = 0.0195 \cdot m_k^2 - 0.1041 \cdot m_k + 0.5795 \qquad (R^2 = 0.9863) \qquad (28)$$

$$\mu_{ak} = 0.0384 \cdot m_k + 0.253 \qquad (R^2 = 0.9841) \qquad (29)$$

$$\mu_{sk} = -0.0015 \cdot m_k^2 + 0.0113 \cdot m_k + 0.2205 \qquad (R^2 = 0.4342) \qquad (30)$$

$$\mu_{rk} = 0.005 \cdot m_k^2 - 0.0202 \cdot m_k + 0.453 \qquad (R^2 = 0.9963) \qquad (31)$$

Statistical analysis indicated that the differences among the static coefficient of friction mean values on various surfaces in the seed and kernel groups were almost insignificant at $p \leq 0.01$. In other words, the values obtained in the calculations were almost the same.

Static and dynamic angle of repose

The angle of repose obtained from the filling method was higher than that obtained from the emptying method for both seeds and kernels. This was because in the filling method, the material was already in the container before measuring took place but for the emptying method was free to move without a container, as observed by Sirisomboon, 2007. The static angle of repose of kernels obtained in the filling method decreased with an increase in the group sample (Fig. 1-9), while the static angle of repose in seeds had a tendency to remain constant. The sphericity of seeds and kernels might have an influence on the static angle of repose, which is important for the design of the shapes of silos and containers where the bulk material is stored, as well as for the determination of belt conveyor width.

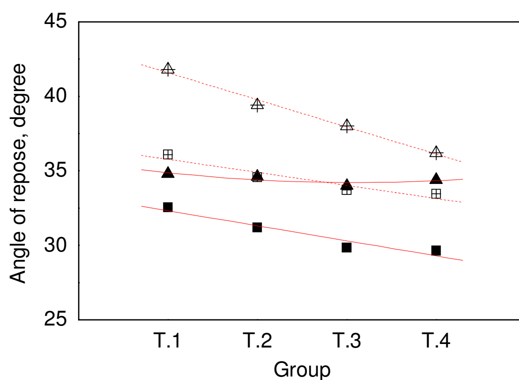

Fig. 1-9 Angle of repose values for *J. curcas* seed (—) and kernel (---) by the filling method (▲) and emptying method (■)

The dynamic angle of repose of seeds and kernels obtained in the emptying method decreased with an increase in the sample groups.

The effect of this is probably due to particle sphericity and the free area at the apex. It was observed that the flow velocity of the particles in both the filling and emptying methods had an influence on the formation of varying angles of repose. The dynamic angle of repose is important for the design of hoppers, funnels, storage bins and conveyors during pressing operations [36]. Constant feeding velocities and angles of repose should be determined before starting feeding operations to avoid possible flow retention due to the formation of varying angles of repose. Deshelling operations for sunflowers for instance affect the angle of repose created by different flow rates. At high feeding rates, seeds

entering the desheller collide with each other instead of colliding with the desheller chamber, therefore inhibiting the deshelling process that could also occur to *J. curcas* seeds [38].

The following linear and polynomial equations show the best fit for the angle of repose of seeds,

$$\theta_{fs} = 0.15 \cdot m_s^{\ 2} - 0.93 \cdot m_s + 35.65 \qquad (R^2 = 0.7200) \qquad (32)$$

$$\theta_{se} = -1.0082 \cdot m_s + 33.325 \qquad (R^2 = 0.9281) \qquad (33)$$

Likewise, the following linear equations show the best fit for the angle of repose in kernels,

$$\theta_{fk} = -1.82 \cdot m_k + 43.4 \qquad (R^2 = 0.9888) \qquad (34)$$

$$\theta_{ek} = -0.8772 \cdot m_k + 36.649 \qquad (R^2 = 0.9079) \qquad (35)$$

Statistical analysis indicated that the differences among the angle of repose mean values in the seed and kernel groups were almost insignificant at $p \leq 0.01$.

Rupture force

The rupture force in seeds and kernels increased with an increase in the sample group. The highest rupture force was applied to T.4 seeds and the lowest to T.1 kernels (Fig. 1-10).

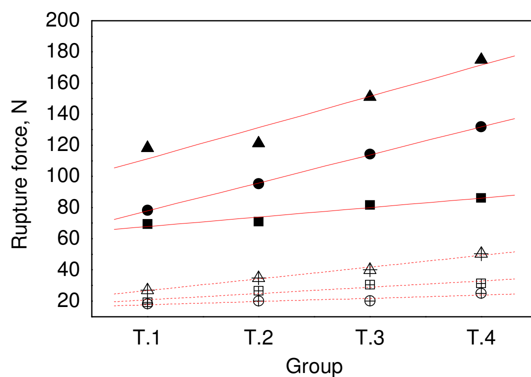

Fig. 1-10 Rupture force values for *J. curcas* seed (—) and kernel (---) under horizontal (●), transversal (■) and vertical (▲) loading, N represents Newtons

High rupture force values were related to the hard shell found in seeds and low values were related to the soft texture found in kernels as noted by Sirisomboon, 2007. Furthermore, rupture forces may also be related to the unit mass and thus matured and/or larger seeds (in this case T.4 seeds) may require higher forces to fracture when observing sunflower [38]. It was also observed that no kernel breakage during the compression of T.3 and T.4 seeds took place, while kernel breakage occurred in the seed groups T.1 and T.2. These results might be due to the inherent “clearance” between shell and kernels for the smaller sample

groups. Similar results were also reported for shea nuts [39]. Additionally, the shell clearance, shell thickness and shell content in seeds might have an effect on the rupture forces as observed in sunflower seeds [40].

Seeds loaded in the horizontal, transversal and vertical directions required from 78.19 N to 131.75 N, from 69.41 N to 86.13 N and from 118.22 N to 174.96 N respectively to induce shell rupture. These results indicated that seeds required less compressive force to deshell when loaded in the transversal direction when compared to the horizontal and vertical directions. The highest compressive force was required when vertically loaded because of the hard polar edges found in seeds (Fig. 1-11). Further, it was noticed that shell breakage in all the seed groups occurred commonly across the length of the seed shell, which might be considered as being the "weak points of the shell" as observed in sunflower seeds [40]. Likewise, probable small variations in moisture content observed between hard and weak shells, as a result of the seed's exposure to different handling operations during the experiments might influence the breaking performance.

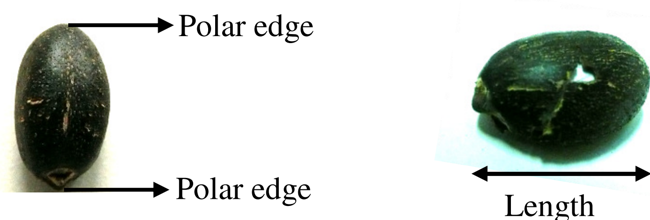

Fig. 1-11 *J. curcas* seed peculiarities. Left (polar edges in seeds), Right (seed showing fracture formed during the compression tests)

The force needed to rupture kernels in the horizontal, transversal and vertical directions required from 18.03 N to 26.93 N, from 19.14 N to 31.32 N and from 26.93 N to 50.29 N respectively. These results indicated that kernels required less compressive force to rupture when loaded in the horizontal direction compared to the transversal and vertical directions. For the vertical loading direction, the force required to fracture the kernels was higher since kernels tended to be compacted, thus causing retarded breakage or failure. Additionally, rupture efficiency is subject to sample classification (i.e. grouping), since incorrect classification might allow small seeds to pass uncracked through the deshelling equipment or even larger seeds might be broken into small fragments instead of being deshelled as observed in other seeds and grains [41].

Deformation at rupture point and deformation ratio at rupture point

The deformation of seeds at the rupture point (Fig. 1-12) (i.e. deformation at first seed breakage) were 0.96 mm, 0.94 mm and 0.84 mm on average in the horizontal, transversal

and vertical loading directions respectively, while the kernel deformations observed were 1.15 mm, 1.44 mm and 1.65 mm respectively.

If the deformation results are compared with the results obtained in the rupture forces, it can be observed that the minimum rupture force required under transverse loading also created the highest deformation in seeds (12).

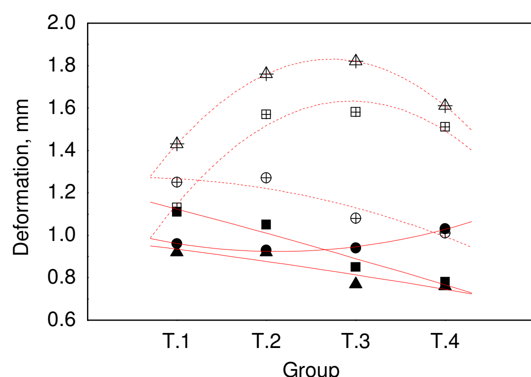

Fig. 1-12 Deformation at rupture point values for *J. curcas* seed (—) and kernel (---) under horizontal (●), transversal (■) and vertical (▲) loading

Moreover, it was observed that deformation in kernels under horizontal loading was the lowest, though the fracture produced was cleaner than in the other loading directions as observed during the experiments with the Instron equipment. Thus, the induced deformation and rupture point applied at the horizontal loading position may be important when considering kernel meal pressing.

The maximum deformation ratio at rupture point in seeds was obtained when the sample was loaded in the horizontal direction (0.12), while the minimum ratio was obtained when the sample was loaded in the vertical direction (0.10). For kernels, the maximum deformation ratio at rupture point was obtained when the sample was loaded in the vertical direction (0.29), while the minimum ratio was obtained when the sample was loaded in the horizontal direction (0.22).

These results indicated that when subject to vertical loading, the seeds underwent a lower deformation in the longitudinal side until fracture, although the rupture force applied was the highest, which may be related to the polar edges found in seeds. Contrary to this, the kernels experienced a high deformation in the longitudinal side when compressed in the vertical direction, which is related to the high rupture force applied to this side.

Furthermore, it was noticed that the standard deviation among deformation values in seeds and kernels was high. This indicated a high variation present between the properties of the samples although their ripening stage was the same as observed by Sirisomboon, 2007.

The Tukey's Test indicated that the deformation values in seeds and kernels were

statistically similar at p≤0.01.

Hardness and energy used for rupture

High hardness values were found in seeds of the groups T.3 and T.4, while lower hardness values were present in the smaller seed groups T.2 and T.1. Hardness during horizontal and transversal loading ranged from 78N to 131N and from 69N to 86N respectively; while during vertical loading the values were much higher, from 118N to 174N (Fig. 1-13).

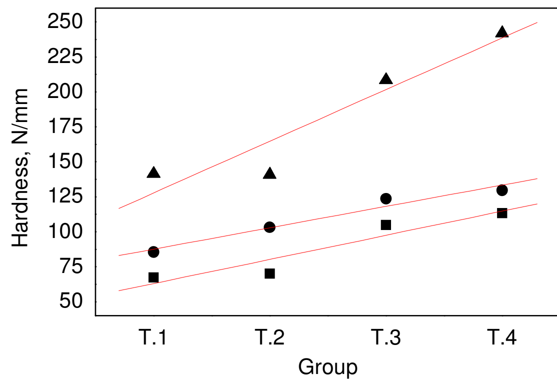

Fig. 1-13 Hardness values of *J. curcas* seeds under horizontal (●), transversal (■) and vertical (▲) loading

These results indicate that seeds were characterised by a harder area at the polar edges of the seeds (vertical position under loading) as also found in the rupture force experiments. This effect of being directionally dependent is known as the "Anisotropy Effect", which can also be found in chestnuts [42]. Likewise, these kernels also demonstrated an anisotropy effect when compressed in different loading directions (Fig. 1-14).

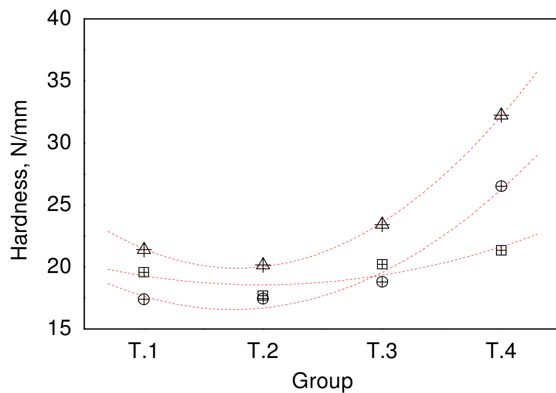

Fig. 1-14 Hardness values of *J. curcas* kernels under horizontal (●), transversal (■) and vertical (▲) loading

The increase of hardness values was related to an increase in the sample group, though the Tukey's Test indicated that hardness values in seeds and kernels were statistically similar at p≤0.01. Further, coefficients of correlation at p≤0.05, between shell content in seeds and

the hardness values obtained in the three loading directions showed an inadequate correlation. Nevertheless, shell thickness values across the length and polar sides in seeds are required in order to find a correlation related to the hardness values in seeds.
The energy required in order to rupture the shell increased when under horizontal and vertical loading in seeds and kernels, while the energy values decreased under transverse loading (Fig. 1-15). It was observed that the energy required for rupture was much higher for seeds than for kernels. Indeed, the energy required to rupture the seed groups in the horizontal, transversal and vertical directions was 77 %, 44 % and 45 % higher than the energy required to rupture kernels respectively. The Tukey's Test indicated that the energy used for rupture in seeds and kernels was statistically similar at $p \leq 0.01$.

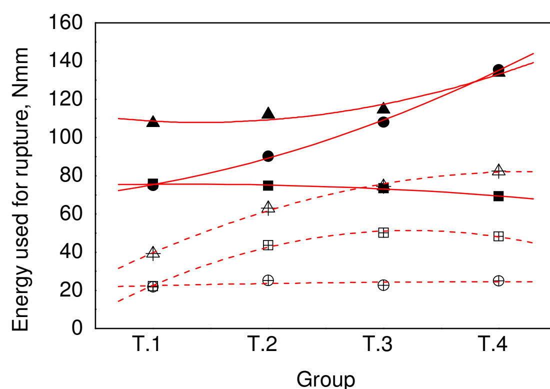

Fig. 1-15 Energy used for rupture values for *J. curcas* seed (—) and kernel (---) under horizontal seed (●), transversal (■) and vertical (▲) loading
The analysis of variance was performed within and between seed and kernel groups to investigate the effect of the loading position on the rupture force, the deformation and hardness at the rupture point, and the energy required to induce rupturing. The calculated F-values showed that the loading direction had a significant effect on the mentioned mechanical properties at $p \leq 0.05$. Nevertheless, especially at the horizontal loading position, the rupture force and deformation ratio at the rupture point did not show any relationship between the seed groups. Likewise, the rupture force, deformation at rupture point, and energy used for rupture did not show any relation between the kernel groups in the horizontal loading direction.
Deshelling machine was constructed at Universität Hohenheim, Institute of Agricultural Engineering (Fig 1–16). The desheller was designed by taking in consideration the physical and mechanical properties described on the results above. Rotating cylinder walls were adjusted between 20 mm to 15 mm decreasing distance and rotational speed of the cylinder was regulated via a frequent converter from 50 to 120 rpm.

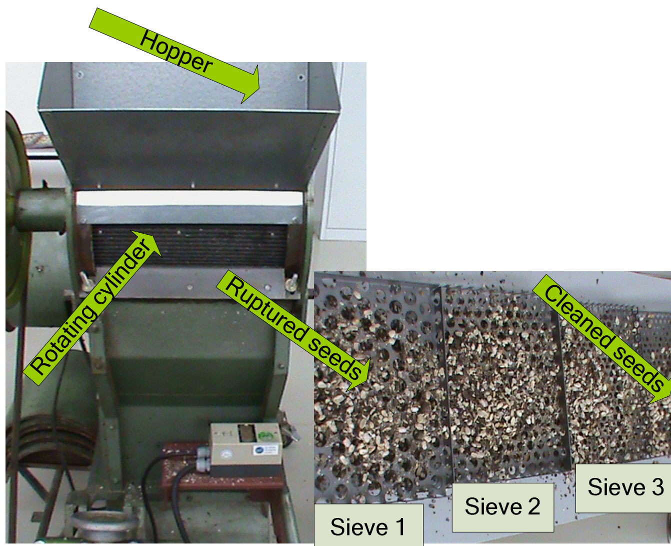

Fig. 1-16 *J.curcas* deshelling machine and cascade sieve cleaning facility
Seeds were poured into the hopper, crashed on the rotating cylinder and than transported on cascade sieves for separation according to their geometrical size. The influence of rotational speed (rpm) on deshelling efficiency was monitored after each interval of increased speed (50, 85 and 120 rpm).

Fig. 1 – 17 Deshelled *J.curcas* seeds with three different levels of rotational speed
J.curcas deshelling efficiency was reduced with the increase of rotational speed from 50 to 120 rpm, more broken kernels were found at higher velocity of rotating cylinder whereas at lower velocity the deshelled kernels were less broken.
Sieve sizes were selected according to the obtained results on *J.curcas* seeds and kernels physical properties (Table 1-2 and Table 1-3). Three different fractions of sieves were separating kernels from shells, the remaining mixture of kernels (broken kernels) and shells were separated via a pneumatic cleaning machine (Fig 1 – 18).

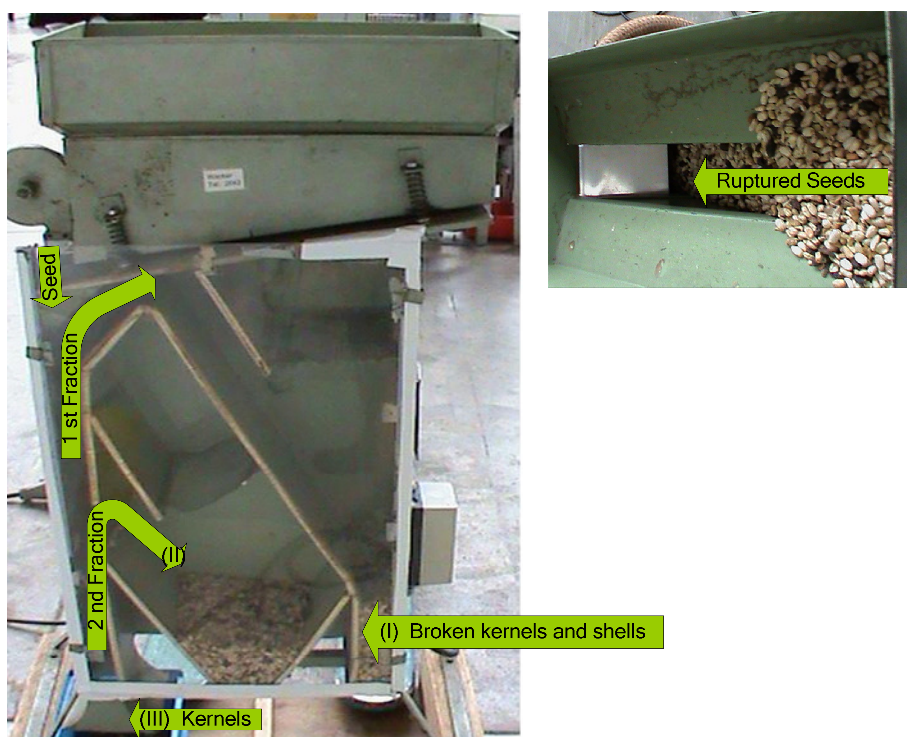

Fig. 1-18 Pneumatic cleaning machine for ruptured *J.curcas* seeds

Figure 1 – 18 shows the principle of seeds cleaning via a pneumatic machine. The ruptured seeds entered from the funnel and than were released on the air channel vessel. The first fraction which were all broken kernels were removed through vessel (*I*), the second fraction which were all kernels were deposited on vessel (*II*). The heaviest fraction of kernels was passing through the entering vessel and was deposited in the end of the air vessel (*III*).

Chemical properties of *J.curcas* seeds and kernels

The oil content of seeds and kernels was analyzed for all four groups (T.1, T.2, T.3 and T.4) with the soxhlet method [24] and the results are presented in Table 1-6. The oil content of kernels is approximately 20 % higher then the oil content of seeds for every group. Group T.4 demonstrates the highest oil content for seeds and kernel (T.4 kernels = 38 %; T.4 seeds = 56 %).

Statistical analyses for seed oil content demonstrates that the composition of oil content compared between groups is expressed with a very high linear correlation (R^2 =0.9771) and mean values according to Tukey's and Fisher tests are significantly different between the groups at $p \leq 0.01$. The oil content of kernels also demonstrates a high linear correlation (R^2 = 0.936) but not all the mean values of the groups (Tukey's and Fisher test) are significantly different, that is group T.3 is not different from group T.4 at $p \leq 0.01$.

Table 1-6 Oil content and dry matter content of seeds and kernels

Property	T.1	T.2	T.3	T.4
Oil content seeds (%)	7.19±2.63a	18.66±3.94b	33.01±0.61c	38.94±0.99d
Oil content kernels (%)	24.26±5.74a	40.15±5.85b	53.20±0.41c	56.61±0.46dc
Dry matter shells (%)	91.065±0.08a	91.06±0.22a	91.30±0.13a	91.24±0.17a
Dry matter kernels (%)	92.45±0.39a	94.52±0.14b	95.75±0.43c	95.89±0.26dc

* Groups with the same letters are not significantly different at $p \leq 0.01$ using Tukey's and Fisher Test

The results show that the dry matter of shells is higher than that of kernels this indicating that kernels contain less moisture content. The dry matter of shells indicates no significant differences between the groups where $p \leq 0.01$ probability, whereas, the dry matter of kernels indicates that groups are significant different where $p \leq 0.01$ using the Tukey's and Fisher tests.

Chemical analyses were completed on *Jatropha curcas* oil extracted from a mechanical screw press and the results were compared with the properties of rapeseed oil and EN 14214 (Table 1-7). The density and kinematic viscosity of J.curcas were lower than rapeseed oil but higher than the European Standard. The gross calorific and iodine values were comparable with rapeseed oil and EN 14214.

Table 1-7 Chemical properties of J.curcas oil seeds compared with rape seed oil and the European Standard Norms 14214

Property	*J. curcas* oil	Rape seed	EN 14214
Density kg/m³	914	920	860 - 900
Kin. Viscosity mm²/s (40°C)	31.2	35.8	3.5 - 5.0
Gross calorific value MJ/kg	39.66	39.6	>35
Iodine value g/100g	100	111	<120
Impurities. C % mass/max	0.11	0.19	<0.30
Water content mg/kg	822.8	609.4	<500
Acid value KOH/g	2.81	1.68	<0.50

Carbon impurities were lower in J.curcas oil compared with rapeseed oil and satisfied the EN 14214 requirements. The water content and acid value demonstrated the highest negative properties compared with rapeseed oil and EN 14214.

Conclusions

The main objective of this study was to determine the physical, mechanical and chemical properties of *Jatropha curcas* L. seeds, as part of an optimization program for deshelling and oil extraction of *Jatropha curcas* L. for direct use in plant oil stoves. The experimental

data obtained in the study suggests a close relationship between $p \leq 0.01$ and $p \leq 0.05$ of the physical properties and the sample groups of *J. curcas* seeds and kernels (9 db. mc.). Indeed, the physical parameters namely, unit mass, thousand unit mass, length, breadth, height, arithmetic and geometric mean diameter, sphericity, surface area, specific surface area, bulk and solid density, volume, porosity, coefficient of static friction on various surfaces and angles of repose correspond to linear and polynomial relationships. Therefore, the design of a desheller system in accordance with the variations and their correlations with the physical properties in seeds and kernels would be advantageous for deshelling efficiency.

No significant difference at $p \leq 0.01$ was found between the sample groups and the variation in the mechanical properties namely; rupture force, deformation at rupture point, hardness, deformation ratio at rupture point and energy used for rupture in seeds and kernels. However, the loading orientation namely the horizontal, transversal and vertical directions had an important effect on the variation of the mechanical properties at $p \leq 0.05$. Differences observed in the rupture forces, hardness and energy used for rupture in seeds and kernels as a function of the loading direction, demonstrated the existence of a certain degree of “anisotropy” in the *J. curcas* structure. Indeed, the dependents for rupture that is force, hardness and energy are at a minimum for the transversal loading direction. Therefore, deshelling of seeds is recommended in the transversal direction.

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Objective 2: Optimization of oil-pressing with respect to oil and press cake

Introduction

Jatropha curcas L. is a drought – resistant shrub/tree belonging to the family Euphorbiaceae (Fig. 2-1) [1, 2]. Cultivated in Central and South America, *Jatropha* was distributed by Portuguese seafarers in Southeast Asia, India and Africa [3]. Propagated by cuttings, it is widely planted for hedging to protect fields from browsing animals. The plant and its seeds are non edible (toxic) to animal and humans; toxicity of seeds is mainly due to the presence of curcine and deterpine [2, 4].

Fig. 2-1 J.curcas fruits and seeds

The distribution of *Jatropha* shows that its introduction has been most successful in drier regions of the tropics. It grows on well-drained soils with good aeration and is well adapted to marginal soils with low nutrient content [2].

Jatropha can be utilized for different purposes, it can be used for erosion control, recreation, fire wood and grown as a natural fence; the bark is rich in tannin and produces a dark blue dye. Leaves have been used for rearing silkworms, in dyeing and in medicine as an anti–inflammatory substance. Seeds have been used as insecticide, soap, and in the production of varnish. Seed cakes have been used as fertilizer, solid fuel, or in biogas production. Non toxic varieties or detoxified press cake have been used as fodder for animals [1, 5]. Despite all these various uses, the application as fuel is probably the most interesting one from both economical and ecological points of view [6].

Four basic methods for extracting vegetable oils from seeds, nuts and fruits have been established [7]. The first method is the basic wet process by which the oil-bearing material is boiled in water leading to partial separation of oil, followed by skimming. The second is the cage-type press in which pressure is applied to a stationary mass by using a system of levers, screw jacks or hydraulic cylinders and the vegetable oil is then allowed to flow

from the compressed mass to collecting rings below. Both these methods are more or less obsolete. The third method is the mechanical screw press and the fourth is by solvent extraction [6, 7].

Mechanical pressing and solvent extraction are the most commonly used methods for commercial oil extraction. Screw pressing is used for oil recovery of up to 90-95%, while solvent extraction is capable of extracting 99 % [6, 8].

The potential uses of extracted oil from *Jatropha* as transesterified oil (biodiesel), or as a blend with diesel have been studied [1, 9-11]. The calorific value and cetane number of *Jatropha* oil are similar to diesel, but the density and viscosity are much higher [12]. In small scale oil pressing units, undesirable residues from plant material can be found in the crude oil. These impurities lead to depositions during combustion in stoves or engines and reduce lifetime and service intervals considerably. Consequently, the contamination of the crude oil by these unwanted impurities should be avoided during production.

The objective of this study was to optimise the mechanical oil extraction of *Jatropha curcas* L. seeds with a cylinder hole screw press. In the scope of this study only the amount of oil recovered was considered whereas the oil quality was not analysed. To optimise the oil yield the influence of different settings of the screw press as well as the resulting dependent factors were monitored with respect to their ability to increase the oil yield. Put more precisely this means that the optimal combination of one of the screws (R8, R11) with a press cylinder (P1, P1.5) and one of the nozzles (R8, R10, R12) was sought. Furthermore the aim was to find out how other factors were dependent on these variables and how they could eventually influence the oil yield. The temperature development was of particular focal interest in the screw press area.

Materials and Methods

Jatropha curcas L. seeds

The seeds for this study were imported from a region in India with the following climatic conditions: An annual precipitation of 1000-1200mm and a temperature ranging from 15-35°C. The seeds were manually harvested during November and December 2007 and stored in jute bags at temperatures ranging from 14-30°C. The moisture content was determined after the seeds were transported to the Institute of Agricultural Engineering at the University of Hohenheim, which was 7.5 % w.b. (wet basis). The average oil content of the seeds was 36%. The trees that bore the seeds were planted 11 to 12 years ago, which have now reached their maximum yield potential at 7500kg/ha/a. Before pressing, the seeds were cleaned with a pneumatic air/ sieve separator.

Screw press Komet D85-1G

For this study a commercially available screw press (Komet D85-1G) by IBG Monforts Oekotec GmbH & Co. KG was used (Fig. 2-2).Technical data for the screw press can be found in Table 2-1.

Fig. 2-2 Screw press Komet D85-1G

Table 2-1 Technical data for screw press Komet D85-1G

Technical Data	
Capacity of input material / time in [kg/h]	10-25
Electric Power of Drive Motor in [kW]	3.0
Weight in [kg] (net only, without input	310
Dimensions in [mm]	
Length	1080
Width	800
Height	600

Independent variables: screw press components

For the experiments two screws with different worm shaft choke widths (R8, R11), two press cylinders with different diameters of the oil outlet holes (P1, P1.5) and three nozzles

with different diameters for the press cake outlet were used (N8, N10, N12). The dimensions of the variable screw press components were measured with vernier callipers (Mitutoyo, model Absolute Digimatic, Japan) with an accuracy of ±0.01mm (Fig. 2-3, Table 2-2).

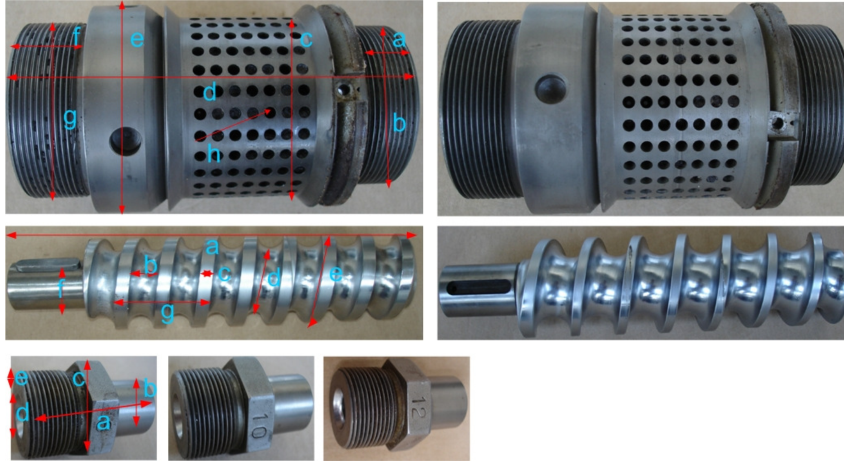

Fig. 2-3 Variable screw press components: press cylinders P1, P1.5; screws R8, R11; nozzles N8, N10, N12

Table 2-2 Specifications of screw press Komet D85-1G components in [mm]

Press cylinder	P1	P1.5
Choke end thread depth	24	24
Choke end thread	68	68
Press cylinder diameter	75	75
Press cylinder length	147	147
Diameter (e)	85	85
Feed end thread depth	30	30
Feed end thread	72	72
Oil outlet hole diameter	1	1.5

Screw press	R8	R11	Nozzle	N8	N10	N12
Length (a)	253	253	Length (a)	50	50	50
Choke worm shaft (b)	16	21.5	Outlet diameter	19	19	19
Worm shaft (c)	8	5.8	Nozzle diameter	32	32	32
Choke ring (d)	41	35.5	Compression size	8	10	12
Worm shaft diameter	56	56	Nozzle wall (e)	9	9	9
Axial worm (f)	30	30				
Pitch (g)	66	62				

Dependent variables

The oil press was modified to accommodate a torque measuring shaft. Rotational speed (ω) can be adjusted between 0 and 600 rpm with a continuously adjustable speed alternator. During the pressing operation, torque (τ) (sensor type: T22/200NM) and rotational speed

(ω) were measured at the torque measuring shaft. Furthermore the temperature was measured at five different points of the screw press (T1-T5). Temperature T5 was measured with an infrared sensor whereas T1 to T4 were measured with thermocouple sensors. The screw press design and installed sensor can be seen in Fig. 2-4.

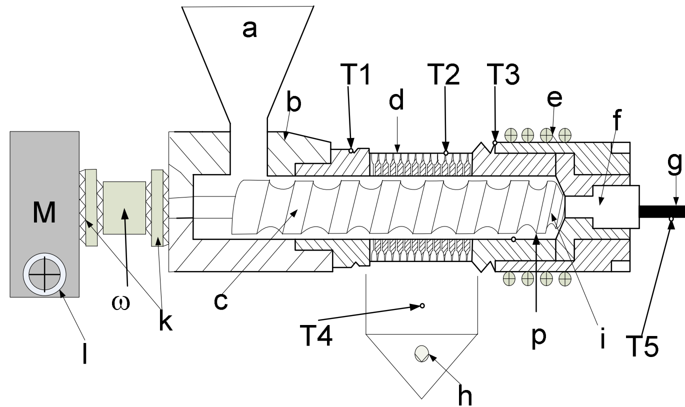

Fig. 2-4 Screw press (cylinder-hole type) for oil extraction and installed sensors, (a) feeding hopper, (b) housing, (c) screw press, (d) oil outlet holes, (e) heating, (f) nozzle, (g) press cake outlet, (i) cohesion zone, (h) oil collector, (k) coupling, (l) speed alternator, (M) motor, (T1-T4) temperature sensors, (T5) temperature sensor (IR), (p) pressure sensor, (ω) rotational velocity

Values were recorded every seven seconds by a data acquisition unit (Agilent 34970A). At the beginning and end of the experiment the scan number of the data bench logger was noted. The total time demand was calculated by multiplying the number of scans by seven seconds. The throughput (Th) in kg/h was calculated by dividing the amount of seeds (S) in kg by the time taken to press them (T) in h:

$$T_h = \frac{S}{T}$$ Eq. 1

The DC Voltage values recorded from the torque sensor, temperature sensor T5 and the pressure sensor needed to be converted into Nm, °C and bar values respectively. The values for applied power P (kW) were calculated from torque τ (Nm) and rotational speed ω (rpm) using the following formula:

$$P = \tau \cdot \omega$$ Eq. 2

Total energy demand was calculated with the following formula:

$$E_t = P \cdot T$$ Eq. 3

Where: Et is the energy demand (kWh), P is power (kW) and T time (h).

It is possible to either relate the energy consumption to the processed seeds or to the

cleaned oil [1]. The pressing of oil seeds does not just involve the recovery of oil but also of press cake, which is a valuable by-product. It is not possible to allocate the energy consumption to oil and press cake individually. Therefore in this study the total energy demand (Et) in kWh was divided by the amount of seeds that were pressed (S) in kg resulting in the energy consumption per amount of pressed seeds (Es) in kWh/kg:

$$E_s = \frac{E_t}{S} \qquad \text{Eq. 4}$$

After each experiment a press cake sample was taken and the residual oil content in the press cake was determined with the Soxhlet method. With help of the residual oil content in the press cake the extraction efficiency (%) could be calculated [2]:

$$O_{R.E} = 1 - \left[\frac{\left(\frac{O_{c.P}}{1 - O_{c.P}} \right)}{\left(\frac{O_{c.S}}{1 - O_{c.S}} \right)} \right] \cdot 100 \qquad \text{Eq. 5}$$

Where: OR.E is oil recovery efficiency or extraction efficiency, Oc.P oil content in press cake sample and Oc.S oil content in the seeds.

Experimental set up

For each experiment 50 kg of *J. curcas* seeds were pressed. The oil was collected in canisters and the press cake in a plastic crate. After finishing the experiment the end products were weighed on digital scales (Sartorius counter scales). Before beginning the pressing process the press head (the casing around the compression zone and the nozzle) was heated with an electrical heating device which is standard equipment of the press. This was done to increase the viscosity of the seed meal to prevent the press from jamming at the beginning of the operation [3]. After preheating, about 5 kg of seeds were pressed using the preliminary settings to be used in the experiment to reach the normal operating temperature.

List of experiments

For this study twelve experiments were conducted. Each screw was combined with both press cylinders. A list of experiments and experimental settings are shown in Table 2-3.

Table 2-3 List of experiments and experimental settings

Experiment	Press cylinder	Screw	Nozzle	Rotational speed (rpm)
R8P1N8ω290	P1	R8	N8	290
R8P1N10ω290	P1	R8	N10	290
R8P1N12ω290	P1	R8	N12	290
R8P1.5N8ω290	P1.5	R8	N8	290
R8P1.5N10ω290	P1.5	R8	N10	290
R8P1.5N12ω290	P1.5	R8	N12	290
R11P1N8ω200	P1	R11	N8	200
R11P1N10ω200	P1	R11	N10	200
R11P1N12ω200	P1	R11	N12	200
R11P1.5N8ω200	P1.5	R11	N8	200
R11P1.5N10ω200	P1.5	R11	N10	200
R11P1.5N12ω200	P1.5	R11	N12	200

Thus four combinations of screw and press cylinders were possible (R8P1, R8P1.5, R11P1, R11P1.5). Each of the four combinations was used with three different nozzles (N8, N10, and N12).

The speed of the larger diameter worm shaft choke (R8) was set at 3.5 on the speed alternator which is equivalent to 290 rpm. For the other screw (R11) the speed was set at 2, which is equivalent to 200 rpm. The speed settings were chosen according to prior experiments. A rotational speed, which is too low for the experiments with screw R8 could have easily led to choking and jamming of the press, whereas high rotational speeds for screw R11 could lead to very low oil yields.

Statistical evaluation

For each of the twelve experiments average values were calculated for the whole of the collected data of the dependent variables.

The statistical evaluation was performed using the software OriginPro 8G. Each of the dependent variables was plotted against each other. Subsequently possible linear and exponential functions were applied to search for correlations. Moreover one way ANOVA tests were performed to determine the influence of the independent variables on the dependent variables. All ANOVA tests were performed at a level of confidence of α = 0.05.

It is theoretically possible to calculate the amount of solids in the oil. After multiplying the extraction efficiency (OR.E) with the initial oil content and the amount of seeds one can obtain the theoretical amount of oil that should have been obtained. This value can be subtracted from the actual amount of recovered crude oil resulting in the amount of solids

present in the oil.

A certain amount of initial seed mass is however lost during the pressing process due to evaporation of water and material getting stuck in the press. This makes the computed values for the extraction efficiency and amount of solids in the oil unsuitable for comparison, since the calculated oil yield is sometimes higher than the actual amount of recovered crude oil. This results in the calculated amount of solids in the oil becoming negative.

For these reasons extraction efficiency was not included in the following statistical evaluation.

Results and Discussion

Influence of independent variables - oil yield

The amount of oil recovered varied from a minimum of 13.2 kg in experiment R11P1.5N12ω200 to a maximum of 16.8kg in experiment R8P1.5N12ω290. The more oil that was recovered from the seeds the less press cake that was obtained. On average 1kg of initial seed mass was lost during pressing. This is a normal process since seed water evaporates during the oil extraction and solid material can get stuck in the press [4] (Fig. 2-5).

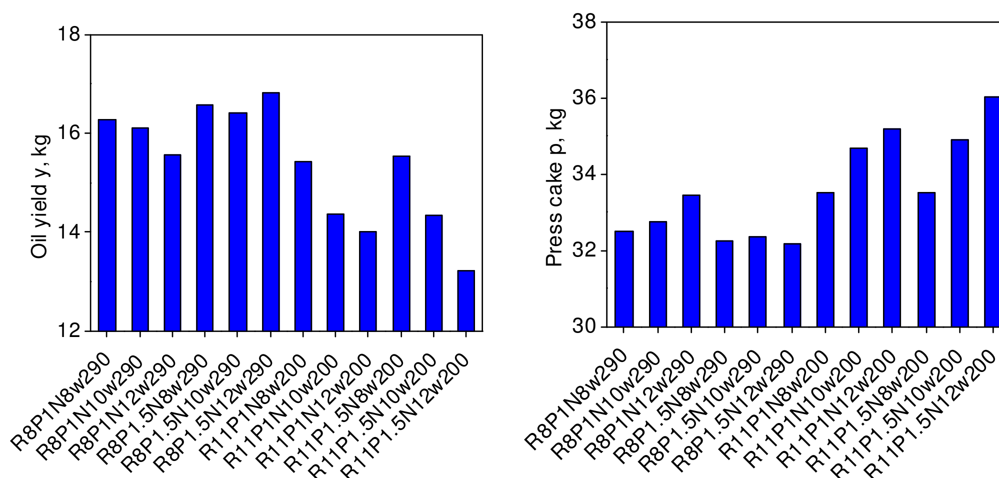

Fig. 2-5 Influence of experiment settings on oil yield and press cake

In case of screw R11 the amount of oil recovered varied from 13.2 kg in experiment R11P1.5N12ω200 to 15.5kg in experiment R11P1.5N8ω200. The experiments with R8 led to greater oil yields ranging from 15.6 kg in experiment R8P1N12ω290 to 16.8 kg in experiment S3.5R8P1.5N12ω290 (Fig 2-5).

At a level of confidence of $\alpha = 0.05$ the average oil yield for the combination of screw R8 is significantly higher than that of screw R11.See Table 2-4.

Table 2-4 One way ANOVA for the influence of screw R8 and R11 on oil yield

	Sample Size	Mean	Standard	SE of
R8	6	16.29	0.43	0.18
R11	6	14.49	0.88	0.36

	DF	Sum of	Mean	F Value	Prob>F
Model	1	9.79	9.79	20.30	0.001
Error	10	4.82	0.48		
Total	11	14.61			

In the experiments conducted with R11 the press cylinder had no recognisable effect on the oil yield, whereas, when using screw R8, the oil yield increased by 2, 1.9 and 8.1%

respectively when the diameter of the oil outlet holes were also increased. It must be noted that these differences did not prove to be statistically significant.

Except for one case (R8P1.5N12ω290) a decrease in nozzle size led to higher oil yields. Decreasing the restriction size by 2mm increased the oil yield by an average of 6.7% when using R11. For those cases in which the oil yield increased when R8 was used the average increase was 2.23%.

An ANOVA test performed with all twelve data points showed no statistically significant differences between the nozzles. The increase in oil yield by decreasing the nozzle size was only significant for the experiments conducted with R11 (Table 2-6).

Table 2-5 One way ANOVA tests for the influence of restriction size (N8, N10, N12) on oil yield for experiments conducted with screw R11

	Sample Size	Mean	Standard	SE of
N8	2	15.49	0.07	0.05
N10	2	14.35	0.01	0.01
N12	2	13.62	0.57	0.40

	DF	Sum of	Mean	F Value	Prob>F
Model	2	3.55	1.78	16.39	0.02
Error	3	0.33	0.11		
Total	5	3.88			

Subsequently performed Tukey's showed that the oil yield is significantly higher when using N8 instead of N12.

The throughput ranged from 6.11 kg/h in experiment R8P1N12w290 up to 10.93 kg/h in experiments R11P1.5N10w200 and R11P1.5N12w200 (Fig. 2-6).

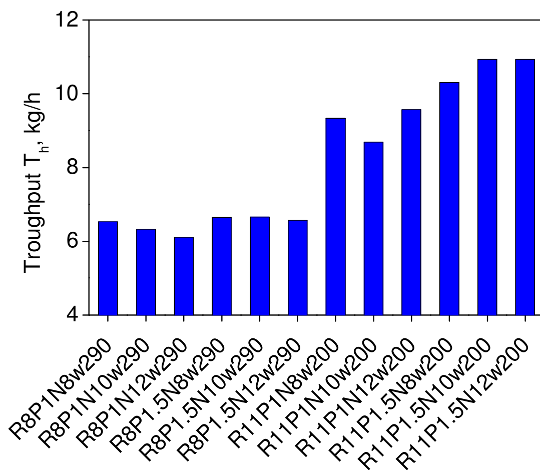

Fig. 2-6 Influence of experiment settings on throughput

For screw R8 the throughput ranged from 6.11 kg/h to 6.66 kg/h while for screw press R11

experiments show that a higher variability of the throughput could be observed, namely from 8.69 kg/h to 10.93 kg/h. The difference between the average throughput for R8 and R11 proved to be statistically significant (level of confidence of $\alpha = 0.05$), Table 2-7.

Table 2-6 One way ANOVA test for the influence of screw R8 and R11 on throughput

	Sample Size	Mean	Standard	SE of
R8	6	6.47	0.21	0.09
R11	6	9.96	0.91	0.37

	DF	Sum of	Mean	F Value	Prob>F
Model	1	36.52	36.52	83.13	0.00
Error	10	4.39	0.44		
Total	11	40.91			

Other screw pressing experiments have shown that by lowering the rotational speed due to a given setting and design of the screw press, decreases in the throughput of seeds and increases in oil yield are possible. [2]. Nonetheless lower throughput rates were also achieved when using screw R8 at 290min-1 instead of R11 at 200min-1 and the oil yields were significantly higher. According to these observations it must be concluded that lower throughput rates and higher oil yields are to be expected when using R8 even at higher rotational speeds.

The throughput decreased when using P1 instead of P1.5. Again the effect was only significant for the R11 experiments: decreasing the oil outlet size decreased the throughput by 0.97, 2.24 and 1.37kg/h respectively. For R8 the throughput decreased marginally by 0.11, 0.33 and 0.46kg/h.

Energy consumption

By combining Eq. 1, 3 and 4 it can be shown that specific energy consumption (Es) is dependent on the throughput (Th). At a given power the energy consumption decreases with increasing throughput:

$$E_s = \frac{P}{T_h} \qquad \text{Eq. 6}$$

The restriction size had no discernible effect on the throughput and thus not on the energy consumption either Throughput was lowest when using screw R8 and press cylinder P1 (6.33 kg/h). Increasing the size of the oil outlet holes increased the throughput. The combination of screw R8 and P1.5 resulted in, on average 6.62kg/h. When using R11 the throughput was higher even though it was run at a lower rotational speed than R8. The throughput was 9.2kg/h and 10.72kg/h respectively.

Consequently the combination of R8 and P1 resulted in the highest energy consumption per kg of pressed seeds that is 0.33 kWh/kg. When using P1.5 the energy consumption decreased by an average of 0.31 kWh/kg. The values for R11 were 0.24 kWh/kg and 0.22 kWh/kg respectively (Fig. 2-7).

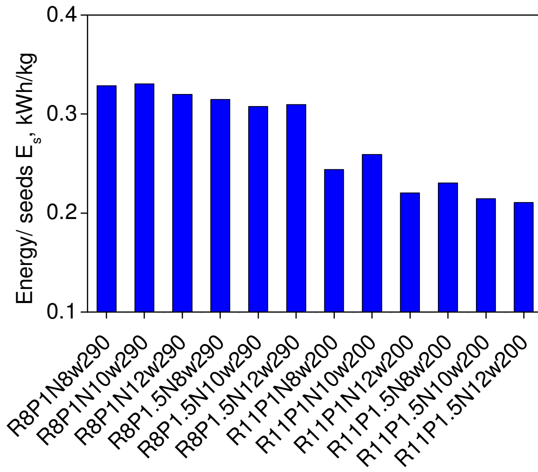

Fig. 2-7 Influence of experiment settings on energy consumption

Influence of dependent variables

During the experiments all parts of the press heated up. A typical picture of the temperature behaviour of the press can be seen in Fig 2-8.

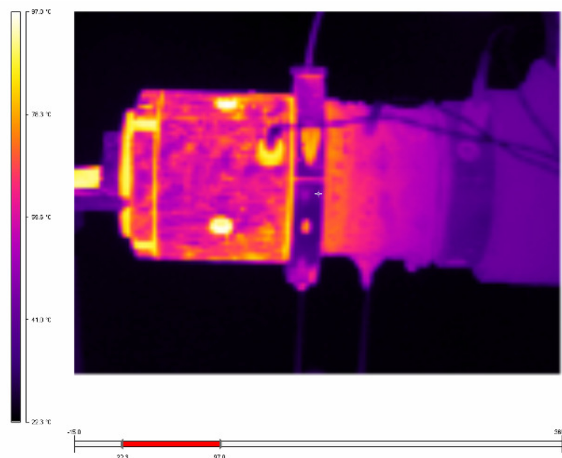

Fig. 2-8 Temperature gradient of screw press from feed section (right) to press cake discharge (left)

Temperatures at the starting point (T1) ranged from 44.4°C to 52.4°C. At the oil outlet (T2), temperatures ranging from 59.8°C to 71.6°C were measured. The hottest part of the press was the compression zone (T3) where the temperatures ranged from 84.5°C up to 101°C. Oil temperatures (T4) were found to be between 65.6°C and 85°C. Press cake temperatures (T5) varied from 38.9°C to 64.1°C (Fig. 2-9).

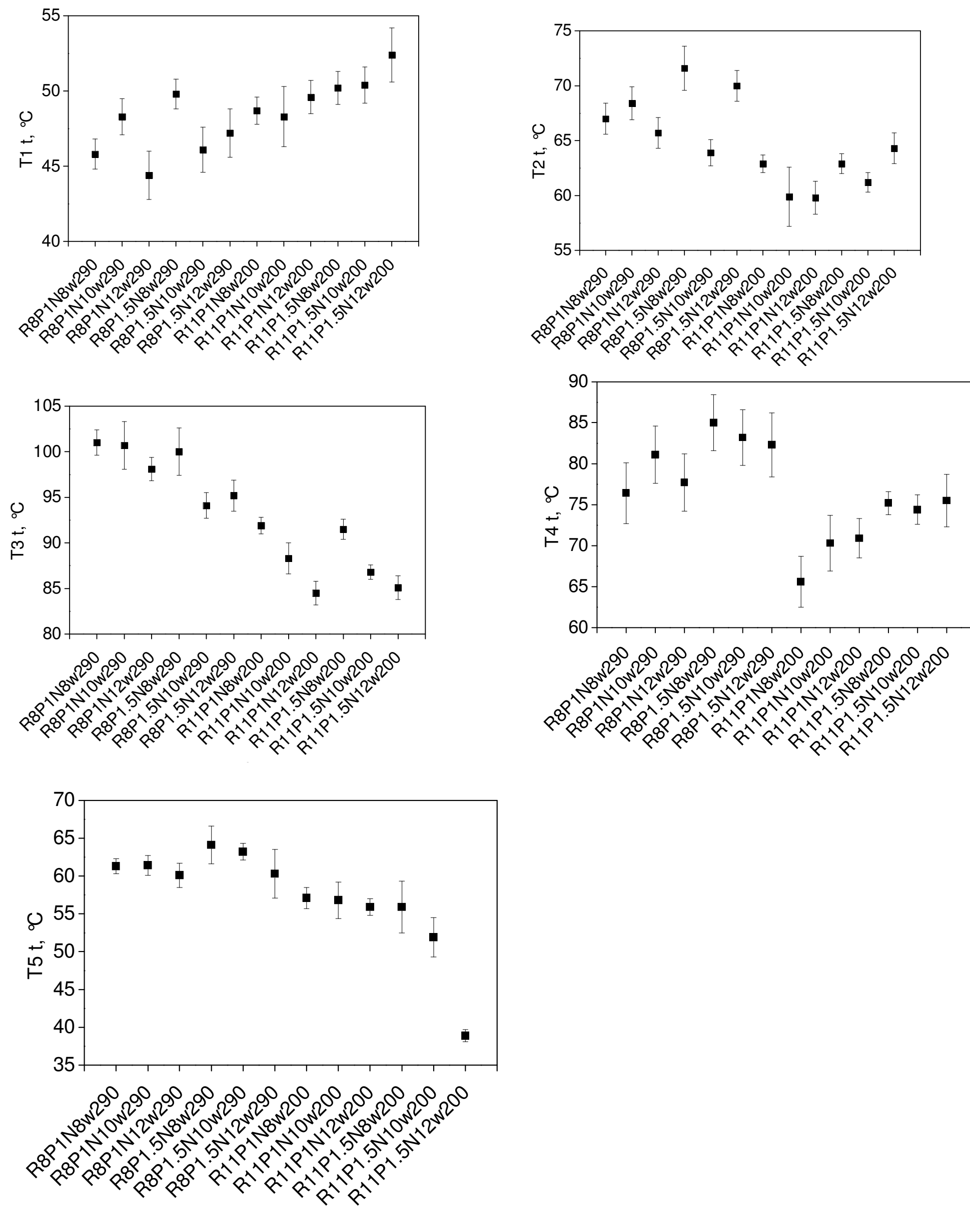

Fig. 2-9 Influence of experimental settings on temperature T1, T2, T3, T4 and T5

Previous screw pressing experiments by other authors have shown that temperatures tend to decrease at higher speed because of a higher mass flow rate [2, 3]. The average values of the measured temperature T2 to T5 were however all significantly higher for R8 compared to R11, whereas T1 was higher when using R11 even though for R8 the speed was set at 290 rpm and 200 rpm for R11.

Decreasing the oil outlet size, only leads to higher oil temperatures (T4). This effect has to be taken into consideration if the oil is to be used as feedstock for biodiesel or as PPO in I.C. engines. Although the threshold temperature at which phosphorus starts to dissolve in the oil is not yet known, a value of 55-70°C is recommended. Moreover reducing the restriction size only slightly, which is statistically insignificant, will increase the

temperature in the compression zone (T3) and that of the press cake (T5). No distinct effect of the nozzle size on temperatures at the beginning of the barrel (T1), in the oil outlet holes (T2) and of the oil (T4) could be observed.

The higher temperature in the compression zone is a result of the increasing pressure due to the further restricted mass flow of the press cake. Hence the rising temperature of the press cake and compression zone can be seen as indicators of a rising pressure.

All temperatures were plotted against each other and against the oil yield. Linear fits were established for all resulting plots. From these results it was not clearly visible how the temperatures were dependent on each other. Correlations were found linking temperatures in the oil outlet holes (T2), to the compression zone (T3) and the temperature of the press cake (T5) to the oil yield (Fig. 2.10).

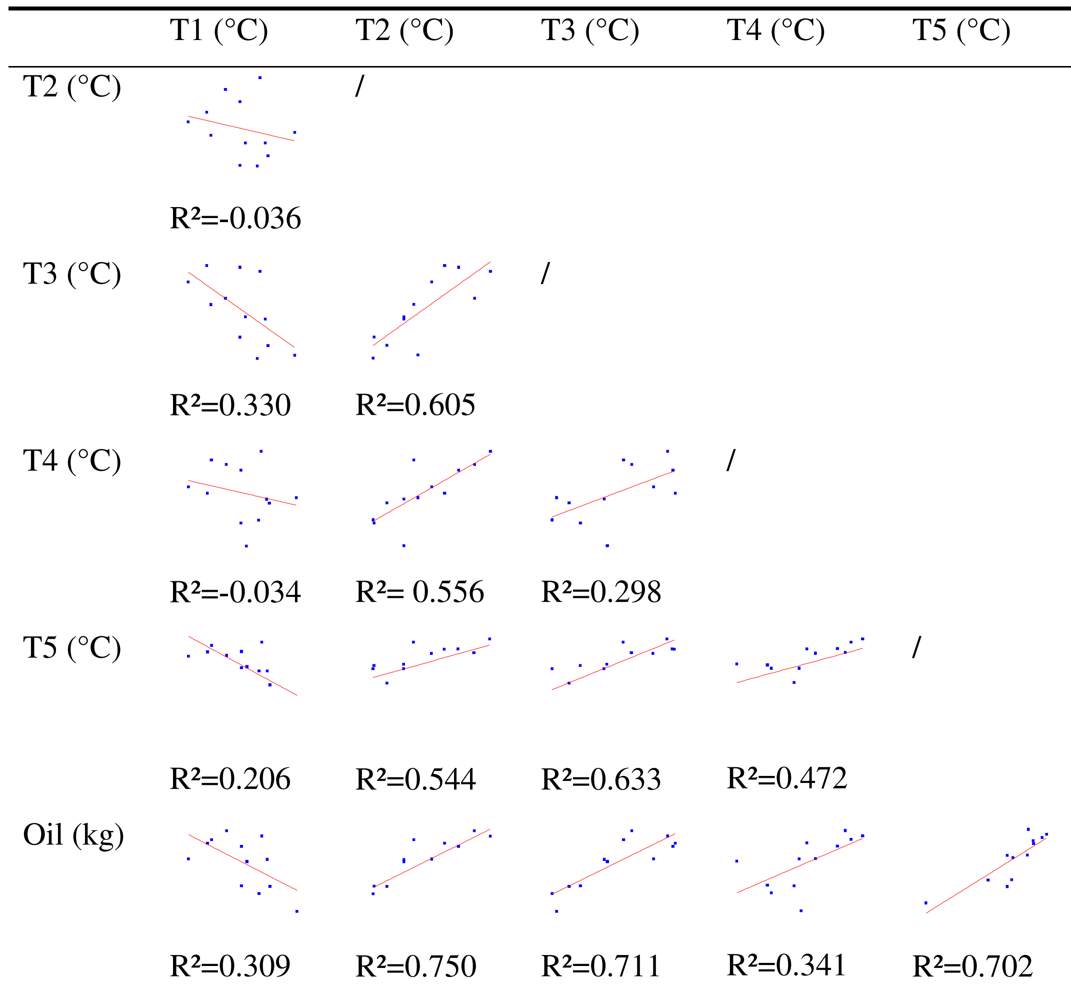

Fig. 2-10 Scatter matrix of temperatures T1 to T5 and oil yield

The equation of the oil yield to temperature of the press cylinder was expressed as a linear function (R^2=0.75) (Fig. 2.11).

$$y = 1.768 + 0.213 \cdot t \qquad \text{Eq. 7}$$

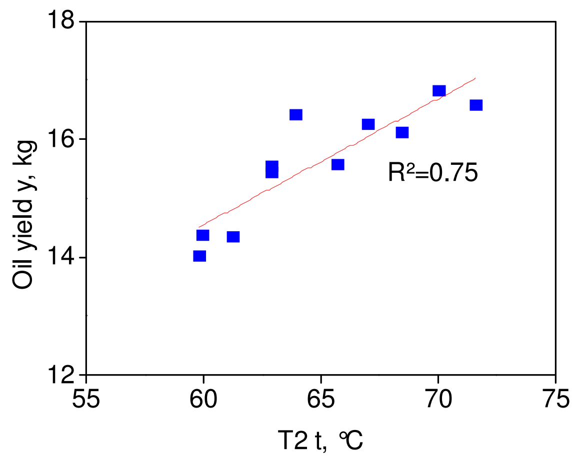

Fig. 2-11 Influence of temperature in the oil outlet holes (T2) on oil yield

Two different functions were able to confirm the dependency of the oil yield by T3. The higher coefficient of correlation was reached using the exponential fit shown in Eq. 9 Fig. 2-12.

$$y = 0.163 + 0.164 \cdot t \qquad (R^2 = 0.711) \qquad \text{Eq. 8}$$

$$y = -775446.18 \cdot e^{\left(-\frac{t}{6.822}\right)} + 16.677 \qquad (R^2 = 0.802) \qquad \text{Eq. 9}$$

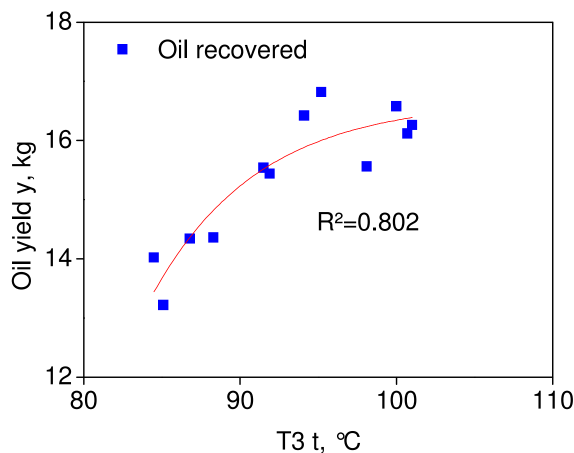

Fig. 2-12 Influence of temperature in the compression zone (T3) on oil yield

The oil yield could also be described as a function of the press cake temperature just after leaving the press (T5). Again the exponential function resulted in the higher coefficient of correlation (R^2).

$$y = 7.051 + 0.146 \cdot t \qquad (R^2=0.702) \qquad \text{Eq. 10}$$

$$y = 0.058 \cdot e^{\left(\frac{t}{14.72}\right)} + 12.333 \qquad (R^2=0.749) \qquad \text{Eq. 11}$$

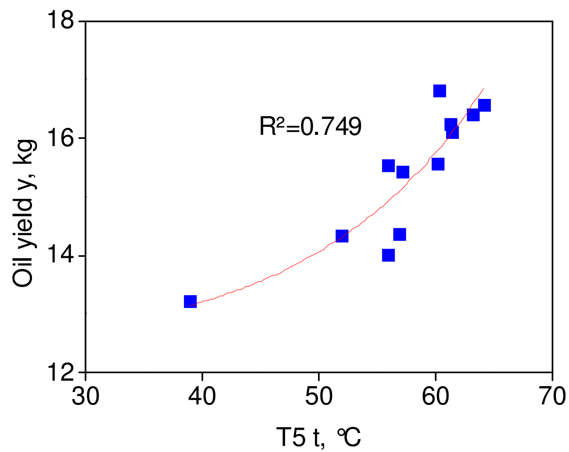

Fig. 2-13 Influence of press cake temperature (T5) on oil yield

Increased temperatures in the oil outlet holes and compression zone indicate an increased pressure. As explained increasing the pressure increases the oil yield. Due to higher temperatures a decrease in oil viscosity will allow it to flow more easily. From the measured values for T2, T3 and T5 conclusions can be drawn about the temperature of the oil in the compression zone. It should also increase when the temperatures in the oil outlet hole (T2), the compression zone (T3) and the press cake (T5) increase. Therefore its viscosity should decrease. The fact that a close correlation could not be found between the temperatures in the oil collector (T4) and the oil yield can be explained by placing the sensor in ambient temperatures.

Conclusion

The aim of this study was to optimise the mechanical extraction of *J. curcas* seeds with a cylindrical hole screw press. The yields obtained from 50 kg of seeds ranged from a minimum of 13.2 kg to a maximum of 16.8 kg. The throughput ranged from 6.11 kg/h to 10.93 kg/h. Oil yields were higher when using R8 instead of R11 and when using P1.5 instead of P1. Decreasing the restriction size also increased the oil yield though this effect was only significant when using screw R11. Throughput was likewise influenced by the screw and the press cylinder in use. Throughput was lower for R8 than for R11 even at higher rotational speeds. Moreover throughput was higher when using P1.5 instead of P1. Consequently the lowest throughput can be expected for the combination of R8 and P1 and the highest oil yield for the combination of R8, P1 and N8.

The temperature behaviour was influenced by the screw, the press cylinder and the nozzle. Using screw R8 leads to higher temperatures. Decreasing the nozzle size increased the temperatures of the compression zone (T3) and the press cake (T5). This increase in temperature could however not be proven statistically. Increased temperatures of the compression zone and the press cake were followed by higher oil yields. The press cylinder with the wider outlet holes (P1.5) resulted in lower oil temperatures compared to the one with narrower outlet holes (P1).

A decreased rotational speed is usually followed by higher oil yields and temperatures as well as lower throughput rates. Notwithstanding these facts oil yields and temperatures are lower and throughput rates higher for R11w200 than for R8w290. Hence it can be concluded that the main influence of the variable screw press components is due to the design of the screw. The press cylinder and the nozzle size also influence the oil yield and temperature behaviour. This effect is less pronounced when they are combined with the screw with the wider worm shaft choke.

Temperatures of the oil ranged from 65.6 to 85°C. Since no quality assessment of the oil was carried out it is not known whether the phosphorus content was elevated. Further studies should focus on determining the threshold temperature at which phosphorus starts to dissolve in oil. This will be necessary when producing oil that will be used as fuel or as feedstock for biodiesel production.

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Objective 2.1: Oil clarification system

Introduction

J.curcas is inedible plant oil, which has oil properties similar to those of diesel oil. Therefore, the use of J.curcas straight vegetable oil (JSVO) also known as Pure Plant oil (PPO) has been developed as a combustible oil [7]. Furthermore, the use of JSVO does not require the transesterification process normally involved in the preparation of biodiesel. J.curcas seeds contain about 30 to 40% of oil from its own weight [8]. These seeds stem from a large tree about 5 meters high, which is native to tropical America [9]. The *Jatropha*'s plant has been commonly used in West Africa as hedging in fields [10]. Moreover, all parts of the plant have particular applications, including its leaves and bark used as insecticide due to its phorbol ester content [11].

J.curcas oil extraction takes place in either decentralized or centralized industrial oil plants. Generally, centralized systems involve the use of solvent extraction followed by a refining process [19, 20]. On the other hand, decentralized systems, also known as cold pressed processes have evolved into established systems in the last few years because the use of expensive and advanced machinery is not required [1].

In remote rural areas the oil used for plant oil stoves should not be highly processed, in order to widen its availability and therefore reduce production costs. However, more impurities can be found in less processed oils [13].

Oil extraction of *J. curcas* in decentralized plants can be summarized in three steps, (i) cleaning the seeds, (ii) extraction of oil, and (iii) oil clarification as showed in Figure 1 (adapted from rapeseed oil extraction). After cleaning the seeds, the oil is extracted by applying pressure with a screw press, which is constructed either with a cylindrical press cage with steel bars or with a perforated press cylinder [1].

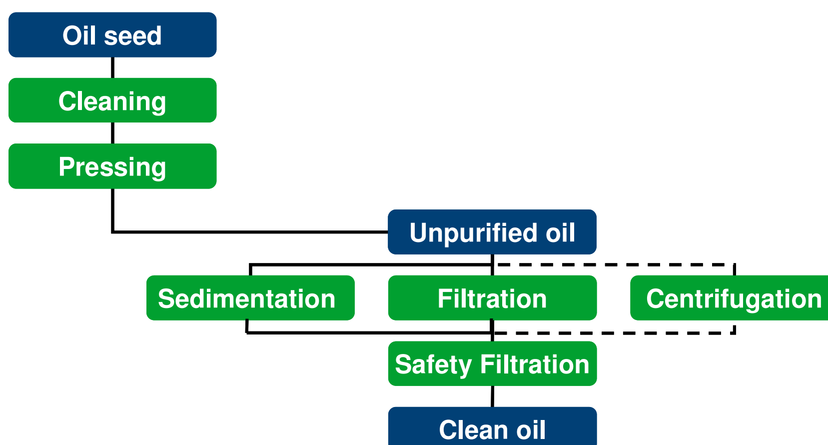


Fig. 3-1 Pure plant oil processing in decentralized industrial plants

The first main stage of *J. curcas* oil clarification is constituted by sedimentation and filtration and in a few cases by centrifugation. The sedimentation time for given oil depends on several factors, i.e. the volume and shape of the sedimentation container as well as on the density and viscosity of the oil, and the density of the particles [22]. However, after the sedimentation process the total contamination value is much higher than the allowable limit. Therefore, it is recommendable to remove the remaining particles from the oil through filtration. [23]. Safety filters with finenesses between 1 or 5 µm have been used for this purpose, i.e. cartridge and bag filters [22]. In cases where an acceptable level of oil clarity cannot be reached by this method, it should then be filtered preferably through a filter press [24]. *Jatropha*´s oil quality is highly influenced by the clarification process. The most relevant chemical properties that can influence the combustion process were found to be the calorific value, boiling point, ignition point, density, iodine value, and saponification value [13]. The viscosity of Pure Plant Oil (PPO) is higher than that of biodiesel and increases with the aging of the oil, although for a given kind of oil is constant [25]. A low iodine number could reduce carbon residues in the engine's (combustion) chamber and some components of plant stoves [26]. The acid value decreases by refining and increases by aging of the oil. This value must be kept low because these acids can attack the metal parts of an engine, causing damage that could prove to be very costly [27]. The press oil contains up to 35% of fine particles, which should be removed in order to increase the gross energy content per liter [27]. In fact, the presence of these contaminants in the oil can lead to an accumulation of residues and thus cause damage to the injection nozzles used in combustion systems [27]. For every kind of technical usage, these solids must be removed from the oil to avoid blockage of mechanical components and to reduce the effect of oxidation [23] and [28]. Likewise, impurities can influence the burning characteristics and lessen storage stability [13].

The aim of this research was to establish the influence of two different clarification systems on the final quality of the *J. curcas* raw oil to be used for direct combustion in plant oil stoves. Thus, the efficiency of continuous and discontinuous clarification methods was analyzed using parameters such as sedimentation time, particle size and total contamination. Besides that, the chemical properties of *J. curcas* oil were analyzed before and after the clarification of the oil in order to both identify the influence of the processes and characterize the constituents in the oil after the sedimentation and filtration processes.

Materials and Methods

Oil extraction

The J.curcas seeds were grown in India. The mature seeds were collected and allowed to sun dry. The seeds were brought to the institute of Agricultural Engineering in the Tropics and Subtropics at the Hohenheim University, Germany. The seeds were stored at room temperature and then cleaned by using a pneumatic machine to remove all foreign matter such as dust, dirt, stones and chaff as well as immature and broken seeds. The selected seeds were pressed with the mechanical cold press oil machine, type OEKO 85D – 1G. *J. curcas* and the oil yield obtained during extraction was about 30 % by weight. The extracted oil was collected in plastic containers and stored at room temperature before the purification process took place. The clarification process of the oil was subdivided in two main stages; sedimentation and filtration.

Sedimentation temperature

The temperature of the oil is an important factor to be considered for an efficient sedimentation process. When the temperature decreases, the rate of settling becomes slower because it is influenced by the density of the particles, as well as by the viscosity and density of the fluid [30, 31]. As a result, the retention time in the sedimentation tanks must increase.

For the sedimentation of the *J. curcas* raw oil, the oil was heated up to around 50°C to simulate the oil's output temperature of the extraction facility. The temperature was measured with a digital temperature sensor, type GTH 1160. The hot raw *J. curcas* oil was then transferred into the sedimentation tanks. The temperature of the oil at the beginning of the settling process was about 50°C and at the end it was at room temperature, about 23°C.

Discontinuous sedimentation system

The discontinuous sedimentation system consisted of a single container oriented either in a vertical or horizontal direction (Fig. 3-3). The containers had the same capacity of approximately 32,000 cm³, and the following dimensions: 600 mm, 270 mm, and 200 mm.

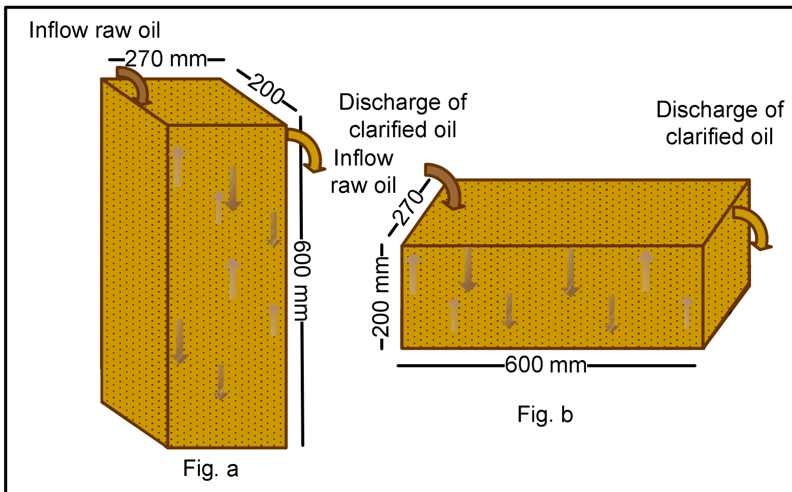


Fig. 3-2 Vertical (a) tank and Horizontal (b) tank respectively

The settling rate was monitored by measuring the height of the interface over a period of time. The particles were allowed to settle by gravity in a period of several days until the clarified fluid was visibly separated from the sediment cake (mud). The sedimentation time was extended until the oil exhibited a clear amber colour. The material used to construct the tank was transparent acrylic glass. A meter was fixed on one of the walls of the tank to quantify the height in increments of the sediment during a given period of time. The removal of the semi-cleaned oil was done manually.

Continuous sedimentation system

Figure 3-4 shows the ideal settling conditions occurring in a continuous sedimentation system. It then follows that a particle enters at the top of the settling zone (point A) and settles with a velocity just sufficient to reach the sludge zone at the outlet end of the tank, at point B.

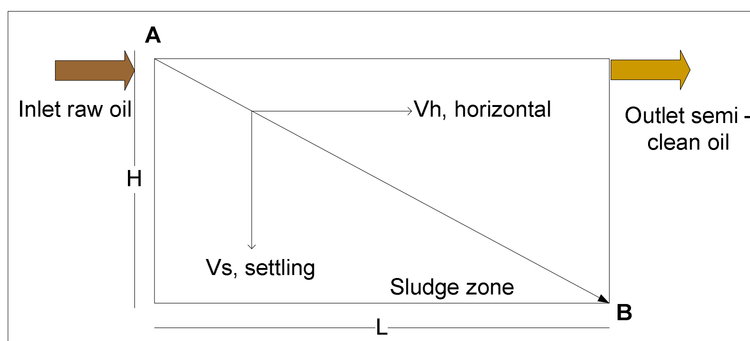


Fig. 3-3 Ideal up flow rectangular sedimentation tank

The velocity components of such a particle are Vh in the horizontal direction and Vs. the settling vertical velocity.

Camp-Hazen described a model for an ideal continuous sedimentation tank and put forward an equation that relates both, the horizontal and the vertical velocities given by the

relationship in Eq.1 [30].

$$A = \frac{Q}{V_s} \qquad \text{Eq. 1}$$

Where: A is the minimum area required for the clarification (cm2), Q the volumetric flow rate of liquid through the tank (m3/s), Vs is the sedimentation velocity (m/s).

This equation shows that the ratio Q/A (overflow rate) should be equivalent to the settling velocity of the smallest particle that is expected to be 100 % removed.

The tanks for the simple continuous system were connected in series by a continuous canal (Fig. 3-5) and the oil was passed with a constant flow rate through the settling system.

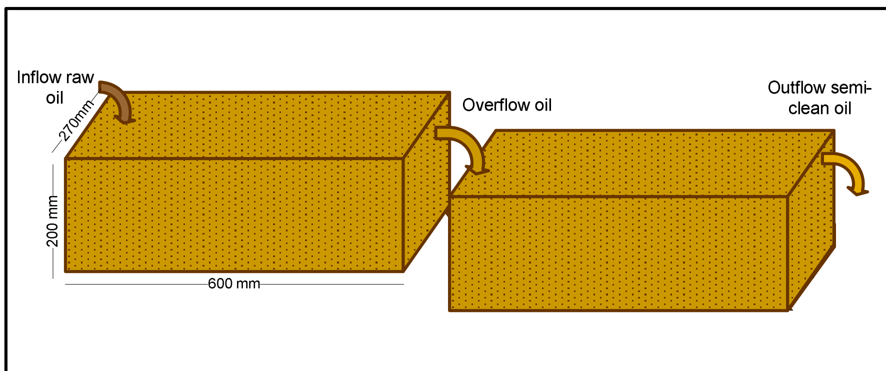


Fig. 3-4 Horizontal tanks for a continuous sedimentation system

The raw oil entered the first tank with a flow rate of approx. 12 l/h and was maintained constant until the oil reached the outlet of the tank. Equation 1 allows us to theoretically determine the flow rate needed for a selected particle size to precipitate at the bottom of the tank. Analyses of total contamination of the oil were performed at the beginning (raw oil) and at the end (semi-clarified oil) of the experiment. The tanks used for the continuous process are the same horizontal tanks used for the discontinuous sedimentation process.

Baffled tank system

The continuous system described above was further improved by introducing baffles within the tanks (Fig. 3-6). The use of baffles is based on the concept of slowing the flow velocity through the tank, thereby taking a longer time for the solids to settle at the bottom of the tank [32]. In addition to decreasing the flow velocity, the baffles help to reduce turbulence inside the tank in such a way that the sludge disturbance is also kept at a minimum. In principle, larger particles usually settle out first and accumulate in the first chambers, while smaller particles usually settle out in subsequent chambers.

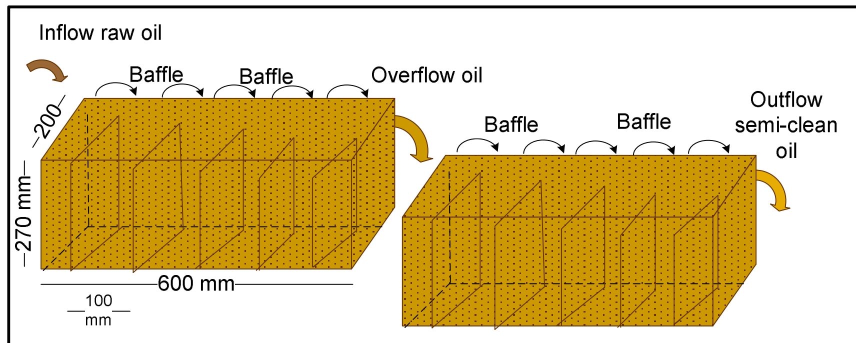


Fig. 3-5 Horizontal tanks with baffles for a continuous sedimentation system

The design of the baffled tank comprised five parallel baffles that were placed in declining order, i.e., the height of the baffles decreased with the distance of the baffles from the inflow wall. The different heights of the baffles allowed the oil to flow smoothly from one compartment to the next. The flat plates were allocated inside each tank, which were equally spaced.

The oil was discharged into the first tank with a constant flow rate of 12 l/h controlled by a valve. The efficiency of the system was determined by comparing the total contamination from the beginning to the end of the process.

Continuous sedimentation system „Weihenstephan Standard“

The design of this sedimentation method was due to the protocol known as the Weihenstephan system designed by the Technologie und Förderzentrum (TFZ) für Nachwachsende Rohstoffe in Straubing, Germany. The method consisted of 4 consecutive fixed funnels, located in the upper part of the system structure where the extraction of the clarified oil and the removal of the solids took place simultaneously (Fig. 3-7). The sediments that settle in the bottom of the tanks were transferred to separated sub-funnels located below each of the main four funnels. To avoid loss of the oil from the transfer of the sludge to the sediment tanks there is a valve between the main funnels and the sub-funnels.

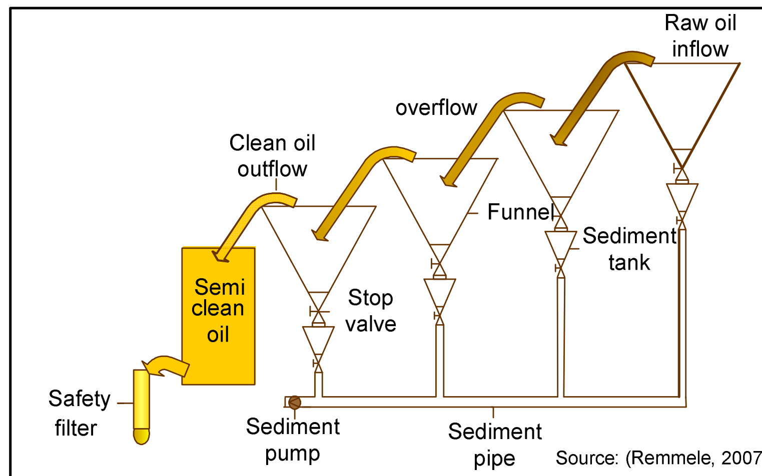


Fig. 3-6 Weihenstephan standard for a continuous sedimentation system

This system allows the raw oil to flow through the four fixed containers that are connected by unions and piping. The second, third and forth containers were filled from the overflow of the previous containers respectively [31, 33].

The efficiency of this clarification method was determined by calculating the total contamination after the oil had left each funnel.

The semi-clarified oil obtained by the continuous and discontinuous methods described above was then safety filtered by using first a bag followed by a cartridge filter.

Filtration

After the first stage of the clarification process, namely, the sedimentation process, the oil can still have traces of undesirable particles. Therefore it is necessary for the oil to be passed through an additional filter with a specific pore size of about 1 to 5 µm [31]. Bag and cartridge filters are used for this purpose, and to achieve better oil quality a two step filtration was performed. The first step was with the filter bag and the second step with the filter cartridge.

The particle size retention of 1 micron (µm) for filters, bags and cartridges is not absolute. In practice even particles of 10-15µm could pass through the bag as well as removing particles smaller than 1µm [34]. This is called depth filtration or cake filtration effect, which consists of a filter cake layer built up inside the bag. Recirculation of the oil took place after the first bag filtration; this recommendation was obtained from Amafilter group Deutschland, GmbH, the filters supplier.

Bag filters (Fig. 3-8) were used for the safety filtration of the *J. curcas* semi-cleaned oil. The principle of operation of the bag filter is as follows. The oil is allowed to flow from inside to the outside of the bag, whereby filtration occurs on the inner face of the bag by diffusion [35]. The bag filter used is manufactured in polyester, with dimensions 178 mm

diameter x 432 mm for the sides, and has a nominal retention of 1micron (µm).

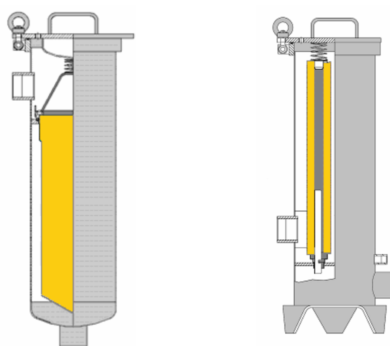


Fig. 3-7 Bag filter (a) and Cartridge filter (b)

A cartridge filter (Fig. 3-8) is characterized as a tubular filter contained in a matching vessel. The vessel or cartridge is usually constructed of plastic e.g. polypropylene or acrylic [35]. The material of the cartridge filter used is cotton with dimensions 64 mm diameter and 254 mm length, and fineness of 1 µ.

Test for the characterization of the *J. curcas* oil

The particle size distribution (PSD) of a fluid defines the relative amounts of particles present, which are sorted according to size. Analyses were performed by the TFZ laboratory on particle size distribution for raw oil, semi- cleaned and filtered *J. curcas* oil. The particle size analyzer used the laser diffraction Fraunhofer method, which is based on an analysis with a laser distribution between 0.1 µm - 8750 µm. This analysis complies with the specifications of ISO 13320-1 "Particle size analysis - laser diffraction methods". The chemical properties were analyzed at the Oil Laboratory of the institute of Agricultural Engineering in the Tropics and Subtropics at Hohenheim University. The samples were prepared following the guidelines laid down by the Deutsches Institute für Normung standards (DIN) for fat and oil derivatives.

Due to a variation in the oil chemical properties it is necessary to separate these into 2 main groups. The characteristic properties are dependent on the oil seed used. In this group density, calorific value, kinematic viscosity, carbon residue and the iodine number are considered. The second group consists of the variable properties that depend on processing, i.e. pressing, chemical extraction, filtering. This second group includes the total contamination, acid value and water content [21].

For our research the most important factor is the total contamination, since its behaviour provides us with an indication of how the purification process was performed.

The results obtained were analyzed according to the “Pre-standard DIN V 51605 for rapeseed oil as fuel”. This pre-norm was created to standardize the quality of the oil

produced for satisfactory engine operation [36]. Despite the fact, that this standard was created for rapeseed oil, the limiting values can be compared with other oils used in engines [21].

The specific gravity of the given oil is the oil weight divided by the same volume of water. The specific gravity for vegetable oils is usually about 0.910 - 0.920 at 25°C [38]. The density of *J. curcas* oil was analyzed; 1 ml of the sample was filled into the measuring cell at different temperatures, ranging from 20 to 90°C. The results are given in g/cm³ [39].

The heating value (HV) or calorific value of a fuel is the amount of heat released during the combustion of a specified amount of it. The calorific value of *J. curcas* oil was determined by employing an oxygen bomb calorimeter. It is measured in units of energy per unit of the substance, usually mass. The values are expressed in kJ/ kg [40].

Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. Kinematic viscosity is the ratio between the dynamic viscosity and the density of the fluid. As the temperature of the oil is increased, its viscosity decreases and is therefore able to flow more rapidly [9] [41]. A Rheometer was used to measure the dynamic viscosity at a temperature of 40°C with constant shear stress of 300 seconds [42].

The iodine value IV is an index of the number of double bonds in oil. It is a term that can quantify the degree of un-saturation of a fat. The IV expresses the number of grams of iodine that will react with 100 g of oil under specific conditions [38].

The iodine number was determined by titration of the sample solution and the blank sample with sodium thiosulfate until the colour changed from blue-yellow into white [43]. For the calculation of the IV the following formula was used:

$$IV = \frac{12.96 \cdot (b - s) \cdot n}{sw} \qquad \text{Eq. 2}$$

Where: IV is the iodine value (g Iod/100g oil), b the amount of sodium thiosulfate used in titration of "Blank" sample (ml), s sodium thiosulfate used in titration of "Sample" sample (ml), n the concentration of the sodium thiosulphate solution (moles/litre), sw the weight sample (g).

Total contamination is of high importance because the presence of larger particles in the oil could cause deterioration of the engine and plug filters.

The sample solution was filtered through a previously dried filter using a watch glass and a vacuum pump with a pressure of 2-5 kPa. The filter was dried again in an exsiccator for 45 minutes. Total pollution was calculated by dividing the difference between output weights by the weighed sample in mg/kg [44].

The carbon residue of a fuel is its tendency to form carbon deposits under high temperature conditions in an inert atmosphere. The carbon content was calculated with the Micro Carbon Residue Tester (MCRT-160).

Test tubes were weighed and placed into the oven for carbonization to take place. After the samples were measured they were weighed again. The calculation for carbonization was performed by taking the difference between output weight and initial weight in percent [45].

The acid value (av) or acidity is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid. The acid number is used to quantify the amount of acid present, in a sample of oil.

The acid value of the *J. curcas* oil was determined by titration testing. The sample was dissolved in a solution of potassium hydroxide solution (0.1 Mol/l) and phenolphthalein was added as a color indicator. The titration took place until the colour of the solution changed to pink. The acid value was calculated using the following formula:

$$av = \frac{56.1 \cdot \mathrm{v} \cdot \mathrm{c}}{\mathrm{sw}} \qquad \text{Eq. 3}$$

Where: ac is the acid value , 56.1 is the molecular weight of KOH., v is the amount of titrant KOH (ml) consumed by 1 ml spiking solution at the equivalent point, c is the concentration of the KOH solution, sw the weight of oil sample used (g) [46].

Usually, only a small quantity of water is present in the oil. The water content should be kept as low as possible to avoid injection problems and engine corrosion [27]. Water content can be reduced through mechanical extraction [13]. It was calculated with the KF Coulometer 831, which displays on its LCD screen a graphical path of the Karl Fischer method applied to determine the water content where µg water is a function of time.

The values are expressed in mg/kg [47].

The experiments and results were repeated in triplicate and a mean value was calculated. The values were rounded off to whole numbers. The results of continuous and discontinuous sedimentation systems were plotted using the OriginPro-8 software. Furthermore, fitting the data to determine the best function to describe the behaviour was performed using the same software.

Results and Discussion

Characterization of the *J. curcas* raw oil

The particle size distribution (PSD) for the raw *J. curcas* oil (immediately after it was cold pressed) is shown in Fig. 3-9. The PSD varied in a wide range, from 4.25 μm to 735 μm, with an average of about 175 μm. This wide particle size distribution of the oil resulted in a broad range of sedimentation times for the particles. The PSD values are given as percentages of the cumulative distribution of the particles. From Fig. 3-9 one can interpret that 50 % of the volume of the particles was smaller than 25 μm. The green line represents the mean values of PSD for the raw oil, and the black and red lines are the maximum and minimum particle size values, respectively.

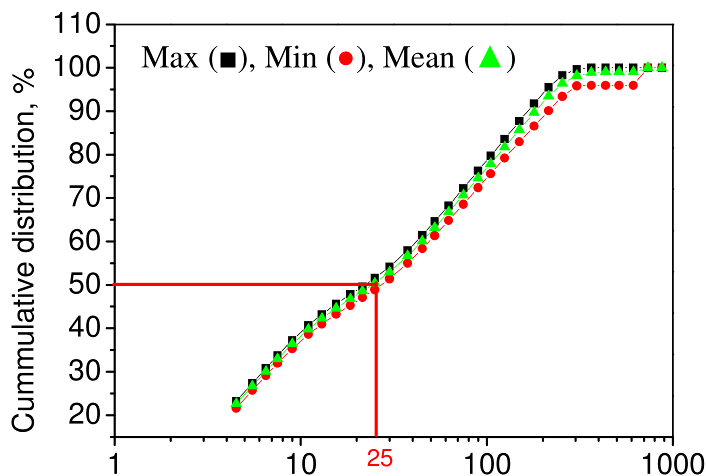


Fig. 3-8 Particle size distribution of *J. curcas* crude oil

There was not a wide variation of the PSD for raw *J. curcas* oil from the mean values. Specifically, for any given particle size the difference between its mean value and its minimum and maximum values varied between 0.6% and 3.3%.

Particle size distribution for the clarified oil in a discontinuous system is depicted in Figure 3-11. The black, red, blue and green lines represent the PSD for the raw, semi-clarified, bag and cartridge filtered oil, respectively. The raw oil's PSD comprises a broader range of particles, i.e., from 1 micron up to 735 microns, compared to the semi-clarified and filtered oils. This is due to the fact that the biggest particles are removed during the sedimentation process. For instance, after sedimentation, the larger sized particles present in the oil were equal to 175 microns.

For the raw oil, 50 % of the accumulated particle distribution corresponds to particle sizes equal to or smaller than 25 microns. For the semi-clarified oil and the filtered oil, 50 % of the accumulated volume corresponds to particle sizes equal to or smaller than 12 and 7.5

microns, respectively. This is a clear indication of the reduced number of larger particles present within the oil after each clarification stage. However, there was no significant difference between oil filtered by the bag and cartridge filter systems.

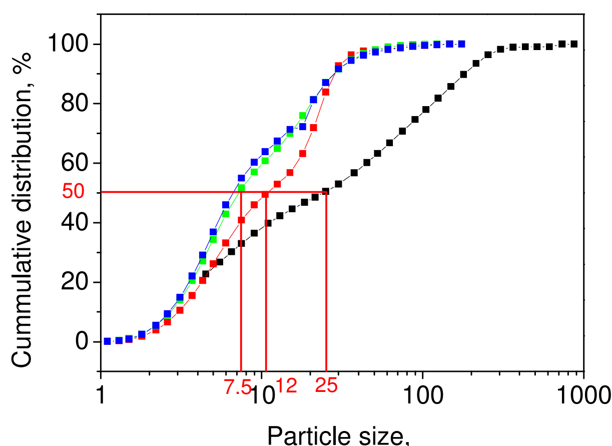


Fig. 3-9 Particle size distribution for the clarified *J. curcas* oil

Raw oil (■), Semi-clarified oil (♦), Bag filtered oil (▲), Cartridge filtered oil (▼)

Particle sizes ≤ to 5.5 microns were still present in the oil at the end of the clarification process.

In case of these particles being present, which may interfere with the operation of plant oil stoves, improvements of the filtration system should then be implemented.

Chemical properties

The chemical properties of *J. curcas* crude oil are presented in Table 3-1. The total contamination values ranged from 2274 to 3682 mg/kg, with an average of about 2978 mg/kg. This result is characteristic of the type of oil press used [28].

Table 3-1 Chemical properties of *J. curcas* raw oil

Chemical	Units	Value	DIN 51605
Total	mg/kg	2978.33 ±	24
Acid value	mgKOH/g	6.09 ± 0.20	max. 2.0
Water content	mg/kg	3679.13	max. 750
Density	kg/ccm	928.87 ±	900-930
Calorific	kj/kg	38696 ±	min.36.000
Kinematic	mm2/s	42.40 ±	max. 36.0
Carbon	mass %	0.80 ± 0.06	max. 0.40
Iodine	g Jod/100g	104 ± 0.58	95-125

Values are means of three replications, ± SD

Approximately 32% of the total volume corresponded to muddy oil. It is important to point out that the settings of the press and its components could influence the total contamination

and the particle size distribution of raw oil more than the properties of the seeds. The previous statement has been reported for cold pressed rapeseed oil [28].

The acid value, water content, and kinematic viscosity values are higher than those expected to comply with the "Pre-norm DIN 51605 for rapeseed oil as fuel"[48]. However, density, calorific value and iodine number results, meet the quality standard DIN 51605, with average values of 928.9 kg/cm, 38696 kj/kg, 104 g Jod/100 g, respectively. For our analysis, only the total contamination, kinematic viscosity and density are of interest since they are influenced by and play an important role on the clarification process of the oil. The influence of these properties on the performance of the *J. curcas* as fuel for plant oil stoves is not within the scope of this work.

Density and viscosity for *J. curcas* oil

Density and viscosity of *J. curcas* raw and sediment oil at different temperatures are depicted in Fig. 3-10. The increase of temperature produces a decrease of density and viscosity for both raw and sediment oil.

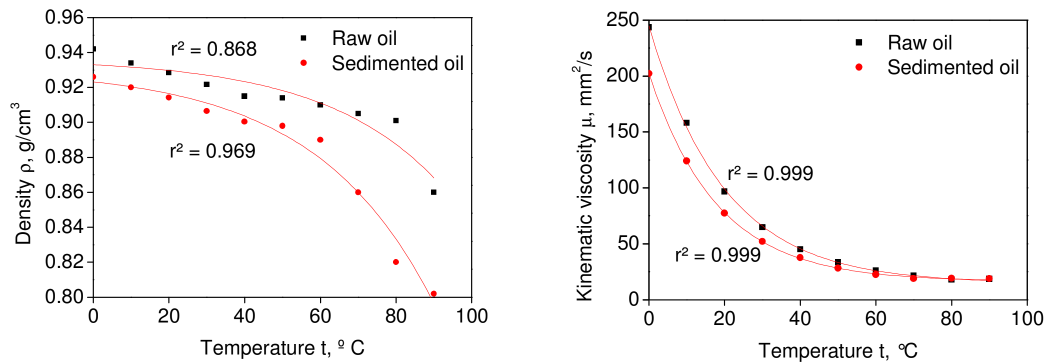


Fig. 3-10 Density □ and kinematic viscosity □ for raw and sedimented *J. curcas* oil

Statistical analysis for raw oil density ρr, reveals a close correlation to temperature that can be expressed as an exponential function ($r^2 = 0.868$).

$$\rho_r = -0.003 \cdot e^{\left(\frac{x}{30.49}\right)} + 0.936 \qquad \text{Eq. 4}$$

Statistical analysis for sediment oil density ρs, reveals a high correlation to temperature that can be expressed as an exponential function ($r^2 = 0.969$).

$$\rho_s = -0.007 \cdot e^{\left(\frac{x}{31.25}\right)} + 0.930 \qquad \text{Eq. 5}$$

Statistical analysis of kinematic viscosity of raw oil μr and oil sediment μs, reveals a high correlation to temperature that can be expressed as an exponential functions ($r^2 = 0.999$).

$$\mu_r = 230.18 \cdot e^{\left(\frac{-x}{19.94}\right)} + 14.59 \qquad \text{Eq. 6}$$

$$\mu_s = 185.79 \cdot e^{\left(\frac{-x}{18.12}\right)} + 16.46 \qquad \text{Eq. 7}$$

There is also a noticeable decrease of density and viscosity values of the sediment oil in comparison to the raw oil. This is due to a lower concentration of solids in sediment oil and larger thermal conductivity of the raw oil compared to the sediment. In this way the oil absorbs more energy from the applied heat yielding a lower viscosity.

Settling time

Figure 3-11 shows the correlation between the height of the interface and the settling time for the raw oil in a test tube. The correlation between the two variables was estimated as being an exponential function which shows a strong correlation coefficient ($R^2 = 0.998$).

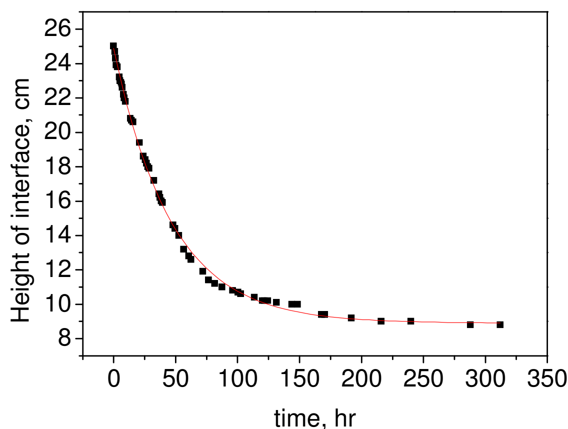


Fig. 3-11 Settling curve for *J. curcas* crude oil: Test tube

The fitted exponential function is presented in Eq. 8, where h is the estimated height of the interface and t represents the settling time. C1, C2 and C3 are constants. The values of these parameters and their standard deviations are given in Table 3-2.

$$\mathrm{h(t)} = C_1 \cdot \exp^{\left(\frac{-t}{C_2}\right)} + C_3 \qquad \text{Eq. 8}$$

Figure 3-2 shows the correlation of the height of the interface and the settling time for the horizontal and the vertical tank, respectively. The best fit was obtained with an exponential function. The fitted functions have the same form of Eq. 8. The correlation coefficients are shown in Table 3-2.

Table 3-2 Parameters and correlation coefficients of the fitted function to the data of height of interface vs. settling time

Settling device	C1	C2	C3	R2
Test tube	15.97 ± 0.10	46.8 ± 0.82	8.89 ± 0.08	0.998

Horizontal	11.94 ±0.33	3.54 ±0.245	2.91 ±0.15	0.961
Vertical	40.10 ± 0.71	21.38 ±1.07	11.98 ±0.52	0.996

The sedimentation time needed to obtain a clear *J. curcas* oil, namely, amber colour in a horizontal tank was about half the time needed for the vertical tank. The required times were 48 and 96 hours, respectively. This difference is mainly due to the shorter distance that the particles have to travel until they settle at the bottom of tank in comparison to a vertical tank with the same capacity.

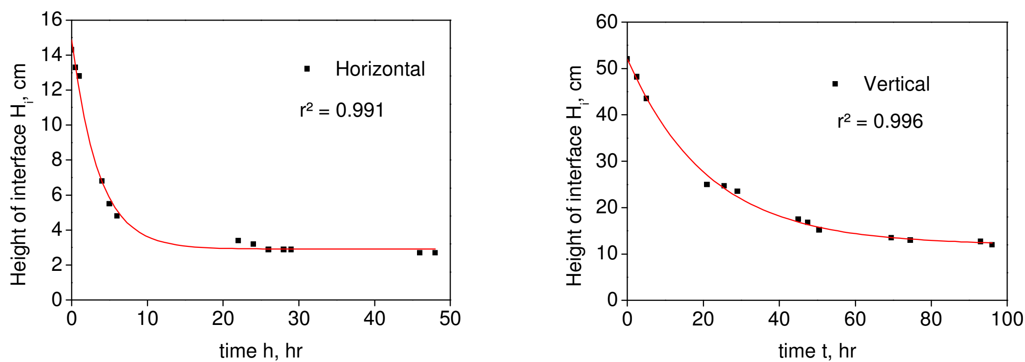


Fig. 3-12 Settling curve for the discontinuous sedimentation process

In Fig. 3-13 one can observe a typical batch settling curve (Kynch theory), where the sedimentation velocity decreases with time. Initially, the particles recover from their initial disturbances induced during the filling of the oil in the tank, and the descending particles becomes evident by observing the highest settling velocity fall of the largest particles and the large concentration of solids at the bottom. When this settling zone at the bottom becomes saturated, the settling velocity slows down because the largest particles have already settled.

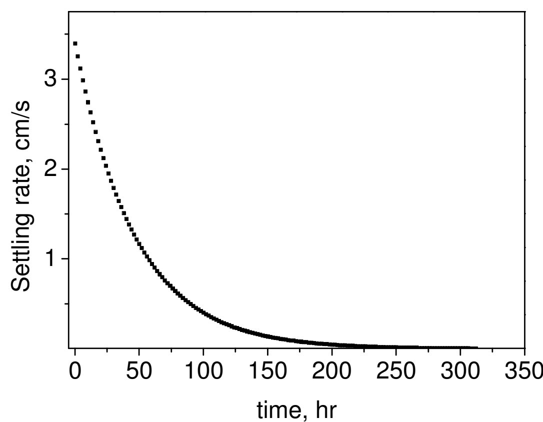


Fig. 3-13 Settling rate for *J. curcas* oil

At this stage, the settling rate decreases and approaches zero, since only the smallest particles remain before descending. Settling velocities ranging between 1·10-7 and 1·10-5

are expected.

In the last stage, there is no a significant decrease in the height of the interface because the remaining particles are of a very small size and will take longer to settle by gravity.

The model presented in Eq. 8 was used to estimate the settling rate based on the observations of height of the interface and the settling time of the *J. curcas* oil at 20 °C. This settling rate was calculated by taking the derivative with respect to time given by Eq. 8, namely:

$$\frac{dh}{dt} = -\frac{C_1}{C_2} \cdot \exp^{\frac{-t}{C_1}}$$

Eq. 9

The horizontal asymptote of the function presented in Eq. 8 was calculated in Eq. 9, in order to find the minimum point that is reached for the height of the interface when t tends to ∞. Given an infinite period of time for sedimentation, there is a point when the height of the interface will only decrease negligibly

$$\lim_{t \to \infty} h(t) = C_1 \cdot \exp(-\infty) + C_3$$

Eq. 10

$$h(t) = C_3$$

In other words, the fitted parameter C3 (Table 3-2) obtained from the availble experimental data is an indicator of the approximate value for which the height of the sediments is not expected to change any longer with time. Therefore, the second stage of the clarification process can be started. For the settling horizontal tank a height of 3 cm was found, whereas for the vertical tank the height was 12 cm.

Total contamination

Total contamination of the discontinuous system for horizontal and vertical orientation is depicted in Fig. 3-14. Statistical analysis for horizontal total contamination Tc.h, reveals a high correlation to time that can be expressed as an exponential function (R^2 = 0.984).

$$T_{c.h} = 64.09 \cdot e^{\left(\frac{-x}{28.25}\right)} + 35.62$$

Eq. 11

Statistical analysis for vertical total contamination Tc.v, reveals a high correlation to time that can be expressed as an exponential function (R^2 = 0.909).

$$T_{c.v} = 54.18 \cdot e^{\left(\frac{-x}{101.52}\right)} + 42.91$$

Eq. 12

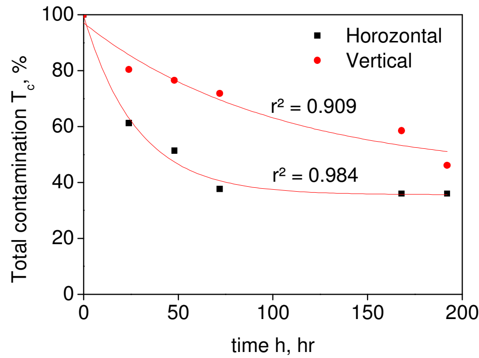


Fig. 3-14 Total contamination Tc for horizontal and vertical clarification systems

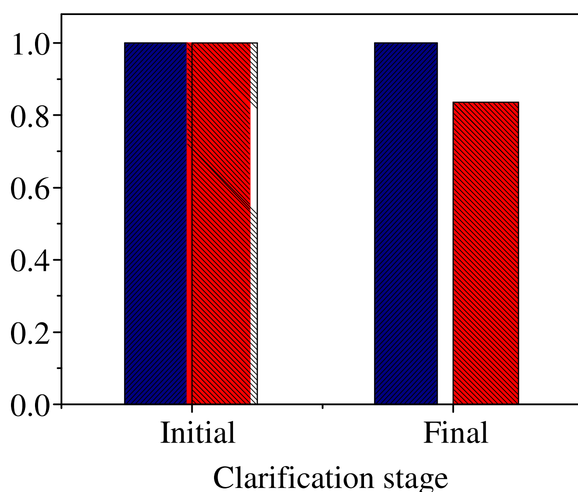
After 190 hr the efficiency of the vertical sedimentation system was about 54 % and that of the horizontal sedimentation system was about 65 %.

Total contamination for a continuous systems

Fig. 3-15 shows as a percentage the results of the total contamination for the continuous sedimentation systems; including the simple continuous system and baffle system.

The simple continuous system operated with a flow rate of 12 l/h but did not show any reduction on the total contamination. The oil at the inlet and outlet of the sedimentation tank had the same total contamination. This was due to the fast oil's flow rate through the tanks. Therefore, the particles did not have enough time to settle at the bottom of the tank before reaching the outflow.

The theoretical flow rate needed for the particles to settle in a continuous system for an area of 2400 cm2 (for both tanks) is given in Table 3-2.



Simple continuous system (■), Baffle system (■)

Fig. 3-15 Total contamination simple continuous and baffle systems

In principle, for particles smaller than 140 microns, a flow rate of 12 l/h was too high to allow the particles to settle. But, the same flow rate would be adequate for the sedimentation of particles larger than 140 microns. However, in practice this was not the case. This could have happened because the particles were moving in irregular trajectories along the tank. Besides that, the flow of oil higher up in the tank caused higher vortices within the fluid. The baffled sedimentation system showed a higher efficiency than the simple continuous system. The clarification process lasted approximately 4.5 hours with a flow rate of 10l/h. The reduction of the total contamination was about 16 % with respect to the initial content. Fig. 3-16 shows the total contamination reduction for the Weihenstephan system as a percentage, where 100 % stands for the raw non clarified oil.

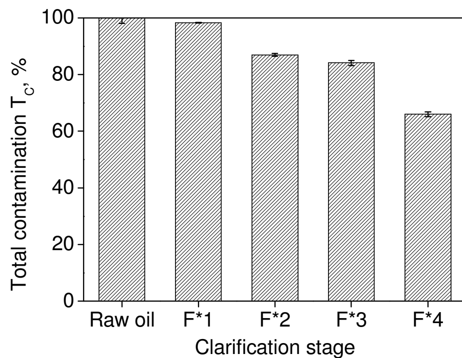


Fig. 3-16 Total contamination TC of Weihenstephan system

The standard deviation was around 13 % and the reduction of the total contamination reduction reached at the end, was about 34 % in 5 hours.

Safety filtration

Fig. 3-17 shows the total contamination along the clarification process including the safety filtration for the Weihenstephan.

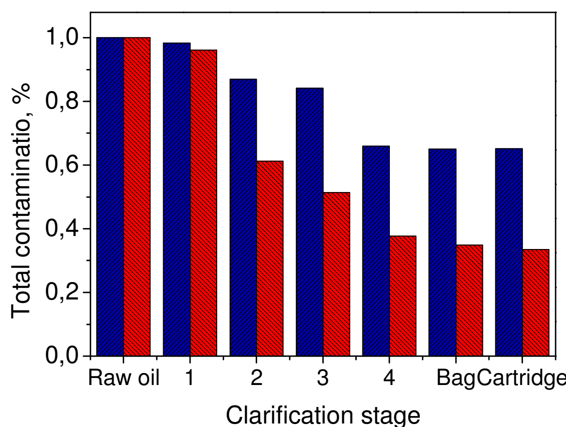


Fig. 3-17 Total contamination for the continuous and the discontinuous system

TCC((■),TCD (■)

The blue bars show the TCC (Total Contamination for the Continuous system) and the red bars the TCD (Total Contamination for Discontinuous system). There was a minimum reduction between the semi-clarified oil and the filtered oil of about 1 to 3% respectively. The filtration between the bag and the cartridge filter did not show any difference.

The semi-clarified oil that was filtered had accumulated a significant amount of sediment within the specification of the filters. This influenced the clogging of the filter pores before the oil could pass through the filter.

Conclusion

The *J. curcas* raw oil showed a broad range of particle size distribution, from 4.25 µm to 735 µm. The sedimentation time therefore presented a wide range since it is dependent on the particles diameter.

The discontinuous sedimentation system in a horizontal tank showed a better performance than the vertical tank since the time required for the removal of the particles is dependent on the geometry of the tank. The horizontal system reached its maximum efficiency on the third day of the sedimentation process, with a 62 % of total contamination reduction. The average total contamination obtained by this method was about 800 mg/kg. This value remained approximately constant indicating that from this point onwards the total contamination reduction was very slow.

The results of the continuous systems showed a large variation of the results concerning the removal of the sediments. Specifically, by using the simple continuous system no clear trend of contamination reduction was found. However, the continuous sedimentation system with baffles performed better than the simple continuous one. This system had better results with a 16 % of contamination reduction in 4.5 hours.

Finally, the Weihenstephan system showed an efficiency of 35 % in only 5 hours. For the same time and for the discontinuous system the reduction was about 1%. It is therefore concluded that the continuous system is more time efficient.

Further tests for different retention times are recommended in order to select a more convenient time for clarification. In general, the efficiency of the continuous systems could be improved by implementing a flow rate regulator. It will also allow the identification of an optimum flow rate for a given tank geometry.

For the optimum selection of the clarification system of oil, the tank geometry, the flow rate, and the sedimentation conditions, are very important to first establish the limit (maximum value) of total contamination of the *J. curcas* oil, which is suitable for plant oil stoves. Having established this limiting value, a target particle size can be defined and consequently the parameters and conditions of the clarification process.

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Objective 3: Preparation of *Jatropha* oil for direct use in plant oil stoves.

Introduction

The usage of energy from plant oil is becoming ever more attractive due to the gradual depletion of fossil fuels thus causing rising prices. Plant oil is now becoming more popular for fuelling I.C. engines and for heat generation. Especially oil from the *Jatropha curcas* seeds is receiving a lot of interest due to its manifold applications [1-3].

For the use of plant oil as household cooking fuel a new technology is emerging. A plant oil pressure stove from Bosch and Siemens Hausgeräte GmbH in Munich (Germany) has been developed [4,5] and recently introduced in the Philippines and Indonesia. Nevertheless, the combustion of crude plant oil in pressure stoves can lead to deposit formation inside the vaporizer [6,7]. These deposits have to be removed at regular intervals as a preventive maintenance requirement.

Very little research work has been devoted to problems arising in the development of plant oil pressure stoves when fuelled with pure plant oil.

Kratzeisen et al. [6] observed that an increased formation of deposits in plant oil pressure stoves was the result of an increased phosphorous content in oil. Furthermore, an increased acid value in plant oils can lead to a rise in deposit formation and accumulation within the vaporizer of plant oil pressure stoves [8]. Kratzeisen et al. [9] has described the deposit formation behaviour of different soybean oils, with different degrees of saturation, during operation in pressure stoves. They found an augmented accumulation of deposits with a higher degree of unsaturation. Furthermore, Kratzeisen et al. [10] found that the content of earth alkaline in plant oil, especially of calcium and magnesium may influence the operation of plant oil pressure stoves. An increased deposit formation inside the vaporizer of plant oil pressure stoves can lead to higher maintenance requirements and therefore rendering it less user friendly. Until now, there are no standards in existence for *Jatropha curcas* oil quality as fuel for plant oil pressure stoves, which would provide threshold values to ensure reliable long-term operation.

The objectives of this study were to establish a model to estimate the deposit formation from *Jatropha* oil quality parameters by applying a multiple regression analysis and to derive a standard for *Jatropha* oil quality guaranteeing a safe application for plant oil pressure stoves.

Materials and methods

Plant oil pressure stove

Tests were carried out with a plant oil pressure stove, Fig: 4-1 ('Protos II', BSH Bosch und Siemens Hausgeräte GmbH). 'Protos II' is an improved version of the previous 'Protos I' plant oil stove which was described in [9, 10].

Fig: 4-1. Plant oil pressure stove "Protos II" from Bosch Siemens Hausgeräte GmbH.

Fuel

Jatropha curcas oil was used as fuel. The origin of the *Jatropha curcas* seeds was in India. The oil was pressed in the laboratories of the University of Hohenheim by a screw press, type Monforts Komet L85. After pressing, the *Jatropha* oil was allowed to settle in a sedimentation tank for 48 hours to separate the solid phase from the oil. After settling, the raw oil was homogenized and analyzed as described in Chapter 1.2.3. Different settings of the screw press resulted in 20 different qualities of test fuel. For evaluation of the regression model, different plant oils (Table 4-5) were analyzed according to standard methods and were tested in the plant oil stove as described in Chapter 1.2.4.

Chemical analyses

The *Jatropha* oil was analyzed for Conradson carbon residue CCR, density D, higher heating value HHV and lower heating value LHV, kinematic viscosity KV, iodine value IV, total contamination TC, acid value AV, phosphorous content PC, calcium content CC, magnesium content MC, sodium content SC, potassium content POC, ash content AC and water content WC according standard methods. Three replications were performed for each test fuel. Mean values were used for further statistical analysis.

Test procedure

The plant oil pressure stove was preheated for 5 minutes to reach a temperature of 550°C at the vaporizer. Then the valve to the plant oil pressure tank was opened to raise the pressure until reaching final operating pressure of 1 bar. The duration for each test was 3 hours. The specific fuel consumption SFC of the plant oil pressure stove was continuously measured by weighing the pressure tank with an accuracy of ±0.5g. SFC was calculated in kg/h. Before starting the operation, the nozzle was cleaned with a heat

resistant steel wire. After turning off and cooling down the plant oil stove, the accumulated deposits were removed from the vaporizer mechanically and weighed with an accuracy of ±0.01g. The deposits DEP were related to the amount of consumed fuel and calculated in g/kg.

Statistical analysis

Deposits DEP, Conradson carbon residue CCR, total contamination TC, acid value AV, phosphorous content PC, calcium content CC, magnesium content MC, sodium content SC, potassium content POC, ash content AC and water content WC were correlated by stepwise linear multiple regression, forward method, using 'SPSS 15'.

Results

Quality parameters of test fuels.

In Table 4-1 the characteristic properties of the applied *Jatropha curcas* oil are shown. Density was 0.914 kg/m³ and was constant for all the test fuels. The higher heating value and the calculated lower heating value varied only slightly as can seen from the low standard deviation. They were on average 39.636 MJ/kg for HHV and 37.139 MJ/kg for LHV. The kinematic viscosity KV did not influence the burning behaviour of the plant oil stove. During operation of the plant oil stove the frame and internal piping as well as the valve were heated up to 70°C. At this temperature viscosities of different test fuels are similar, therefore exhibiting almost identical flow characteristics. Under climatic conditions representative of the regions of application the viscosity of plant oils is an important parameter. In general, kinematic viscosity must be low enough to enable the oil to flow under pressure from the tank to the plant oil stove. The iodine value IV was on average 104 ± 2 g I2/100 g. The influence of the iodine value on the formation of deposits was investigated by Kratzeisen et al. [19], who found that a higher iodine value of plant oils can lead to a higher amount of deposits. Differences of IV of test fuels were marginal, so no influence of raised deposit formation by IV was expected. The variable properties are scattered around mean values as shown in Table 4-1. An accumulation of deposits DEP was in the range between 0.93 g/kg and 2.11 g/kg and DEP were found to be in the pyrolysis zone, where both, liquid and gaseous fractions of the evaporating plant oil were present [20]. Consistency of these deposits was soft and could be easily removed. So it can be stated, that the second generation of plant oil stove ´Protos II´ was able to run with unfiltered *Jatropha curcas* oil. The specific fuel consumption SFC was in the range between 209 g/h and 237 g/h. This means that the power output varied only marginally when different test fuels were applied. Generally the combustive behaviour of the plant oil

stove was satisfactory and all tests runs could be completed without failures.

Table 4-1. Characteristic properties, variable properties and test results from burning of test oil; mean values ± SD, n= 20.

Characteristic properties of *Jatropha* oil			
Parameter	Mean	Min	Max
D, kg/m³	0.914±0.000	0.914	0.914
HHV, MJ/kg	39.636±0.117	38.734	40.322
LHV, MJ/kg	37.139±0.115	36.227	37.816
KV, mm²/s	77.018±1.368	75.250	80.140
IV, g I2/100 g	104±2	100	108
Variable properties of *Jatropha* oil			
CCR, %	0.270±0.070	0.162	0.482
TC, mg/kg	1334±324	966	2109
AV, mg KOH/g	6.7±1.5	4.6	11.0
PC, mg/kg	42.2±12.4	20.5	67.6
CC, mg/kg	17.8±3.6	11.8	25.0
MC, mg/kg	12.8±4.1	7.0	24.3
SC, mg/kg	0.9±0.5	0.5	2.5
POC, mg/kg	21.9±10.4	9.4	48.3
AC, %	0.033±0.031	0.002	0.111
WC, %	0.109±0.022	0.083	0.180
Combustion characteristics			
DEP, g/kg	1.31±0.35	0.93	2.11
NCF, 1/h	0.73±0.42	0.00	1.33
SFC, kg/h	223±7	209	237

Multiple regression analysis

Table 4-2 shows the quality parameters of four different models for the prediction of deposits DEP. From models 1 to 4, the parameters AV, WC, AC and finally MC were included stepwise in the regression model. An adjusted R2 increased thereby from 0.356 to 0.820 for models 1 to 4. Model 4 was the most complex and with an error probability of P<0.000 was also the most significant.

In Table 4-3 the values for the regression equation for predicting the dependent parameter deposit DEP and the Beta values are shown. The Beta values are standardized and

therefore useful, for the evaluation of those values, which have the greatest effect on the predicted value.

For model 3 the formation of deposits is influenced ´by the acid value but almost with the same level of water content WC and contributes to the ash content, which is 1.6 times higher.

Table 4-2. Regression coefficient R2, adj, R2, Standard error SE, F-value and significance P of multiple linear regressions model (1-4) of deposits DEP vs. plant oil quality parameters.

Model	Parameter	R2	adj. R2	SE	F-value	Significance
1	AV	0.390	0.356	0.277	11.521	0.003
2	AV, WC	0.716	0.682	0.195	21.392	0.000
3	AV, WC, AC	0.819	0.785	0.160	24.081	0.000
4	AV, WC, AC, MC	0.858	0.820	0.147	22.683	0.000

Table 4-3. Coefficient of multiple linear regression models (1-4) B, SE, Beta, t-value and significance of deposits DEP vs. plant oil quality parameters.

Model	Parameter	B	SE	Beta	t	Significance
1	Constant	0.3712	0.2832	-	1.311	0.206
	AV	0.1398	0.0412	0.625	3.394	0.003
2	Constant	-0.7366	0.3205	-	-2.299	0.035
	AV	0.1597	0.0293	0.713	5.450	0.000
	WC	0.8921	0.2021	0.577	4.410	0.000
3	Constant	-0.5624	0.2700	-	-2.083	0.054
	AV	0.1271	0.0264	0.568	4.814	0.000
	WC	8.1112	0.1682	0.525	4.811	0.000
	AC	3.9843	1.3213	0.353	3.015	0.008
4	Constant	-0.735	0.261	-	-2.820	0.013
	AV	0.059	0.041	0.265	1.440	0.170
	WC	0.985	0.176	0.637	5.597	0.000
	AC	5.070	1.320	0.449	3.841	0.002
	MC	0.032	0.015	0.278	2.039	0.059

Table 4-4 shows the parameters of test fuels which were not included in models 1 to 3. Furthermore the Beta-In-values with the corresponding t-value and their significance are given. The Beta-In-values indicate the beta weight of the variables if they would be included in the model.

The Beta-In-values of the excluded parameters of magnesium content MC in model 3 at 0.278 was below the Beta value of ash content AC at 0.353. However, the significance of magnesium content at 0.059 was marginally below the rejection criteria of 0.05. Including magnesium content MC into the model resulted in a slight adjustment of R2 from 0.785 to 0.820.

Table 4-4. Beta In, t-value and significance of excluded oil parameters of multiple linear

regression models (1-3) for deposits DEP vs. plant oil quality parameters.

Model	Parameter	Beta In	t	Significance
1	CCR	-0.023	-0.106	0.917
	TC	0.352	2.026	0.059
	PC	-0.416	-2.150	0.046
	CC	-0.334	-1.530	0.144
	MC	-0.363	-1.334	0.200
	SC	-0.010	-0.019	0.985
	POC	-0.803	-2.494	0.023
	AC	0.442	2.523	0.022
	WC	0.577	4.414	0.000
2	CCR	-0.077	-0.504	0.621
	TC	0.328	3.014	0.008
	PC	-0.055	-0.292	0.774
	CC	0.070	0.362	0.722
	MC	0.090	0.390	0.702
	SC	0.327	0.899	0.382
	POC	-0.234	-0.739	0.470
	AC	0.353	3.014	0.008
3	CCR	-0.060	-0.479	0.639
	TC	0.192	1.279	0.220
	PC	0.185	1.107	0.286
	CC	0.180	1.139	0.273
	MC	0.278	2.039	0.059
	SC	0.196	1.741	0.102
	POC	-0.035	-0.127	0.901

For further consideration and for evaluation model 3 was selected. The regression model generated from the acid value AV, water content WC and ash content AC parameters were expressed by Equation 1:

$$DEP = -0.5624 + 0.1271 \cdot AV + 8.1112 \cdot WC + 3.9843 \cdot AC \quad (1)$$

A highly significant ($p < 0.000$) adjusted R2 of 0.785 for Model 3 indicated that deposits DEP could be estimated from these parameters with reliable accuracy. Furthermore, the advantages of model 3 in comparison to model 4 were that all predictors were determined

by simple standard methods.

Model validation

For validation of model 3, twelve different unrefined plant oils were used as test fuels as shown in Table 4-5. The acid value AV, water content WC, and ash content AC of test fuels were analyzed by standard methods, and the deposits were predicted by using model 3, and measured after burning of test fuels in the plant oil pressure stove was complete. Fig: 4-2 shows the correlation between the predicted and measured deposits. The coefficient of regression was $R^2 = 0.756$.

Table 4-5. Acid value AV, water content WC, and ash content AC of Test fuel for validation of model 3, n=3.

Number	Test fuel	AV, mg KOH/g	WC, %	AC, %
1	*Jatropha* oil 1	4.10	0.083	0.030
2	*Jatropha* oil 2	8.49	0.064	0.008
3	*Jatropha* oil 3	8.52	0.101	0.011
4	*Jatropha* oil 1	9.01	0.105	0.054
5	*Jatropha* oil 2	6.49	0.101	0.020
6	Palm oil 1	12.17	0.158	0.016
7	Palm oil 2	9.38	0.174	0.049
8	Palm oil 3	9.81	0.195	0.080
9	Cottonseed oil	0.97	0.087	0.004
10	Sunflower oil	2.49	0.083	0.001
11	Coconut oil 1	7.71	0.072	0.018
12	Coconut oil 2	3.88	0.104	0.038

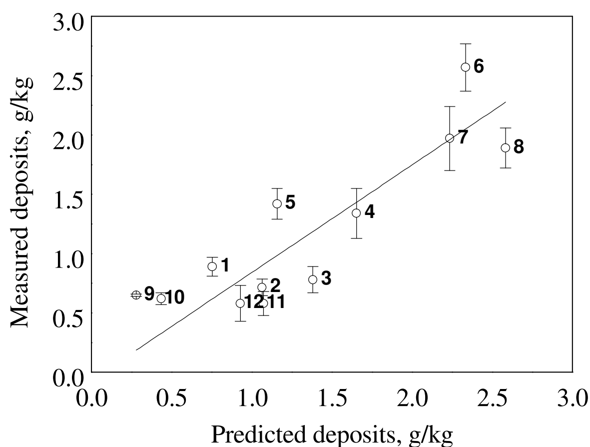

Fig: 4-2. Correlation between measured and predicted deposits (model 3), numbers identify

test fuels according Table 4.5.

Jatropha oil standard for use in plant oil pressure stoves

In Table 4-6 the recommended quality parameters for *Jatropha* oil, which will guarantee a reliable operation of plant oil pressure stoves, were suggested. Acid value AV lower than 6.00 mg KOH/g, water content WC less than 0.15% and ash content AC below 0.10%. When these threshold values are considered, accumulated deposits can be removed easily and cleaning time will be tolerable.

Table 4-6. Recommended threshold values for *Jatropha* oil for use in plant oil pressure stoves.

Parameter	Abbreviation	Unit	Range
acid value	AV	mg KOH/g	<6.00
water content	WC	%	<0.15
ash content	AC	%	<0.10

Even though model 3 was applicable for different kinds of plant oils, the application of this model can only be recommended for crude *Jatropha* oil. As different plant oils require different production processes, oil components could be present which were not considered by model 3.

Conclusion

The purpose of this study was to investigate the influence of various *Jatropha* oil parameters on deposit formation in plant oil pressure stoves by applying a multiple regression analysis. A model based on the parameters acid value AV, water content WC and ash content AC was useful for the prediction of deposit accumulation inside the vaporizer of the plant oil pressure stove. Threshold values are recommended for safe operation: acid value AV lower than 6.00 mg KOH/g, water content WC lesser than 0.15% and ash content AC below 0.10%. If these recommendations are adhered to, an uninterrupted and reliable operation of plant oil pressure stoves with low maintenance requirements can be achieved.

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Objective 3.1: Gaseous emissions of a plant oil stove when fuelled with oil from *Jatropha curcas* .

Introduction

Interest in *Jatropha curcas* has increased noticeably due to the multifunctional characteristics of the oil plant. Of particular interest is the high oil content of the seeds which can be used for biodiesel production (Makkar et al. 2008).

The oil “is used for illumination (it burns without emitting smoke) and as a lubricant for making soaps, candles, and varnish” (Makkar et al. 1997). Its oil can be used to substitute kerosene and diesel and as a substitute for fuel wood for household cooking devices. It has been promoted to make rural areas self sufficient in fuels for cooking, lighting and motive power.

The use of plant oil as fuel for cooking purposes can decrease the exposure of indoor air pollution to inhabitants in rural communities. However, nothing is known about the emissions of plant oil pressure stoves when fuelled with *Jatropha curcas* oil. Therefore the objectives of this Study were to determine the effects of emissions when toxic and non toxic *Jatropha curcas* oil, varieties were burned in plant oil pressure stoves and to detect the differences between compounds from *Jatropha curcas* oil, toxic and non toxic, which

remain in the emission after burning in a plant oil pressure stove.

Materials and methods

Test Oils

Jatropha curcas toxic variety and *Jatropha curcas* non toxic variety of oil were used for the study. The physical and chemical characteristics of the oils are shown in Table 5-1.

Table 5-1. Specification of the oils

Parameter	Unit	*Jatropha curcas* oil	*Jatropha curcas* non oil
Kinetic Viscosity at 40°C	mm²/s	78.96	77.58
Free fatty acid content	%	2.98	0.87
Iodine value	-	101	103
Carbon content	%	77.1	77.2
Hydrogen content	%	11.8	11.7
Oxygen content	%	11.3	11.2
Nitrogen content	%	0.1	0.13
Water content	%	0.822	0.393
Phosphorus content	mg/kg	48.7	5.9
Calcium content	mg/kg	20.3	2
Magnesium content	mg/kg	14.0	1
Ash content	%	0.011	0.003
Total contamination	mg/kg	1094	123
Higher heating value	MJ/kg	39.423	39.555
Lower heating value	MJ/kg	36.921	37.050
Phorbolester	mg/g	5.66	n.d.
6:0 Caproic acid	%	-	-
8:0 Caprylic acid	%	-	-
10:0 Capric acid	%	-	-
12:0 Lauric acid	%	<0.1	<0.1
14:0 Myristic acid	%	<0.1	0.3
16:0 Palmitic acid	%	14.5	10.7
16:1 Palmitoleic acid	%	0.9	0.6
18:0 Stearic acid	%	6.9	6.6
18:1 Oleic acid	%	43.2	36.6
18:2 Linoleic acid	%	33.7	44.4
18:3 Linolenic acid	%	0.3	0.3
20:0 Arachidic acid	%	0.1	0.2
20:1 Gadoleic acid	%	<0.1	<0.1
22:0 Behenic acid	%	<0.1	<0.1
22:1 Erucic acid	%	<0.1	<0.1
24:0 Lignoceric acid	%	<0.1	<0.1
24:1 Nervonic acid	%	<0.1	<0.1

Test procedure.

The combustion of two different types of plant oils was carried out with a plant oil pressure stove (‘Protos II’, BSH Bosch und Siemens Hausgeräte GmbH), presented in Fig: 4-1. For each type of oil three samples of emissions were taken for the duration of 40 min/oil. The sampling system was set up according to guidelines VDI 2457 part 3, as shown in Fig: 5-1. The sampling was carried out with one sampling tube connected between a device for collecting emissions and a pump and volume meter. A plant oil stove and device for CO

measurement are also shown.

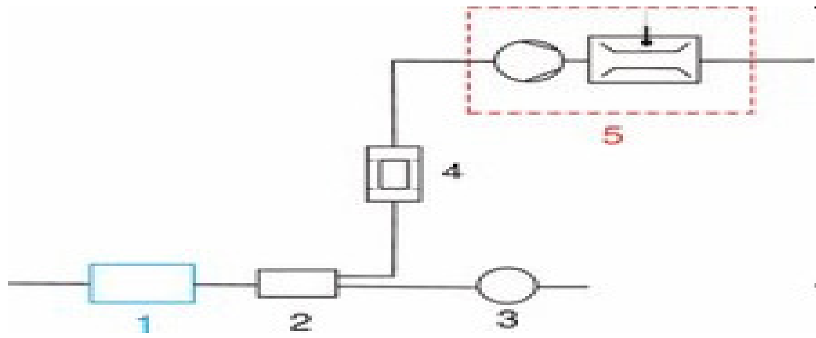

Fig: 5-1. A sampling system with: (1) plant oil stove, (2) device for collecting emissions, (3) CO measurement, (4) adsorption tube and (5) pump and volume meter

The exhaust to be examined was drawn through the adsorption tube, which was filled with activated carbon (Activated Charcoal Type G coconut shell charcoal). The sampling tube consisted of a sampling- and control section. The substances to be sampled were adsorbed in the sampling section. A gas detector pump type “accuro” was used for sampling. During the experiment, the emission of gases CO and O2 were measured (according DIN 30-1-1). The oil consumption C and power P were calculated by Eq. (1) and Eq. (2)

$$C = \frac{m_b - m_a}{t}$$

m_b = Mass oil at the beginning [kg]

m_{ba} Mass oil at the end [kg]

t = time [h]

$$P = \frac{\Delta m \cdot LHV}{\Delta t}$$

Δm = Delta mass oil [kg]

LHV = Lower heating value [MJ/kg]

t = time [s]

Chemical analyses

The activated charcoal filter from the tubes was analyzed by the GC-MS. When using the GC-MS, each chemical compound was classified for both its relative retention time and mass spectrum. This mass spectrum provides a unique fingerprint of the chemicals resulting from its fragmentation on entering the mass spectrometer (Lommen, 2009).

The measurements were performed by an Ion trap Polaris Q evaluation. Identification of GC/MS differences was undertaken by searching the NIST 2005 Mass Spectral Library. The NIST MS Search Program contains integrated tools for GC-MS De-convolution, MS Interpretation and Chemical Substructure Identification.

This library contains 190,825 spectra of 163,198 different chemical compounds, 147,194

chemical structures, and 27,750 replicate spectra. The library of retention index values contain 121,112 Kovats Retention Index values for 25,893 compounds on non-polar columns, 12,452 of which are compounds represented in the Electron Ionization Library. The MS-MS Library contains 5,191 spectra of 1,943 different ions (1,671 positive and 341 negative ions) (http://www.sisweb.com/software/ms/nist.htm).

Data handling and alignment

MetAlign software handles a broad range of accurate mass and nominal mass GC/MS and LC/MS data. It is capable of automatic format conversions, accurate mass calculations, baseline corrections, peak-picking, saturation and mass peak artifact filtering, as well as alignment of up to 1000 data sets. A 100 to 1000-fold data reduction can be achieved. MetAlign software output is compatible with most multivariate statistics programs (Lommen, 2009).

Chromatographic data generated by the software Xcalibur are files with the extension (*.raw). These files can be converted by using the software Xconvert into files with the extension (*.netCDF) and imported into the MetAlign software for further processing. After optimizing the settings according to the specific chromatographic conditions, this software was able to compare samples based on the ions detected in an unbiased and unsupervised manner by performing the following steps:

data smoothing by digital filters related to the average peak width,

estimation of local noise as a function of retention time and ion trace,

baseline correction of ion traces and introduction of a threshold to obtain noise reduction,

calculation and storage of peak maximum amplitudes,

between-chromatogram alignment using high S/N peaks common to all chromatograms ('landmark peaks'),

iterative fine alignment by including an increasing number of landmark peaks with lower S/N.

For each mass trace the noise is estimated as a function of time. The background elimination algorithm applied is not a simple threshold initiated data reduction but utilizes a baseline shape independent series of linear corrections on individual mass traces. All mass peaks in all datasets are used in an alignment algorithm to obtain a predefined data matrix ('aligned peaks' vs. samples) which can be exported in a format compatible with most multivariate software packages. An univariate approach to a simple two group differential problem (t-test) can be undertaken by metAlign resulting in the exporting of differential datasets to MS-data formats for direct visual validation (Lommen et al, 2009).

The settings, used for this study, with corresponding values are shown in Table 5-2. A more detailed description of MetAlign buttons and parameters can be found in the manual, which can be downloaded from http://www.metalign.nl (Lommen et al. 2007).

Table 5-2. Settings in MetAlign

Setting	Value
Maximum amplitude	500000
Peak slope factor	1
Peak threshold factor	2
Average peak width at half height	5
No scaling	-
No Pre-align Processing	-
Maximum shift per 100 scans	35
Significance percentage	95
Minimun ratio between means	2
Minimun S/N ratio	2
Either present in Group 1 or Group 2	Yes
Select min. nr. per peak set	1

Data evaluation

differences between toxic and non toxic

Fig: 5-2 presents an overview when comparing the samples with each other from two types of plant oil. Each sample of *Jatropha* non toxic oil is compared with three samples of *Jatropha* toxic oil: J. NT 1 with J. T 1, 2, 3; J. NT 2 with J. T 1, 2, 3; J. NT 3 with J. T 1, 2, 3. Then, each sample of *Jatropha* toxic oil was compared with three samples of *Jatropha* non toxic oil: J. T 1 with J. NT 1, 2, 3; J. T 2 with J. NT 1, 2, 3; J. T 3 with J. NT 1, 2, 3.

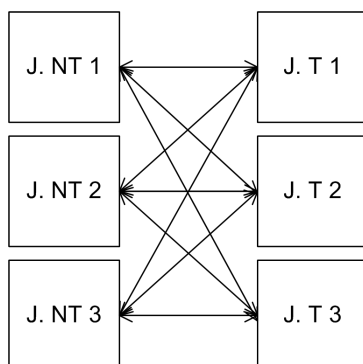

Fig: 5-2. Comparison of the samples between two different types of plant oil

Results

Consumption and Power input

The calculated values of oil consumption C and power P for *Jatropha* toxic and *Jatropha* non toxic oils are given in Table 5-3Table 2.

Table 5-3. Oil consumption and power of plant oil stove.

Type of Oil	Consumption, kg/h	Power, kW

Jatropha toxic	0.23	2.33
Jatropha non toxic	0.24	2.44

Jatropha non toxic oil

MetAlign has been designed to distinguish between and filter out statistically significant differences, pre-defined classes of full scan LC or GC mass spectrometry data sets. Basically the software extracted all masses from full scan GC–MS or LC–MS data sets, sorted out which masses were the same in all data sets and then allowed for selecting differences.

Differences might be selected using the univariate statistics module as an option, which looks for unique signals present in the sample of interest and which are below a given noise threshold in background-related samples (Lommen et al. 2007).

This method enabled the alignment of a set of 9 GC-MS's where up to 7686 mass components were detected. All peaks were normalized according to the highest peak 292153 from the second sample of *Jatropha* non toxic oil.

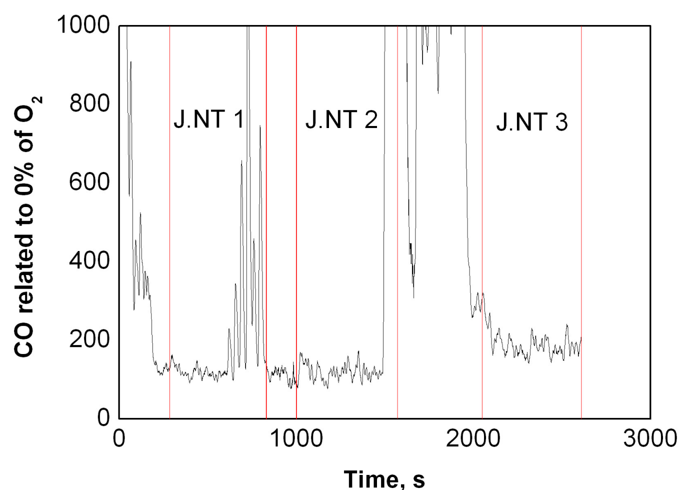

Fig: 5-3. Emission of CO gas during combustion of *Jatropha* non toxic oil.

The uneven fluctuation of CO gas emission (Fig: 5-3) demonstrates poor performance of the stove. Currently, stabilization indicates the right moment take three J. NT samples.

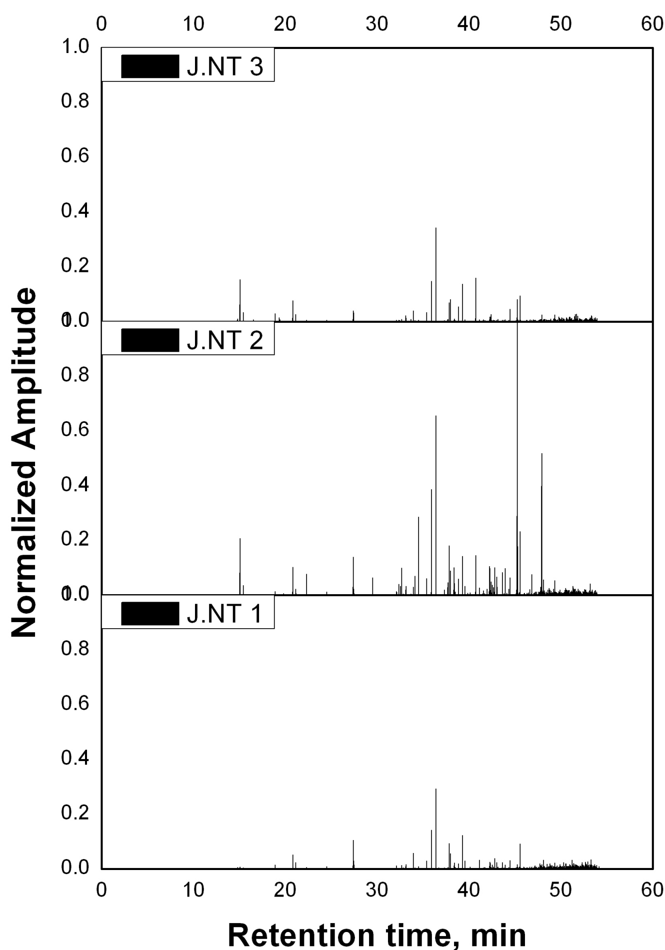

Fig: 5-4. Baseline corrected chromatogram from three samples of *Jatropha* non toxic oil.

In Fig: 5-4 the base peak chromatograms from three samples of J. NT oil showed very similar profiles. J. NT 1 had similar peaks with J. NT 3. Two peaks were clear in J. NT 2 but absent in another two samples, J. NT 1 and J. NT 3.

The type of analysis, if only two groups of data sets are to be considered, is facilitated by MetAlign. This software includes the option to perform a student t-test which selects significant peaks throughout the analysis, at a confidence level of 95%. The results of such analysis, in which two samples of oil were compared, are shown in Fig: 6-7 and Fig: 6-10. A number of significant differences were revealed between samples when observing the samples of oil, which had a high level of statistical confidence.

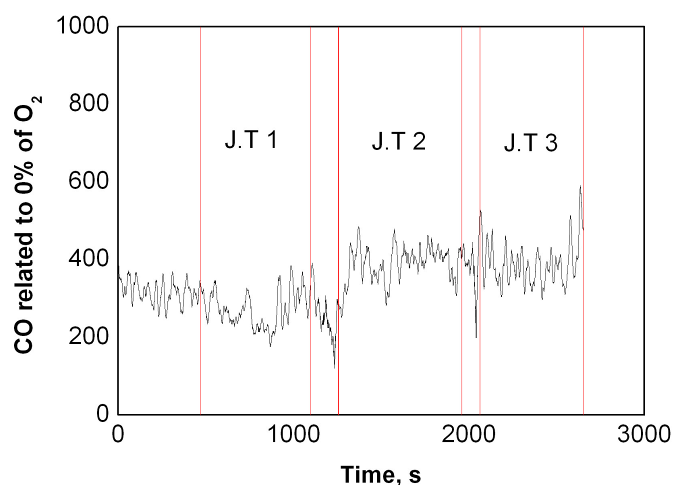

Fig: 5-5. Emission of CO gas during combustion of *Jatropha* toxic oil.

Mostly uniform fluctuations (Fig: 5-5) have shown a good performance of the stove and the right moment of removing the samples with the tubes.

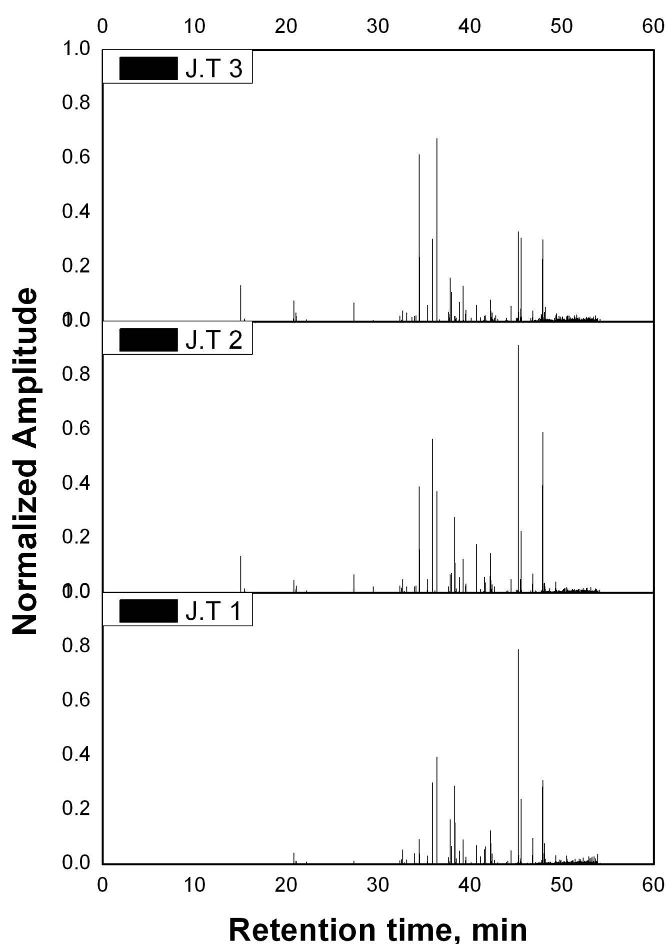

Fig: 5-6. MetAlign-assisted baseline correction of chromatogram, from three samples of *Jatropha* toxic oil.

In Fig: 5-6 similar significant peaks were noticeable in all three samples of *Jatropha* toxic

oil. For J. T 2 and J. T 3 it was obvious that one lower peak was similar but was not present For J. T 1.

Jatropha toxic oil versus *Jatropha* non toxic oil

In Fig: 5-7 the main peak differences between *Jatropha* toxic and *Jatropha* non toxic oil can be seen.

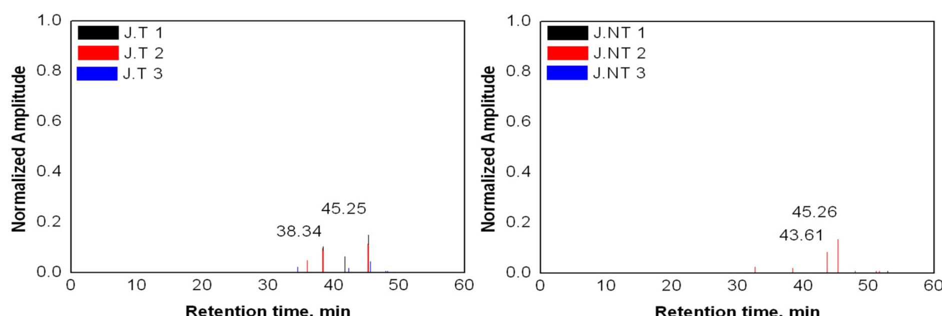

Fig: 5-7. Comparison between *Jatropha* toxic and non toxic oil.

Identified significant peaks in Fig: 5-7 (left panel) are labelled as follows: In J. T 1, two peaks at 38.34 min (Cyclohexane,1-(cyclohexylmethyl)-4-(1- and at 45.25 min (Hexadecanoic acid, butyl ester).These two peaks appear at the same retention time in J. T 2. In Fig: 5-7 (right panel) the most intense differential peaks of J. NT 2, at 43.61 min (4b-8-Dimethyl-2-isopropylphenanthrene) and at 45.26 min (not identified).

Most significant peaks of compounds between oil types

Table 5-4 illustrates the most significant differential peaks between the applied oils. The resulting chemical compounds matched the compounds in the NIST database.

Table 5-4. The list of most significant differential peaks (chemical compounds with retention times)

Jatropha toxic	*Jatropha* non toxic
38.34 min Cyclohexane,1-(cyclohexylmethyl)-4-(1-	43.61 min 4b-8-Dimethyl-2 isopropylphenanthrene
45.25 min Hexadecanoic acid,butyl ester	45.26 min not identified

For each of the applied oils and *Jatropha* non toxic oil, *Jatropha* toxic oil and chemical compounds with retention times corresponding to the most significant peaks are described below. The gas chromatograph was not calibrated with the different compounds, so that concentrations of the detected chemical compounds were not known.

Jatropha non toxic oil

15.06 min Ethyl Benzene is irritating to the eyes and skin but has not been identified as a

hazard. Inhalation of high concentrations may cause CNS excitation followed by depression. Prolonged exposure may lead to functional pulmonary changes and hepatbiliary complaints.

45.55 min Cyclohexane,1,1'-(1,2-dimethyl-1,2-ethane) Cyclohexane is a CNS depressant and may produce mild anesthetic effects. Exposure through inhalation can cause headaches, nausea, dizziness, drowsiness, and disorientation.Very high concentrations may cause unconsciousness, convulsions, and death. Prolonged exposure may produce liver and kidney damage. Cyclohexane is not a carcinogen or a developmental toxicant.

47.90 min Decanedioic acid, dibutyl ester is a clear liquid. It is most often used for making plastics, for packaging food and to enhance flavour in foods and drinks. Such products are described as non-hazardous.

Table 5-5 introduces all chemical compounds, which were present and identified in the applied oils.

Table 5-5. Main chemical compounds present in the samples of different plant oils

Compound	JNT			JT		
	1	2	3	1	2	3
Cyclopentane,1-pentyl-2-propyl-	■	■		■	■	■
Phenol,4-(1,1,3,3-tetramethylbutyl)-	■	■	■	■	■	■
Quinoline,2-(1-methyl-2-(indol-3-yl)ethyl)a	■	■		■	■	
Hexadecanoic acid,butyl ester	■	■		■	■	■
Hexane,3,3-dimethyl	■					
1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo(5.3.	■					
Diethyl Phthalate	■		■			■
p-Anisic acid,4-cyanophenyl ester	■		■			
Dodecane,2,6,10-trimethyl-	■					
1-Methyl-1-(8-pentadecyl) oxy-1-silacy	■					
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	■		■			
1H-Phenanthro(9,10-d)imidazol-2-amine	■					
Cyclohexane,1,1'-(1,2-dimethyl-1,2-ethane)	■	■				
Decanedioic acid, dibutyl ester		■		■	■	■
Butane,1-nitro-			■			
Hexane,2,2,5-trimethyl-			■			
Oxalic acid,allyl octyl ester			■			
Sulfurous acid,2-ethylhexyl hexyl ester			■			
2-(4-Cinnolinyl)malononitrile			■			
Acetic acid,(3,4-dimethoxyphenyl)(trimetl)			■			
Cyclohexane,1,1`-(1-methyl-1,2-ethanediy			■			
Butylated Hydroxytoluene						■
Phenol,4-(1,1,3,3-tetramethylbutyl)-						■
Hexadecane						■
Quinoline,2-(1-methyl-2-(indol-3-yl)ethyl)a						■
Ethyl Benzene		■	■		■	■
Cyclohexane,1-(cyclohexylmethyl)-4-(1-me				■		

For some compounds, toxicology has not been fully investigated. For Cyclopentane,1-

pentyl-2-propyl- no data on long-term exposure, genotoxicity, mutagenicity, carcinogenicity and reproduction toxicity have been found. For Butylated Hydroxytoluene BHT the major routes of exposure are inhalation and contact with the eyes and skin.
Very high concentrations of Cyclohexane 1,1-1,2-dimethyl-1,2-ethane may cause unconsciousness, convulsions, and death. Significant exposure to Phenol,4-1,1,3,3-tetramethylbutyl by absorption through the skin, by inhalation, or by ingestion can lead to death within minutes. Ingestion of even 1 g of phenol has been reported as lethal.
Oxalic acid, isobutyl nonyl ester is a toxic organic compound, which can lead to death by cardiovascular collapse is corrosive to tissue and the nervous system causing convulsions and comas.

Phorbol esters

The content of the phorbol ester in the *Jatropha* toxic oil was 5.66 mg PE/g oil. After combustion of *Jatropha* toxic oil in the plant oil pressure stove, phorbol ester could no longer be detected in the flue gas. During combustion, plant oil flows into the vaporizer, where temperatures of 600°C can be reached. The high temperature was sufficient to completely decompose the phorbol esters.

Conclusion

The purpose of this study was to determine the effect of gaseous emissions when toxic and non toxic varieties of *Jatropha curcas* oil were burnt in a plant oil pressure stove. Differences between compounds, which remain in the emission after combustion, were investigated. Different hydrocarbon compounds could be detected in the exhaust of the plant oil pressure stove for both, non toxic and toxic variety plant oils. The high temperatures reached during decomposition and combustion of *Jatropha* oil in the vaporizer completely destroyed the 5.66 mg/g of phorbol ester and therefore the phorbolester could no longer be found in emissions.

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Objective 4: Analysis of *Jatropha* seed shells as an energy source

Introduction

During the processing of plant oil from *Jatropha* seeds, usually the whole seed (kernel and shell) was used. The press cake, which accrues as a by-product is rich in protein and could be used as animal feed after the detoxification process. However, due to the high content of press cake shell, the amount of crude fiber was also high. This could be a disadvantage for the use as animal feed. Therefore, the production of a high value, protein rich animal food, from the press cake of *Jatropha* seeds without shells was preferred. The expanding areas of plantations of *Jatropha curcas* in India, China and Indonesia will probably lead to an increased amount of *Jatropha curcas* in the future. The shells of the seeds will most likely serve as an alternative fuel for these countries, which are a by-product of *Jatropha* oil processing and could not be used for other applications until now. The thermal energy released during combustion can be applied in many ways for example for the drying of *Jatropha* nuts or for the production process of biodiesel from *Jatropha* oil. The calorific value of the shells is between 16-17 MJ/kg and thus is comparable to rice husks or moist wood, which are the main energy sources in rural areas of developing countries till this day. The *Jatropha* seed was cracked by a desheller and separated by using an air separator into kernels and shells. The material from the deshelling process of the *Jatropha curcas* nut is a free flowing material comparable to rice husks.

Initially, it is not intended to produce pellets or briquettes to achieve a reduction of volume or an increase of energy density. That however excludes the transportation of this alternative fuel over long distances. Generally combustion units for such fuels have already been established, yet have to be optimized regarding the combustion process and the

exhaust gas quality [1].

Furthermore, the operation of a combustion unit affects the efficiency and emergence of toxic exhaust gas components. Hence ash content and ash quality, which are largely responsible for failure-free operation, were included in the analysis. In this study the physical and chemical properties of *Jatropha* shells as fuel have been investigated, leading to the design of a robust functional combustion unit.

Analysis of fuel characteristics of *Jatropha* seed shells

Calorific value and water content

Table 6-1: Net and gross calorific value and water content of *Jatropha* seed shell

NCV ± SD (MJ/kg)	GCV ± SD (MJ/kg)	Water content ± SD %	Methode
16.520 ± 0.127	17. 980 ± 0.127	8.96 ± 0.32	DIN 51900

The calorific value is a measurement to characterize the energy content of fuel as net and gross values. The net calorific value is the energy content of a completely burned fuel without the latent heat of water vapour from moisture and hydrogen. The net calorific value is used for technical processes, as the vapour in the flue gas is usually not condensed to prevent deposition of sulphuric acid and tar. Both acids could corrode the metal parts of the furnace and chimney.

Particle Density

Table 6-2: Particle density of *Jatropha* seed shell.

	Testing result	Method
Particle density, g/cm³	0.963 ± 0.087	DIN CEN/TS 15150

Fig: 6-1. *Jatropha* shell after deshelling with different settings. Left side: sample 1. Right side: sample 2.

Grain size distribution of *Jatropha* shell

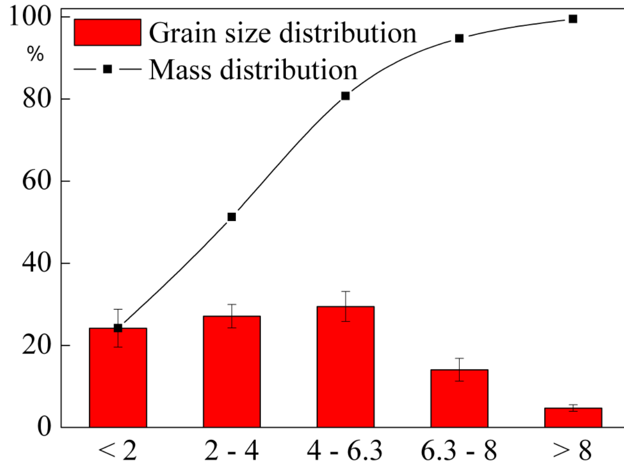

Fig: 6-2. *Jatropha* shell grain size distribution, sample 1

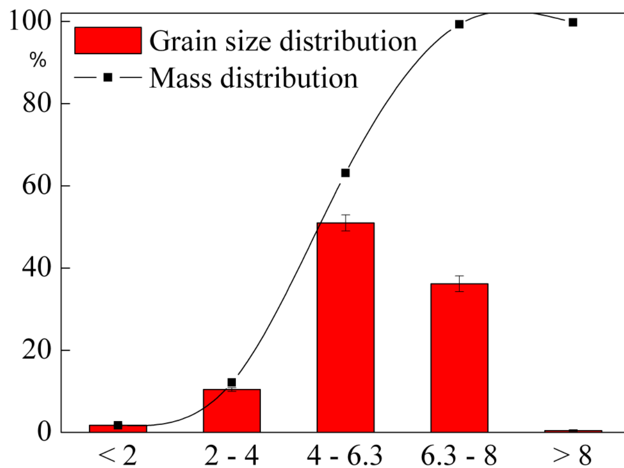

Fig: 6-3. *Jatropha* shell grain size distribution, sample 2

Bulk density

The shape of *Jatropha* shell influences its bulk density as shown in Table 6-3. Such a low bulk density of *Jatropha* shells is uneconomical for long-distance transportation. *Jatropha* shell requires large storage capacities and its bulk density determines the dimensions of the hopper and combustion chamber.

Table 6-3: Bulk density of *Jatropha* seed shell.

	Sample 1	Sample 2	Testing method
Bulk density*, kg/m³	250.79 ± 0.54	329.26 ± 2.16	DIN CEN/TS 15103

Bulk density**, kg/m³	228.23 ± 0.49	299.77 ± 1.97	

*delivery condition

** dry matter

Angle of repose

The angle of repose affects the design of different parts of the furnace, specially the storage container. *Jatropha* shells normally flow under gravity.

Table 6-4: Angle of repose of *Jatropha* seed shell.

	Sample 1	Sample 2	Testing method
Angle of repose, °	44.96 ± 0.39	44.28 ± 0.22	DIN EN 12047

Volatile matter and ash content

The proximate analyses show the contents of volatile matter, fixed carbon and oxidized ash as shown in Table 6-5. Volatile matter is the part of the fuel that escapes in the form of gas or vapour when exposed to heat. Fixed carbon burns in the solid state after exclusion of the volatile matter. Ash is the non-combustible portion that remains after complete combustion. Compared to wood the ash content of *Jatropha* shells is twice as much as the ash content of wood. Therefore, *Jatropha* shells are considered as being a lower-rank fuel. The higher ash content requires an ash removal system. A high content of volatile matter requires secondary combustion inside the furnace in order to minimize losses of the combustible flue gas.

Table 6-5: Proximate analyses

Testing parameter	Testing result	Testing method
Volatile matter*, %	61.33	
Fixed carbon*, %	29.27	DIN 51 720
Oxidized Ash*, %	3.764	

*db

Ultimate analyses

The results of the ultimate analyses as shown in Table 6-6 can be used for calculating the flue gas composition. Carbon comprises both fixed carbon and volatile matter which is combustible to CO2 during complete combustion. Hydrogen in the fuel is burnt to water and appears as vapour in the flue gas. The sulphur content is important for estimating the harmfulness and corrosiveness of the combustion products of the fuel. Sulphur is combustible to oxides SO2, when these are dissolved in water whereby sulphuric acid is formed and is deposited in the exhaust system.

Table 6-6: Ultimate analyses

Testing parameter	Testing result	Testing method
Carbon content*, %	50.9	DIN 51 732
Hydrogen content*, %	5.8	
Nitrogen content*, %	0.8	
Oxygen content*, %	39.5	
Phosphorus content*, mg/kg	884	DIN EN 14 107
Chlorine content*, %	0.120	DIN 51 577-3
Sulphur content*, mg/kg	827	DIN EN ISO 20884
Silicon*, mg/kg	423	XRF (only detectable elements)
Sodium*, %	0.127	
Magnesium*, %	0.436	
Aluminium*, mg/kg	267	
Potassium*, %	1.15	
Calcium*, %	0.922	
Manganese*, mg/kg	33	

*db

Ash analysis

The ash content and composition have a crucial influence on the operating performance, burning and emission behaviour of fuel as well as the design and arrangement of the combustion system.

Main elements of the ash are potassium, calcium, magnesium, sodium and silicon. Aluminum, phosphorus, sulphur, chlorine, manganese and iron contribute to less than 1%. There are also traces of copper and zinc (Table 6-7).

Table 6-7: Composition of ash (only detectable elements).

Testing parameter	Testing result	Testing method
Silicon, %	2.1	DIN 51 729-10
Sodium, %	2.2	
Magnesium, %	19.5	
Aluminium, %	0.4	

Phosphorus, %	9.9
Sulphur, %	2.7
Chlorine, %	0.9
Potassium, %	24.0
Calcium, %	37.5
Manganese, %	0.2
Titan, %	<0.1
Chrome, %	<0.1
Manganese,	0.2
Nickel, %	<0.1
Barium, %	<0.1
Iron, %	0.5
Copper, mg/kg	<0.1
Zinc, mg/kg	<0.1

During combustion, physical changes of the ash will take place. Depending on the temperature, the ashes either start to sinter and stick together or even start to melt totally, which results in adhesion, deposits and blockage in the grate and firing chamber. A blockage in the grate can hinder the airflow through the grate (in a cross-flow system) and consequently the combustion of the fuel.

This is a problem especially for fuel with a low ash softening temperature, which has to be included in the arrangement of the combustion and design of the firing zone. The ash melting behaviour is characterized by the softening temperature, hemispherical temperature and fluid temperature as shown in Table 6-8. The ash melting behaviour is not constant, for it depends on the ash composition and therefore on the fuel composition. The alkali metals potassium and sodium lower the ash melting temperature and the earth alkali metals raise it. To minimize slagging, the combustion temperature must not exceed 900°C.

Table 6-8: Ash melting behaviour

Testing parameter	Testing result	Testing method
softening temperature, °C	980	
hemispherical temperature, °C	> 1550	DIN 51 730
fluid temperature, °C	> 1550	

Construction of test firing and its operation in terms of efficiency and complete

combustion

To keep the fuel consumption as low as possible a high combustion efficiency is required. Only locally available materials and unspecified constructional steels should be used. The construction has to be adapted to meet the limited resources and manufacturing techniques available on site, with no specific welding, machining or casting methods to be used.

The combustion shall have an emission which is as low as possible and meet the German emission standards. In order to achieve this, an optimized combustion chamber and combustion air flow should provide complete combustion.

Systematic of small scale combustion units

According to Hartmann et al. (2007) three main combustion principles can be defined namely, underfeed, transversal and tilting furnace. Except for the underfeed furnace there are variants such as, with grate, without grate, different cooling systems and suitability for various biomass fuels.

Regarding the requirements of *Jatropha* shell combustion as mentioned and considering the slagging of the ashes, the tilting furnace with a bowl burner is the most suitable for combustion of *Jatropha* shells.

In the tilting furnace the *Jatropha* shells are fed by a screw conveyer and fall through a pipe into the bowl. The bowl has a grate, which can be pulled out. Primary and secondary air is guided through holes on the bottom and the side into the burning chamber, which has a cooling effect. The ash falls through the grate and can be removed.

In order to achieve a high efficiency and low emissions the furnace technology has to be built according to the special characteristics of the biomass. *Jatropha* shells have high volatile matter content. For complete combustion, the most important factors are air supply, sufficient retention time of the fuel gas/ air mixture in the reaction zone, good mixture of the fuel gas and combustion air and a sufficiently high combustion temperature. There is a primary air supply in the fire bed for the pyrolysis and a secondary air supply to achieve complete combustion of the gas.

Materials and Methods

Shell Furnace

The combustion unit has been designed according to the principle of the tilting furnace/without grate/bowl burner according to [2]. Paying particular attention to the intended site of operation of the combustion unit (tropical and subtropical countries) complex mechanical parts have now been omitted in the construction to provide a reliable

combustion unit, which can be easily operated and maintained. The burner is initiated by adding a predetermined amount of *Jatropha* shells through the dosing screw. These shells in the burner bowl are ignited manually using firelighters. Fuel and combustion air are then continuously added and adjusted until the desired power has been reached.

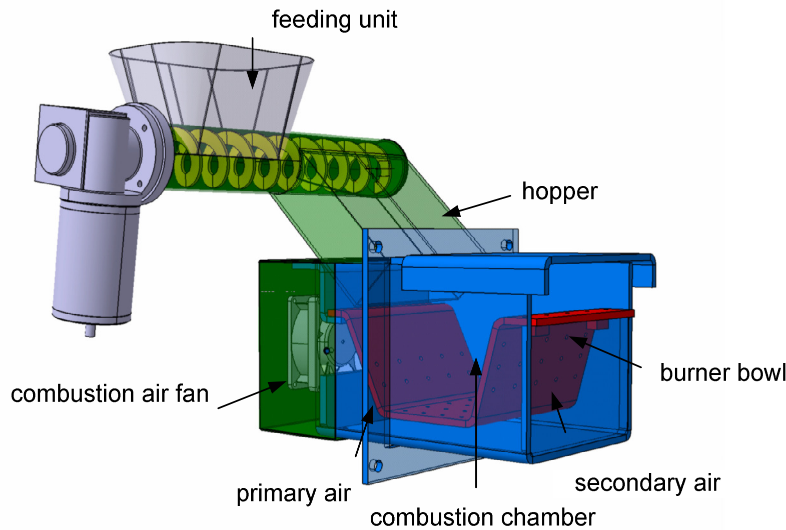

Fig: 6-4. 3-D Model of the combustion unit designed via CATIA VR5.

Fig: 6-4 shows a 3D model of the combustion unit constructed with the CAD-software CATIA. The combustion chamber is the main unit consisting of angled stainless steel sheet, to which the feeding unit is attached together with the hopper and combustion air fan components. The burner bowl can be slid in and out and the cap is removable, which facilitates rotational cleaning of the combustion chamber, whereby the combustion unit is cleaned of possible combustion residues.

The combustion unit as a whole is mounted in a brickwork combustion chamber in which the combustion of the flue gases takes place. To keep the construction of the combustion unit as simple as possible, automatic control of the combustion process has been excluded. The adjustment of the fuel mass flow and the combustion air is performed empirically by controlling the flue gases. The drive for the feeding unit and the fan for the combustion air run on 24 volt.

Fig: 6-5 and Fig: 6-6 show the fabricated prototype of the *Jatropha* seed shell burner.

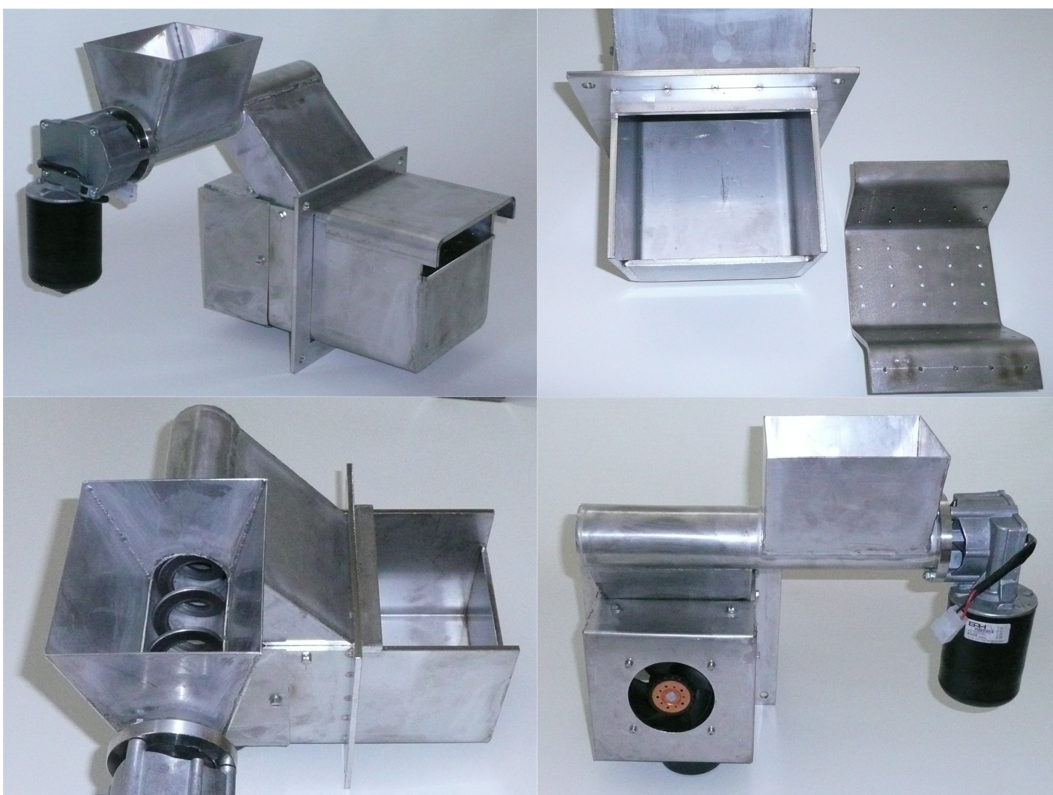

Fig: 6-5. Fabricated shell burner.

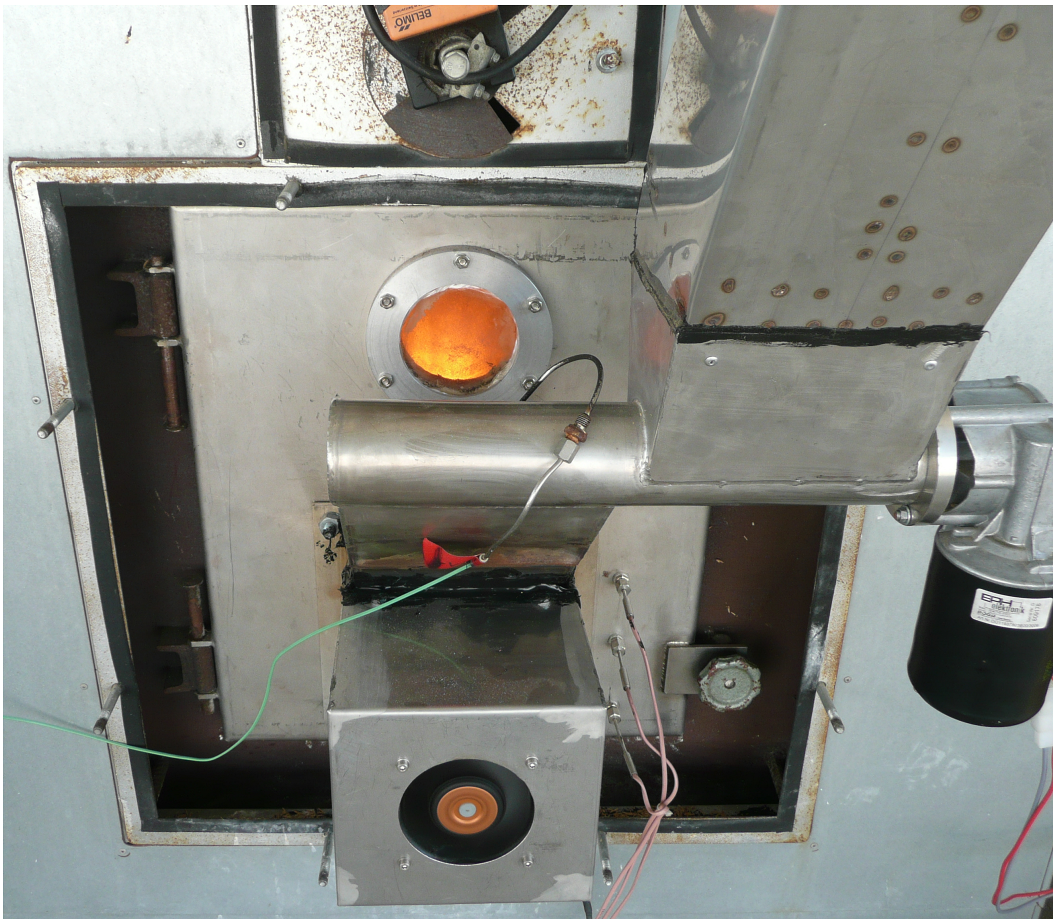

Fig: 6-6. Combustion unit during operation.

Feeding rate

The feeding rate was determined by measuring the weight of the *Jatropha* shells per unit time, when filling the funnel.

Thermal output

The thermal output PTH of a furnace describes the energy which is available for the preheating of air or water. The thermal output was calculated from the furnace efficiency, the feeding rate of the fuel and its net calorific value.

$$P_{th} = \frac{\eta \cdot \dot{m} \cdot NCV}{3.6}$$

Emissions

The carbon monoxide content indicates the efficiency of the combustion process. The carbon monoxide content in the flue gas became increasingly diluted with a rising excess-air coefficient. The results have to be converted to the same basis for all tests. German legislation on air quality control allows a basis of 13% oxygen content of the flue gas from biomass furnaces. The basis used in this study was 13% of oxygen. It delivers the pure carbon monoxide content that can easily be related to any other standards. The carbon monoxide results can be demonstrated with regard to a 0% oxygen content basis using the equation:

$$MC_{CO} = CM_{COfl} \cdot \frac{0.21}{0.21 - MC_{O_2 fl}}$$

In order to estimate the combustion quality the CO-concentration was quantified continuously in the dry exhaust air. The CO2-concentration was calculated. Additionally the hydrocarbon concentration was determined in the wet exhaust with a flame ionization detector. The O2-concentration was defined with an electrochemical measuring method.

Combustion efficiency of *Jatropha* seed shells

The combustion efficiency of the *Jatropha* shells was analyzed by determining the content of the remaining combustible compounds of the ash samples.

The ash of the *Jatropha* shells, which remained in the combustion chamber after conducting the experiments, was collected and combusted at 550°C until a constant weight was reached. The combustion efficiency was calculated by applying the following equation:

$$\eta_{cmb} = \left(1 - \frac{\frac{MC_{a\,js}}{1 - MC_{cm\,test}} - MC_{a\,js}}{MC_{cm\,js}} \right) \cdot 100\,\%$$

Furnace efficiency

Furnace efficiency was calculated by using the following equation:

$$\eta_{FE} = \left(1 - \frac{(q_{th} + q_c)}{(NCV \cdot \dot{m}_F)} \right) \cdot 100\,\%$$

with chemical exhaust gas losses:

$$q_c = (V_{fg} \cdot C_{CO} \cdot CV)$$

and thermal exhaust gas losses:

$$q_{th} = (\dot{m}_{fl} \cdot C_{p,fl} \cdot (T_{fl} - T_{amb}))$$

Results

Feeding rate

Feeding rate of *Jatropha* seed shell during operation of the seed shell burner was adjusted by a potentiometer to vary the speed of the screw conveyor. Fig: 6-7 shows the linear correlation between the voltage from the drive motor and feeding rate.

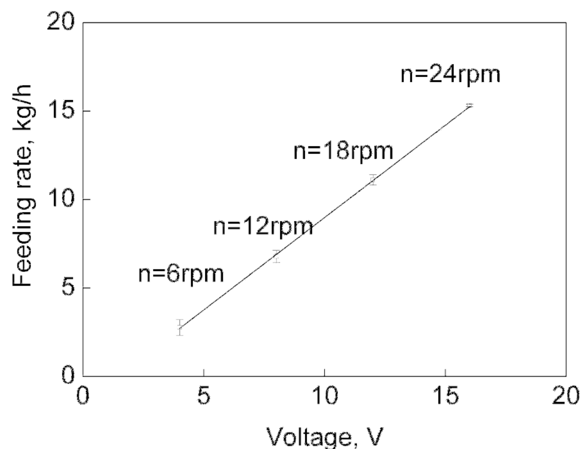

Fig: 6-7. Characteristics of feeding screw dependent on the rotational speed.

Thermal output

In Fig: 6-8 the correlation between the thermal output and the feeding rate of *Jatropha* seed shells is shown. Thermal output was between 11 kW and 28 kW for a feeding rate of *Jatropha* seed shells of 2.9 kg/h to 7 kg/h, respectively. For thermal power of more than 30 kW the quality of combustion decreased and emissions like carbon monoxide in the flue gas increased.

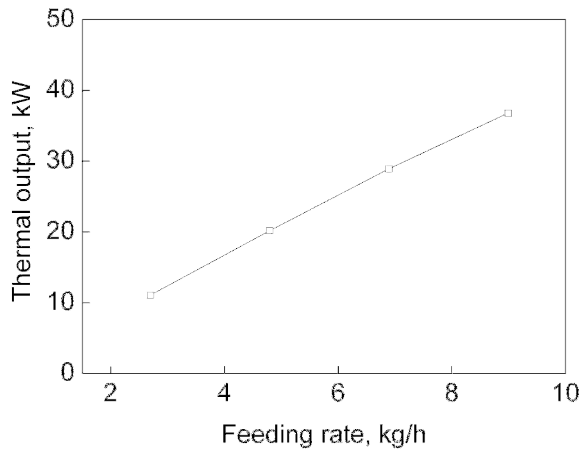

Fig: 6-8. Effect of feeding rate on thermal output of *Jatropha* seed shell burner.

Emissions

Fig: 6-9 shows the carbon monoxide content as well as the oxygen and carbon dioxide content when varying with time, during the combustion of *Jatropha* seed shells with an approximately thermal output of 20 kW. The first 15 min shows the ignition period until the burner had reached a stable operating temperature, as well as stable combustion conditions and concentrations of carbon monoxide. Emissions did not vary with time, which was a result of a stable burning process and continuous feeding with *Jatropha* seed shells.

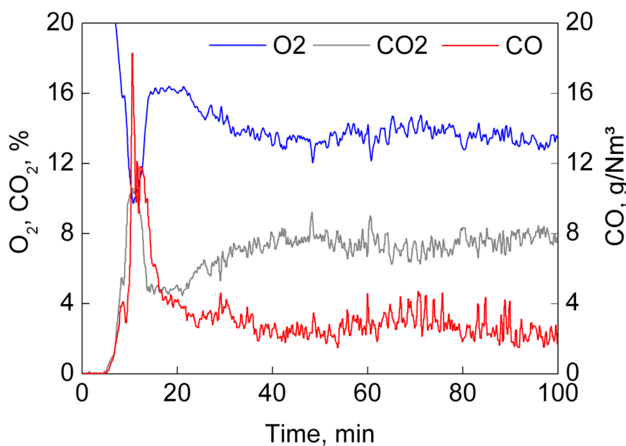

Fig: 6-9. Emissions and oxygen content during operation of *Jatropha* seed shell burner, 20.2 kW.

Furnace efficiency

Fig: 6-10 shows the furnace efficiency of the combustion unit depending on the feeding rate. Furnace efficiency varied between 87% and 91%. With higher feeding rates and increasing power of the combustion unit, the efficiency decreased. The main reason for this was an increased content of carbon monoxide in the flue gas. This was the result of an incomplete combustion of the *Jatropha* seed shells during the operation with high feed

rates.

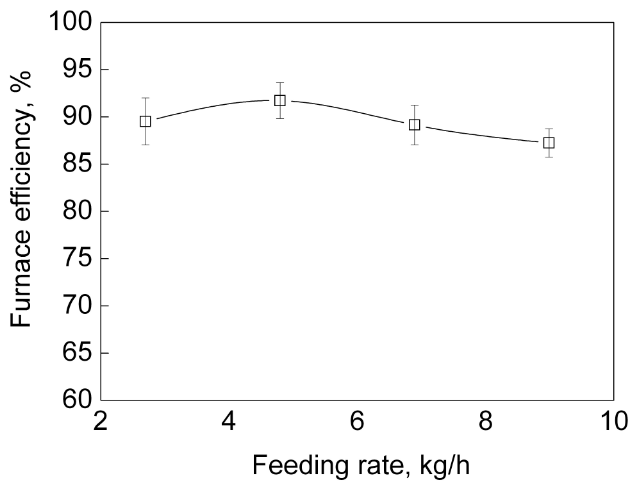

Fig: 6-10. Effect of feeding rate on combustion efficiency of *Jatropha* seed shell burner

Combustion efficiency

Fig: 6-11 shows the combustion efficiency of *Jatropha* seed shell depending on the feeding rate. The combustion efficiency was almost the same and ranged from 98.2 to 99.2. The colour of ash inside the combustion unit, which was directly removed from the grid was grey and white. This denotes an almost completely burnt out shell. Additional burning of these coarse ashes shows a content of combustible matter of less than 0.01%. The reason for the decreased furnace efficiency was a discharge of partly burned *Jatropha* seed shells out (external to) of the combustion chamber. With increasing power of the combustion unit the air flow of combustion air also increased. This led to the discharge of the shells. These shells still contained combustible matter and therefore reduced the furnace efficiency. This behaviour limited the maximum power output of the combustion unit to approximately 30 kW.

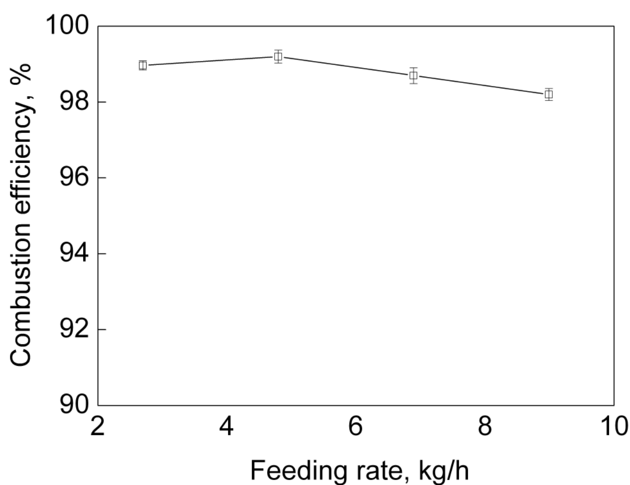

Fig: 6-11. Effect of feeding rate on combustion efficiency of *Jatropha* seed shell burner

Temperature of *Jatropha* seed shell burner

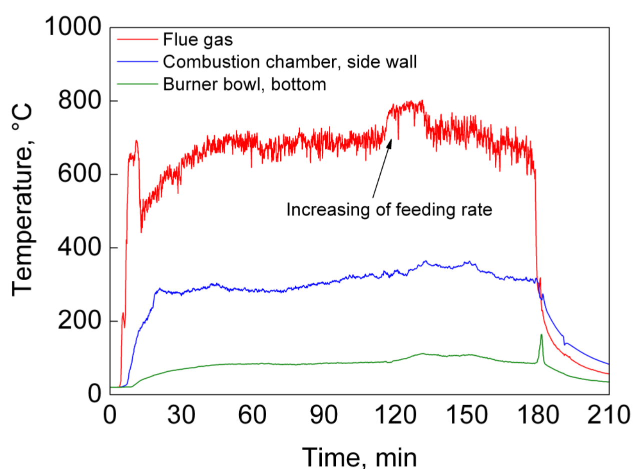

Fig: 6-12. Temperature profile of the *Jatropha* seed shell burner

Composition of combustion chamber ash

During the experiments it could be observed that an agglomeration of ash had melted on the grate in the combustion chamber. An ash bed with an approximate height of 2.5 cm had accumulated on the grate during combustion. The ash could be removed easily after the tests. A clogging of the air supply holes on the grate was not evident. Table 6-9, shows the composition of the *Jatropha curcas* ash. The Ash demonstrates a high content of plant mean nutrients like phosphorous, potassium, magnesium and calcium and therefore can be applied as a substitute for crop cultivation fertilizer.

Table 6-9: Composition of ash from combustion chamber after burning of *Jatropha* seed shells (only detectable elements).

Testing parameter	Testing result	Testing method
Calcium, %	34.8	DIN 51 729-10
Potassium, %	25.9	
Phosphorus, %	16.0	
Magnesium, %	14.9	
Sodium, %	2.6	
Silicon, %	1.6	
Iron, %	1.6	
Sulphur, %	1.2	
Aluminium, %	0.4	
Chlorine, %	0.3	
Manganese, %	0.2	
Titan, %	0.1	

Chrome, %	0.1
Nickel, %	0.1
Copper, %	0.1
Zinc, %	<0.1
Barium, %	<0.1

Conclusion

The combustion of *Jatropha* shells is possible without previous processing e.g. pelletizing, whereby the fuel costs can be kept low. However, the fuel should be expended locally at its point of origin, in order to avoid transportation costs. The concentration of carbon monoxide is below the norm of existing regulations. However, the next stage will be to introduce a means of controlling combustion quality. The problems of softening and fusion of the ashes in the combustion unit investigated did not occur.

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3.3 University of Hohenheim, Stuttgart (Economics Group: Inst 490e; Germany 6)

Financial Assessment of the *Jatropha* Value Chain in Southwest China

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- 2.3 Scope of the study
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3 Analysis of the JCL value chain
- 3.1 Expected benefits of JCL production in China
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Appendices

Summary of the findings

Since the 1990s, the government of China has shown a strong interest in the development of the biodiesel sector as an alternative to fossil fuels. Development activities took off in 2006 with the large-scale planting of *Jatropha curcas L.* (JCL) in the provinces of Sichuan and Yunnan. There are many expected benefits from JCL, such as increasing energy security, the reclamation of degraded lands, rural development, and food security. Areas in the southwest of the country where first targeted as they have most barren lands classified as a non-arable land.

Significant actors in the JCL development include state energy companies, local governments, and forestry authorities. Private firms are involved and provide seedlings and establish the plantations under the rules set by government officials. Most of the activities have been entrusted to local governments, however, there are also a few privately-owned domestic and international energy companies participating in the JCL development. Local households were only involved as suppliers of hired labor to work in the nurseries and plantations. The development activities reached a peak in 2007-2008, while activities came to an abrupt halt in 2009 because of financial constraints at the level of plantations.

Our cost-benefit assessment shows that under current prices all segments of the JCL production chain fail to produce a high quality product in a profitable manner. The estimated profits are, however, highly sensitive to changes in the cost of variable inputs such as feedstock, labor, fertilizers, chemicals and costs of transportation. At the level of seed processors, the economic viability is additionally determined by the prospective capacity of the plant, the choice of production, and the production efficiency.

The financial assessment and value chain analysis undertaken in this study were much constrained by the fact that plantations have not reached maturity and yields were low, and that seed processing and detoxification units are presently not operating. As a consequence, the computations had to be based on certain assumptions and the results of this preliminary investigation need to be interpreted with caution. Nevertheless, the report showed that there are many factors currently constraining the development of the *Jatropha* based biofuel sector in China. The detoxification of *Jatropha* kernel meal and protein isolate is a promising strategy to improve the profitability of the *Jatropha* processing units but without additional efforts to raise *Jatropha* seed production efficiency and reduce input costs, this bio-refinery activity might not be a profitable venture in the near future in China.

Acknowledgement

This research was conducted within the project "Efficient oil extraction and use, and production of feed grade protein concentrate and seed meal from *Jatropha curcas* seeds for inclusion in livestock feeds." Funded by the Federal Ministry of Education and Research (BMBF) and Ministry of Science and Technology (MOST), the project aimed to enhance the economic viability and sustainability of *Jatropha*-based biofuels production systems by introducing innovative industrial and livestock production systems.

We thank our Chinese counterparts at the Sichuan Agricultural University (SCAU) and the College of Animal Science (Zhejiang University, Hangzhou) for their kind support throughout the research. We are also grateful for the support of the Department of Biology at Panzhihua University during the data collection period.

We are grateful to all the Chinese MSc students from SCAU for their patience during the interview period in China as well as the farm households who we interviewed. Special thanks go to Mr. Lin Han, a graduate from Universität Hohenheim, for managing the first round of data collection in China. During the second round we received great help from Mr. Dong Min from the Southwest Forestry College in Yunnan province and his MSc students and thank them for their valuable time and friendship.

We would like to express our deep appreciation to the Chinese officials, especially the Sichuan Forestry Department, for their kind cooperation during our data collection in the field, and the forestry bureaus in Panzhihua prefectures, Renhe district, and Yanbian County for making their time available for the interviews. We also express our gratitude to all key informants from other sectors, including afforestation companies, energy companies, and individual households for sharing their opinions with us.

Prasnee Tipraqsa
Thomas Berger

Abbreviations and acronyms

BCR	Benefit-Cost Ratio
CDM	Clean Development Mechanism of the Kyoto Protocol
CNESP	China National Energy Strategy and Policy 2020
CNOOC	China National Offshore Oil Corporation
CNPC	China National Petroleum Corporation
CO_2	Carbon dioxide
d.wt.	Dry weight basis
FB	Forestry bureau
FYM	Farm yard manure
g	Gram
g/cm^3	grams per cubic centimeter
GVC	Global Value Chain
hp	Horsepower
hr	Hour
IRR	Internal rate of return
JCL	*Jatropha curcas* Linnaeus
JPPO	*Jatropha* Pure Plant Oil that being extracted and filtered
kg	Kilogram
kJ	Kilo joule
KW	Kilowatt (x 10^9)
KWh	Kilowatt hour
l	Litter
MJ	Mega joule. One mega joule, MJ, is one million joules
masl	Meters above sea level
Mt	Million tons (x 10^6)
n.a.	not available
NDRC	National Development and Reform Commission of China
NPV	Net Present Value
PE	Phorbol esters
Phz	Panzhihua prefecture
PPP	Purchasing Power Parity
RMB	Currency in mainland China

SCAU	Sichuan Agricultural University
Sch	Sichuan province
SFA	State Forestry Administration of China
SINOPEC	China Petrochemical Corporation
SOHO	Private company who can participate in a government bidding process
UHOH	Universität Hohenheim, Stuttgart, Germany
Ynn	Yunnan province
ZJU	College of Animal Science, Zhejiang University, Hangzhou, China

Conversion factors

International dollar			unit
1	PPP$ equivalent to	3.44	RMB (PPP factor, 2005)
1	PPP$ equivalent to	0.89	EUR (PPP factor, 2005)
1	USD equivalent to	1.24	Eur (10/27/2008)
1	USD equivalent to	6.84	RMB (10/28/2008)
1	Euro equivalent to	8.75	RMB (10/25/2008)
Energy			
1	Btu equivalent to	1,055.00	joules (1.055 kJ)
1	Btu equivalent to	1.06	kilo joule (kJ)
1	MJ equivalent to	1,000.00	kilo joule (kJ)
1	MJ equivalent to	947.87	Btu
1	kWh equivalent to	3.60	mega joule (MJ)
1	hp equivalent to	0.75	kilowatt (kW)
1	hp equivalent to	2.68	mega joule (MJ)
1	l of diesel equivalent to	36.00	mega joule (MJ)
Area			
1	ha equivalent to	15.00	mu
1	ha equivalent to	0.01	km2
Volume			
	JPPO density equivalent to	0.92	g / cm3 of water
	JCL biodiesel density equivalent to	0.88	g / cm3 of water
1	oil barrel equivalent to	158	ltr

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Introduction

The Chinese government has shown large interest in building up national biodiesel supply as an alternative to petroleum and natural gas that are largely being imported. As a result, a special Energy Forest Program has been launched to establish large-scale plantations of *Jatropha curcas* Linnaeus (hereafter referred to as JCL) in the degraded mountainous areas of southwest China. Growing JCL on marginal lands avoids competition for arable land with food grains and contributes to solving the problem of sloping barren lands.

In addition to the growing demand for fuels in China, demand for livestock products has increased remarkably in both rural and urban areas during the last two decades. By using livestock feed more efficiently, livestock output from China now reaches a world market share of about 50% (American Soybean Association, 2009; Bruinsma, 2003). This has intensified the use of protein feed resources, particularly obtained from soybean, rapeseed and cottonseed.

To secure this feed supply for the livestock industry, policy makers need to critically assess available options. Importing feedstuff or investing in feed production abroad would ease the shortage of production resources in China, but would create dependency on other countries and expose the sector to price fluctuations in world markets. To produce the feed domestically, the feed industry would need alternative energy and protein crops, and efficient production technologies.

JCL might be an attractive option also in this respect. The crop has a high content of inedible oil that is used for producing biodiesel. The oil cake, a waste product of the oil extraction, is rich in protein but toxic for animals. Recently developed detoxification technologies make the crude protein from seed meal suitable for animal feeding.

The emerging JCL sector in China is therefore shaped by the three factors of energy, environment, and agriculture. Based on this potential, the Chinese government has made large investments in JCL in only 4 years. It established large-scale plantations of millions of hectares, mostly in the rural areas in southwest China. In spite of the large-scale investment in JCL plantations and oil extraction facilities, so far biodiesel has not been produced from JCL in China. As a consequence, the potential of JCL biodiesel and detoxified seed meal for livestock feeding has not been exploited yet. This study analyzes the main factors of JCL oil extraction for biodiesel and seeks to shed light on the question as to why biodiesel from JCL has not materialized in southwestern China.

The specific objective of this study is to understand the various constraints along the value chain of JCL. The study analyzes in detail the segments from JCL seed production to seed processing. At each stage of the JCL chain, we analyze the cost structure and value adding to products and by-products. From this we assess the competitive advantage of JCL as compared to other types of feedstock. Our results lead to suggestions on how to improve the competiveness of the JCL chain.

The remainder of the report is organized as follows. Chapter 2 provides a description on JCL, the study area, and the methodology. Chapter 3 provides an analysis of the JCL value chain while Chapter 4 analyzes the costs and benefits at each segment of the chain. Both chapters first provide a framework used in the analysis and then present the results. Chapter 5 discusses the results. The report ends with conclusions.

Background

Description of Jatropha* curcas *Linn.

Botanical description

Jatropha is a genus of a medium-large shrub belonging to the Euphorbiaceae family. The *Jatropha* genus contains about 170 known species (Heller, 1996). JCL is native to countries in Central and South America such as Mexico, Brazil, Peru, Nicaragua, Argentina, and Bolivia (GRIN). In English JCL is commonly known as physic nut. Achten et al. (2008) reported JCL trees have a lifespan of about 50 years.

The tree normally starts to bear fruit in the second year and reaches maturity at year five on average. The size of JCL trees varies with location but normally reaches a height of between two to eight meters. The tree has a smooth bark with sturdy branches. The leaf has four to six lobes that have a length of four to fifteen cm; the length of the leaf is about equal to its width. The root system consists of three to four lateral roots and a deep vertical taproot (Heller, 1996).

Figure 1: Characteristics of *Jatropha curcas* Linnaeus
Source: Own survey, 2009

The flowers are terminal monoecious. Flowers are unisexual, that is, there are male and female flowers. The ratio of male and female flowers ranges from 13:1 to 29:1 (Raju and Ezradanam, 2002). Rainfall induces flowering in JCL. Jones and Miller (1992) reported that the tree can flower two to three times per year, although Raju and Ezradanam (2002) reported only one time of flowering per year. The tree bears an inedible capsule-

shaped fruit, 3-4 cm in length and 2.5-3 cm in width. The fruit takes three to four months to mature (Jones and Miller, 1992). The immature capsule is subsphaeroidal and the mature seed is oval-shaped. Mature fruits are either brownish or black with cracks.

Each fruit contains two to four kernels (seeds). Kernels are rich in oil, though there is much variation in oil contents, ranging from 20 to 50 percent. The kernels have an average size of 1.5-2.0 cm long and 1.0-1.2 cm wide and have an elliptical shape and black color (Heller, 1996). The above morphology of the tree and its fruits varies with the water regime (Achten et al., 2008; Openshaw, 2000).

JCL can easily establish itself on infertile soils and tolerates drought. The tree requires a minimum amount of water of only about 250 mm per year, while the maximum annual rainfall that the tree can tolerate is about 3,000 mm (Achten et al., 2008).
The climate-tolerant range of the tree is much influenced by the humidity. The tree has a wide distribution area ranging from low altitudes to relatively high altitudes; the maximum altitude is about 1,800 masl (Heller, 1996). In China, Ye and Li (2006) reported that JCL in the southwest region could be found with an average annual rainfall over 500 mm, annual temperature higher than 19 ºC, and annual sunshine hours of more than 1,700 hrs. The vertical distribution of JCL in China is limited to an altitude below 2,000 masl, and most JCL plantations are concentrated at altitudes of around 1,600 masl.

Pests and diseases

Pest and pathogen problems in JCL include millipedes, termites, grasshopper, stem and leaf borer, beetles, and caterpillars. There is also a suspicion that JCL is a host for transmitting the super-elongation disease in cassava (Heller, 1996). For a continuous JCL plantation in India, Kaushik (2006) reported on the scutellarid bug and the capsule borer. Some scholars have mentioned that irregular tending practices and other practices could increase the susceptibility of JCL monoculture to pests and pathogens (Sharma, 2007).
In addition, Hannan-Jones and Steve Csurhes (2008) mentioned other potential pest found in JCL, including the *Jatropha* mosaic virus (JMV) that has recently been found in southern India, as well as different types of fungus that can cause damping off and root rot, and leaf spot.

Seed yield

Systematically measured seed yields of mature JCL trees have not been reported. Seed yields in the literature show a large variation, but are mostly based on estimates rather than

measurements. The seed yield varies with planting density, age of the trees, attributes of the site, and the management, especially in monoculture JCL plantations. The cultivated tree density reported in the literature varies from 1,100 to 3,300 stems/ha. The reported seed yield from cultivated plantations ranges from 0.1 t/ha/year to 15 t/ha/year (Heller, 1996). Openshaw (2000) reported a variation in seed yield, ranging from 0.4 to 12 t/ha/year. In southwest China where wild JCL are distributed, JCL seed yield is estimated to be 2.25 t/ha/year (survey, 2009). The seed yield per stem ranges from 1.5 to 3 kg/stem/year (Heller, 1996).

Regarding JCL seed viability, Hannan-Jones and Csurhes (2008) reportet that fresh JCL seeds have higher levels of viability, but low levels of germination, suggesting innate (primary) dormancy. In addition, Dagar et al. (2004) reported that the germination rate of JCL seed correlates with the size and weight of the seed as shown in the table below.

Table 1: Correlation between JCL seed weights and germination rate

Seed size (mg)	**Seed weight (mg)**	**Germination rate (%)**
100-201	**167**	**0**
201-300	**241**	**0**
301-400	**368**	**20**
401-500	**471**	**30**
501-600	**560**	**60**
601-700	**648**	**85**
701-800	**724**	**100**

Source: Dagar et al. (2004)

Uses of *Jatropha curcas* Linn.

Various uses of JCL are reported in the literature, including: (1) the direct use for crude oil for diesel engines; (2) JCL crude oil for transesterification to produce biodiesel, or for blending it with petroleum diesel; (3) the use for lighting (as lamp oil); (4) fuel for cooking stoves; (5) production of soap; and (6) production of bio-pesticides.

In addition to the use of crude oil, the tree itself is reportedly used for multiple purposes. In many developing countries, a hedge of trees is used as a fence to prevent the browsing of cattle around homesteads, and to prevent soil erosion from sloping fields (Henning, 2004).

Various parts of the JCL tree are also used for either medicine or poison in many countries. People also like to play with the latex from the tree to blow air bubbles, just for fun.
In addition, there are several applications of the byproducts from processing JCL seeds, such as the byproducts from oil processing. The seed meal, a byproduct from pressing the oil from the seeds, is used as fertilizer in agriculture. The seed meal together with filtered cake—obtained from purifying the extracted oil—together with shells from fruits and seed hulls and dried branches are used as fuel by households (to replace charcoal, which is more expensive) or sometimes used to produce biogas. Glycerin and Glycerol, a byproduct obtained from in the transesterification, is used in various industries such as cosmetics and pharmaceuticals.

Study area

The study area for this research project is located in Sichuan province. This province is one of the main locations for large-scale JCL plantations in China. The project focuses on the Panzhihua prefecture, one of largest prefectures of Sichuan province. To get an overview of the JCL development in the larger region, the study area was extended to some parts of Yunnan province, which neighbors Sichuan.

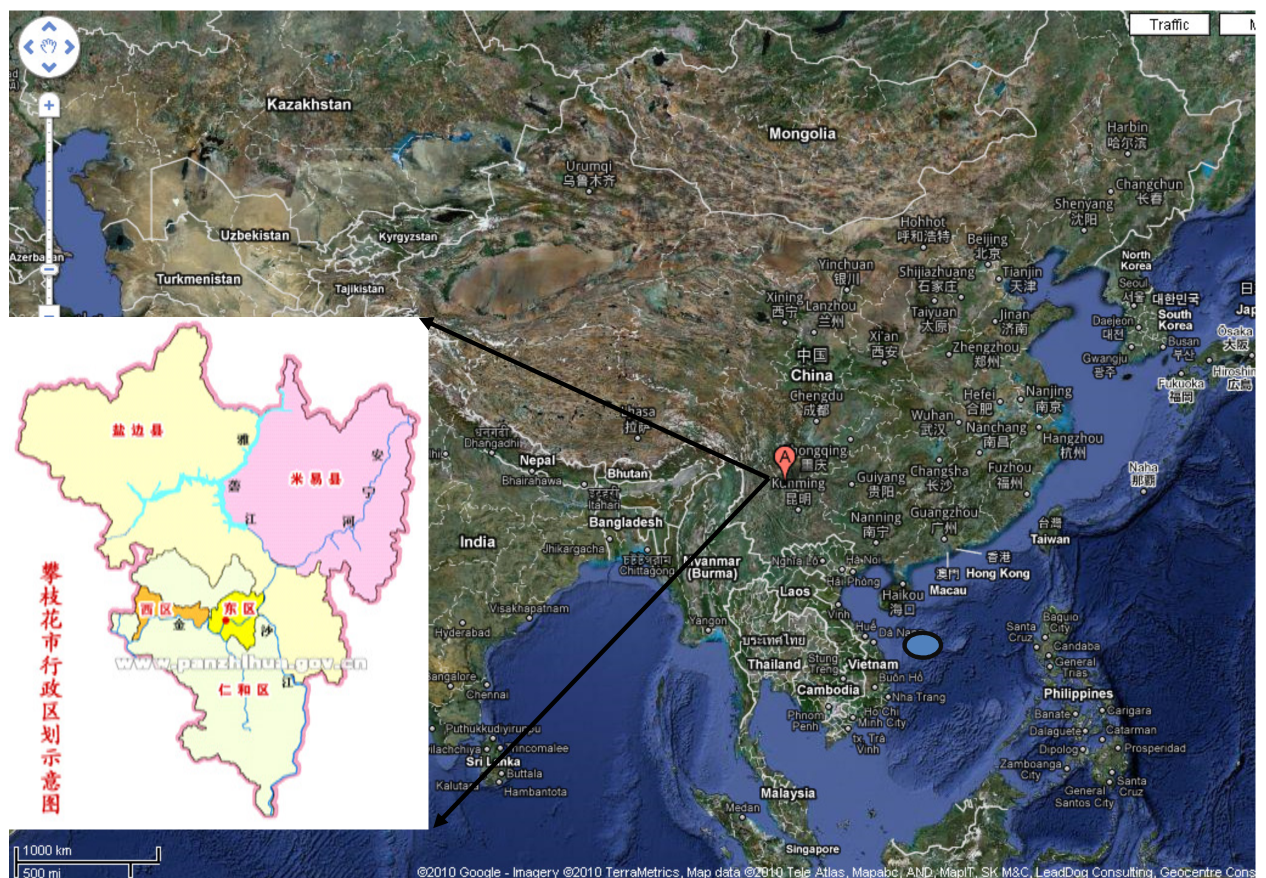

Figure 2: Map of the study area in southwest China

Source: http://www.panzhihua.gov.cn/ ; http://maps.google.com

Panzhihua prefecture is located at latitude 26°05′ to 27°21′N and longitude 101°15′ to 102°08′ E. The prefecture is located in the Jinsha river valley in the upper reaches of Yangtze River, southwest of Hengduan Mountain where the Jinsha and Yalong rivers meet. The average altitude is 1,400 masl. The prefecture is located in the most southwestern tip of Sichuan province as shown in the figure above. The administrative area of Panzhihua is divided into two counties (Yanbian and Miyi county) and three districts (East district where the Panzhihua government is located, West district, and Renhe district), as shown in Figure 2.

Climate

Panzhihua is located in a typical dry and hot valley area. The weather condition is influenced by a combination of a south sub-tropical climate and a north temperate climate, known as the “three-dimensional southern-based sub-tropical climate". The climate is characterized by a long summer, unclearly demarcated in four seasons, but clearly demarcated between a dry and a rainy season. The climate has a high evaporation and a rainy peak from June to October. There is a large difference between average day and average night temperatures. The sunshine hours vary from 2,300 hours to 2,700 hours per

year. The annual average temperature is between 19.7 ℃ and 20.5 ℃. The hottest month is

May and the coldest month is either December or January. The rainy season starts from June and lasts until October. From November to May of the following year is the dry season. Frost-free periods can last up to 300 days.

Land use

Panzhihua has a total area of 7,440 km2. Only about 4 percent of its total area is classified as arable land, 2 percent is water area, and 6 percent is wasteland; the rest includes various other categories (including grassland and forest). Under the current land use classification system, 64 percent of the total area is covered by forest. Yet the actual area under forests is about 59 percent (4,384 km2). According to local forest authorities, a large part of the

declared forestland is barely covered with forest. The land use policy of the Panzhihua government therefore allocated this land to large-scale JCL plantations. The Panzhihua government launched a biomass/biodiesel project with the objective of planting *Jatropha* on 200,000 ha in this barren land. The planting started in 2006 (Wang 2006).
Land used for farming is not always classified as arable land. Local farmland is also included in the categories of grassland, forestland, wasteland and water areas for fishing. The main agricultural products produced in this area are wheat, rice, sweet potato, maize, soybean, sugarcane, peanut, rapeseed, tobacco, tea, vegetables, fruits (mango, longan, apples, pears, bananas, grape, oranges) and meat (chicken, duck, goose, pork, beef, and mutton) (Panzhihua Statistic Yearbook 2007).

The land allocation policy for Panzhihau at the present has been implemented 20 years ago. According to this land policy, each villager could receive 0.5 mu of arable land (~333 m2). This would suggest that households with many members receive more farmland than those with fewer members. However, as this policy was implemented 20 years ago and household sizes have changed much since, the allocation principle no longer reflects current household sizes and a redistribution of farmland has not taken place.

Socioeconomics

In 2007, Panzhihua had a population of 1.06 million. Most urban residents concentrate in the East and West districts as well as in the city center of Renhe district. Of its total population, 47 percent are rural residents and 53 percent are urban residents. Among the administrative areas, the city of Panzhihua is comparatively well developed in terms of secondary and tertiary industries. Secondary industries include manufacturing of processed materials; tertiary industries include providers of services, transportation and finance. The growth of these industries attracts many rural residents to the city.

More than 50 percent of the rural population is engaged in arable farming, fishing, and animal husbandry. These rural residents distribute unevenly in 78 townships which are part of Yanbian county, Miyi county, and the rural area of Renhe district. From 2007, a further administrative area reform has been undertaken at the township level. Following this reform, some townships with a small area and small population will be combined into new townships and given more rights of self-government. Land will be allocated to the minority autonomous townships from the Panzhihua government.

The average annual income of the rural population was about 2,300 RMB per capita (~263 Euro), which is below the Chinese poverty line (Sichuan statistics bureau,

2004). The average per capita daily income for rural residents in 2003 was below 1 USD. The main source of protein for rural residents is the own livestock rather than what they buy from the market. The selling of animal products occasionally generates extra income. Because of the low income from agriculture, the share of non-farm employment in the household income has increased. Farming is no longer the main source of income for most rural households. The efforts put in agricultural production are merely enough for meeting their daily household needs. During the household interviews of this project, rural residents expressed their desire for more convenient forms of transportation and water supply and for better access to agricultural markets in urban areas.

Scope of the study

This study focuses on two main issues. First, it assesses the production costs and benefits for processing JCL seed meal as a crude protein for use in the animal feed industry. Second, the study identifies factors that affect the development of the JCL industry.
We anticipated that in the near future it will be possible to detoxify JCL seed meal for feed industry in China. This research focuses on the supply chain of JCL from seed production, to oil industry, to animal feed production. As a feed industry using detoxified JCL seed cake does not exist at present in China, we compared the potential of JCL to existing sources of protein feeds.

Data collection

Data for this project were collected from various sources described in the following.

Data collected from semi-structured interviews

Data collected from semi-structured interviews are divided in interviews with the farm households, and interviews with the state authorities and private entrepreneurs engaged in JCL in southwest China.

Although the interviews were conducted at different times, the preparations for the interviews were similar. In the first stage, a list of actors involved in JCL industry was compiled from public sources (Internet) and with the help of the Chinese counterpart from Sichuan Agricultural University (SCAU). Later, a list of key informants was finalized, and necessary contacts related to the Chinese authorities were established with the support of SCAU. At household level, a list of key informants was obtained through a stratified random sampling approach (see more details Han, 2009). Key informants included individual farmers and households engaged in the JCL nurseries and plantations,

afforestation companies, energy companies, local forestry authorities, and research institutions.

Because of various groups of key informants, different types of questionnaires were prepared. The various questionnaires were then refined through discussions with the Chinese counterpart, after which they were translated into Chinese. The questions were explained to the Chinese enumerators and pre-tested with a few key households; some questions were discarded and others reformulated. After that, a group of enumerators contacted the key informants to arrange an interview. This preparation process took about three weeks.

Semi-structured interviews were conducted twice as a first round of interviews was interrupted by a large earthquake in Sichuan on 12 May 2008. The first round was between April and May 2008. At the household level, data were collected from the farm households in one village (Lixin village in Zongfa Township, Renhe district, Panzhihua prefecture). Apart from interviews with households engaged in JCL, the interviews were conducted with various key informants, including nurseries producing JCL seedlings, and officials from local forestry bureaus who are engaged in JCL production. During this survey period, two MSc students from SCAU and one Chinese MSc student from Hohenheim University (UHOH) conducted the interviews. The household interviews were conducted during this period because it was spring and most households took some time off from their routine work. The interviews started at the end of April but came to an abrupt stop on 12 May. Continuing the survey after this date was impossible.

The second round of the survey was conducted from August to September 2009. It involved interviews with officials from forestry bureaus, private companies engaged in JCL nurseries and plantations, and energy companies. The procedures for preparing the interviews were similar to the first round of interviews. Enumerators included Chinese scientists from the Southwest Forestry University in Yunnan province. The interviews took one month to complete.

Not all key informants that we contacted were available for an interview. Private companies and government officials appeared to be hesitant to share information during the interviews. This resulted in a sparse set of data. However, the different backgrounds of the key informants provided a broad perspective on the various issues.

Data generated within the project

Technical coefficients for JCL seed meal processing were obtained from the various workpackages of project at UHOH and the College of Animal Science (at Zhejiang University, Hangzhou, ZJU).

Data collected from other sources

To fill some of the data gaps, data were also collected from the following sources:

(1) Data from published and unpublished sources, including reports distributed by experts and research organizations. These data were particularly useful for providing the information related to the value chain analysis.

(2) Publicly accessible data were important for this study because JCL development in China is relatively new and few data have appeared in the scientific literature. This source was particularly useful for the analysis of the value chain, in which some leading firms interact with other actors in various segments of the feed sector. This source includes local and international newspapers, websites, libraries, and other sources.

Analysis of the JCL value chain

The of value chain approach was originally developed from an interest in the flow of agricultural commodities and local production systems as influenced by national authorities. The concept was originally developed during the 1960s and 1970s and was extended several times to address additional issues and criticisms (Gibbon and Ponte, 2005; Raikes et al., 2000). In 2000s, the value chain approach became known as Global Value Chain (GVC) analysis (Gibbon and Ponte, 2005; Kaplinsky, 2000; Van Dooren and Zarate-Hoyos, 2003). In line with the GVC concept, each analytical component is explained below.

Value chain configuration

The value chain configuration consists of two elements: (a) an input-output structure, and (b) geographical coverage. The input-output structure refers to the configuration of a specific chain of subsequently connected value-adding activities. Geographical coverage refers to the spatial dispersion of activities or actors in the value chain (Gibbon and Ponte, 2005).

Governance

Within the GVC concept, governance is defined as the relationship between firms, including the institutional mechanisms that are used to accomplish non-market coordination in the chain (Gereffi et al., 2005). In line with this, the leading firms define parameters that structure the production process. The leading firms do not necessary have a large market share, but firms could just control certain functions that facilitate them to control the participation of other actors with different function in the chain (Gibbon and Ponte, 2005). According to this definition, if some firms work according to the parameters set by leading firms, the chain is recognized as a producer-driven chain. Based on this trend, GVC distinguishes between producer-driven chains and buyer-driven chains (Gereffi et al., 2005).

In many cases, the complex relationship among firms (a local cluster) cannot be captured with the traditional governance distinction. In addition, different forms of coordination can be applied at different segments along the value chain. Various new forms of governance have therefore been proposed in revisions of the GVC concept. In spite of these revisions, the overall form of the governance can still be translated into the original distinction between producer- and buyer-driven chains.

Institutional framework

This component is used both to examine how a firm can expand or how it can integrate into upstream and downstream activities. This component is also used to describe the influence of the state and international policies at different segments of the value chain.

The analysis in this chapter describes the three analytical key components in line with the GVC framework. The first part provides a general description of the value chain in order to give an overview of the structure of the value chain. The description includes geographical coverage, and the actors involved in the chain. In the second part, the governance structure and its dynamics within the value chain are described. The last part of the analysis provides information about the institutional framework that shapes the JCL value chain in China.

Because the JCL industry is still in its infancy (it only took off in 2006), some of the actors we interviewed were involved at the start of the development while actors in further segments along the value chain are just anticipations. To capture the full chain, we had to combine figures from various sources that are related to JCL development in China.

Analysis of costs and benefits

The analysis is divided in two major parts: an analysis of the seeds production sector and an analysis of the seed processing sector. The seed production sector includes seed compilation, seedling production, and seed plantations. The seed processing sector includes oil extraction, oil processing, and the detoxification of seed meal.

Production capacity

The analysis of the present production capacity of the seed production sector is based on the real situation based on data obtained from the firm interviews. The production capacity was analyzed at various production scales. Where actual production did not exist, a large-scale production capacity was assumed. Assumptions about production capacities are based on expert opinions and other available studies. Assumptions related to the production capacity are made explicit in the results section.

Production process

For the seed production sector, the analysis was based on the data collected from the field surveys (2008-2009). The processing of JCL seeds was assumed to be a continuous process (that is, from seed selection, to oil extraction, to oil processing). The detoxification unit for

JCL seed meal was assumed to be part of this continuous process and is considered to be an additional unit installed in the biodiesel production plant.

Cost of production

The total production cost was calculated from capital cost, operation cost, and production cost. These costs are based on the data collected from the survey (2008-2009). Where data for estimation of costs were unavailable or impossible to obtain, assumptions were made based on expert opinions and other studies. These assumptions are made explicit in the appendix section, which also specifies prices used in the cost calculations.

Capital cost: represents the cost of constructing a new production site (i.e nursery, plantation, seeds processing plant), processing equipments for all the processing unit (i.e. pre-treatment unit, processing unit, separation unit, purification unit, storage for feedstock and goods produced, waste treatment unit, and auxiliary facilities). This category includes the cost of purchased or rented land, and auxiliary facilities. These auxiliary facilities refer to necessary buildings for production and management units, irrigation systems, power grid, electricity generators, roads, equipments for the functioning of the unit.

Operation cost: is defined as expenditures for routine operations. It includes salary for permanent staff, overhead, inventories, project management, inspection of the authority, publication and exchanges for an appraisal, research and training, and other administrative expenses.

Production cost: The production cost refers to the daily operation for producing goods. This cost includes direct and indirect costs. Direct production cost refers to the cost of raw materials, chemicals, consumable items related to goods produced and packaging, labor fees, maintenance and repairs, indirect taxes and duties, and transportation. The indirect production cost includes expenses for preparing the land and production plant, depreciation of capital, insurance for capital, duties and indirect taxes occurred from producing goods. The prices used for the calculations are market prices in China, obtained from interviews and market surveys. If it was not possible to get the price of some commodity then the price was obtained from secondary sources such as market reports.

As the production cost is a function of production capacity, production efficiency, and the production technology, various assumptions were made that are specified in the appendix section. In processing stages that are based on chemical reactions, a fraction of main chemicals and outputs was defined and the quantity estimated from a thermodynamic model called UNIQUAC (Universal quasi-chemical). UNIQUAC was chosen to predict the

activity coefficients of the main chemical components. Yet, some of the interaction parameter coefficients were not available, the output fractions were estimated using the extrapolation of some relative volume and output fractions that were obtained from the laboratory.

Total production cost was calculated as the sum of capital cost, operation cost, and production cost. Economic cost was defined as the sum of capital cost and operation cost.

Benefit of production

Total benefit is defined as the sum of revenues generated from all good produced and sold annually, plus the total income from other sources, including direct and indirect subsides. The net benefit was estimated from the total benefits less annual production cost each year. Tax needed to be paid annually was calculated from a sum of tax from annual revenues from good produced, which was assumed to be between 6 and 7 percent. In addition, tax is paid annually for owning fixed capital, which was assumed to be 1 percent of the capital cost.

Financial indicators

Cash flow refers to the annual cash flow, defined as total benefit less the production cost of each year. The analysis assumed the absence of debt capital. The opportunity cost of equity capital was assumed to be 12 percent.

The Net Present Value (NPV) is defined as the sum of the present values of the project cash flows. Each of the cash that flows in and out is discounted to its present value of the project. These discounted cash flows are summed over the lifespan of the project. The formula to calculate NPV is given in the following equation:

$$\frac{R_t}{(1+i)^t}$$

Where,

$t =$ the time of the project

$i =$ the discount rate, here we assumed to be 12%

$R_t =$ the amount of cash that flows in – (minus) the cash that flows out at time t.

The Internal Rate of Return (IRR) is defined as a rate of return for using capitals throughout the period of the project and capital. IRR is therefore used to measure the profitability of investments.

The benefits-cost ration (B/C ratio) is expressed as: the sum of discounted benefits over the time span of the project / the sum of all discounted cost over the time span of the project.

The break-even price is defined as the price for which the revenue from the major produced goods is the same as the total production cost. This price therefore indicates the minimum required selling price of the detoxified protein produced to cover all costs (capital, operation, and variable costs).

Further details about the calculation of the cost-benefits analysis are given in Gittinger (1984).

Sensitivity analysis

Sensitivity analysis was performed to examine the magnitude of effects of some selected variables on the financial viability of the production activities. The analysis is done by developing an empirical model describing the relationship between input and output variables.

Variables included in the sensitivity analysis include the prices of raw materials, products and by-products. By varying the value of one variable while keeping the other variables unchanged, we determined its effect on the benefits of the firm (cash flow after tax).

Analysis of the JCL value chain

This chapter analyzes the segments in the JCL value chain, and the potential of adding value to the chain by extraction of crude protein for the feed industry. More specifically, the objectives are:

1) to understand the structure of JCL seed production and seed processing and how each sector allocates its resources along the supply chain; and
2) to understand how the current firms and actors in JCL seed production and processing and animal feed industry are positioned in the value chain.

The first section of the chapter describes the settings of the JCL production in China. This is followed by a description of the JCL configuration. After this, the governance structures of the leading firms are examined.

Expected benefits of JCL production in China

Chinese rural households have been using JCL for various purposes for more than 50 years, though records about its contributions hardly exist (own survey, 2008). The government got interested in JCL in the 1990s. Its interest stemmed from the oil crises in the 1970s, 1990s and energy crisis in the 2000s (various media sources). Insecurities in the energy supply threatened economic development (various media sources). The interest of the government was also triggered through adverse effects from environmental pollution and a decline in functioning of environmental services stemming from the rapidly growing economy since the 1980s (various media sources). A third contributing factor behind the recent interest in JCL was a wave of high prices for food commodities in the 2000s as the planted area of food grains is reported to be in decline, creating serious concern about food security in China (Sichuan statistics bureau, 2004; State Statistical Bureau of China, various issues).

These factors created favorable conditions for the development of the JCL energy forest program on marginal lands in southwest China. The growing of inedible oil grains on degraded lands for fuel production is seen as a solution to reduce the dependence on petroleum and natural gas. It also avoids competition for arable land with food grains and contributes to solving the problem of sloping barren lands. Ideally, using the seed meal as an input for the livestock industry could further contribute to a secure supply of animal

feeds for the rapidly expanding livestock sector. The emerging JCL sector in China is therefore shaped by the three factors of energy, environment, and agriculture.

Numerous benefits are anticipated from the development of the JCL sector, including: (1) a source of renewable energy to secure future national energy demands, (2) technology development related to energy and utilization of JCL byproducts, (3) improvements in environmental quality through the CDM mechanism, (4) securing human food production from rehabilitating degraded lands, (5) improving energy use efficiency of rural households, (6) enhancing the health status of rural residents through a better energy supplies, (7) reinvigorating agro-industry and the development of rural economies, and (8) expansion of international trade by exploiting China's competitive advantage.

Configuration of the JCL value chain

Production area

Production area of seed suppliers to nurseries

In about 2005, the political conditions were favorable for a JCL industry in China to take shape, and consequently, a program to cultivate JCL took off in 2006. Small and medium sized nurseries supplied the large scale JCL plantations in the initial stages of development. The seeds propagated by these nurseries predominantly came from natural forests, especially from the area along the Jinsha river basin (金沙江), in the most western part of the headwaters of the Yangtze River in southwest China. With regard to the JCL seed collection from natural forests, these forests can be divided into those in the Southwest region and those in the Southeast region as shown in the figure below (Ye et al., 2009). The stars in the figure indicate the provinces where JCL is commonly distributed.

Figure 3: Distribution JCL in natural forests in China

Source: Ye et al. (2009)

The southwest region has a hot monsoon climate and stretches from the southwest of the Yunnan-Guizhou Plateau to the so-called three river valley (the Nu, Jinshajiang, and Lancang rivers). This area includes the west of Panzhihua in Sichuan, most of Yunnan and the southwest of Guizhou province. In Sichuan province, *Jatropha* is popular in the Panxi area (city of Panzhihua and the Liangshan Yi Autonomous Municipality), including Panzhihua, Yanbian, Miyi, Ningnan, Dechang, Xichang, Huili, Jinyang Yanyuan (Ye et al., 2009).

Figure 4: Wild JCL along the roads and in the wilds in Sichuan

Source: survey, 2008

In Yunnan province, *Jatropha* is widely grown in the Chuxiong Yi Autonomous City, Dali, and Honghe, located around the three river valley in the west and southwest of Yunnan (Zhang et al., 2001). In the southwestern Guizhou province, the valleys of the Nanpan, Beipan and Hongshui rivers has the largest natural distribution of *Jatropha*, which includes Wangmo, Anlong, Zhenfeng, Ceheng, and Luodian. In the southeast region, the

climate is tropical and subtropical. JCL trees are naturally found in Fujian, Guangdong, Guangxi, Hainan and Taiwan along the southeast coast (Ye et al., 2009).

Production area of the nurseries

Nurseries providing the seedlings for the JCL plantations are usually located near the plantations (survey, 2008 and 2009). Most nurseries In Sichuan province are located in Panzhihua prefecture. The size of the nurseries varies considerably (survey, 2008 and 2009). The smallest nursery operates an area of about 0.067 ha (1 mu), while the largest nursery operates an area of 1.4 ha (21 mu).

Figure 5: JCL nursery in Zongfah township, Panzhihua, Sichuan

Source: survey, 2008

Production area of the plantations

Although JCL is found in natural forests across China, JCL plantations are concentrated in the provinces of Sichuan and Yunnan. The local government together with the local forestry bureau set criteria for what types of land to convert to JCL plantations. General criteria for establishing JCL included barren mountainous areas, availability of water resources, and no competition with local farming activities. In addition, soil quality,

altitude, moisture, and other related factors were taken into account (survey, 2008). The size of plantations ranges from 55 to 100 ha. Early JCL plantations were established in 2006. Table 2 below shows the distribution of JCL; these data need to be interpreted with care as they are solely based on official figures.

Table 2: Distribution of JCL seeds production in some area of Sichuan and Yunnan provinces

Location	Natural forest areas (ha)	Plantation areas (ha)	Source
Sichuan province, total	9,333 [a]		Phz FB, 2009
(1) Panzhihua prefecture	n.a	31,333 [b]	Renhe FB, Phz 2007
		20,000 [c]	Phz FB, 2009
(2) Miyi county, Pzh	n.a	n.a	Renhe FB, Phz, 2007
(3) Renhe district, Pzh.	52 [d]	467 [d]	Renhe FB, Phz, 2007
(4) Yanbian county, Pzh.	128 [d]	380 [d]	Renhe FB, Phz, 2007
Yunnan province, total	18,000	83,667	Ynn FB, 2009
(1) Shuangbai county , Chuxiong prefecture		18,333 [e]	2009

Notes:

a = productive area is 1,333-2,000 ha

b = developed in 2007-2008 as a production and demonstration base for biodiesel production by Petro China

c = mostly planted in 2006, invested by Petro China

d = estimated from an average planting space of 2x3m, density = 1,667 stems/ha

e = developed in 2006-2008, invested by Yunnan Shenyu New Energy

Source: Own survey 2008-2009

Figure 6: *Jatropha* plantations at a demonstration site in Yiabian country, Sichuan, planted in 2006

Source: Survey, 2008

In the Panzhihua prefecture, JCL plantations are only found in Renhe district and Yanbian county, which together occupy most of the collective forestland (survey, 2008). In Panzhihua, an area of about 85.49 km2 was afforested with JCL in 2007 (Panzhihua Statistical Bureau, 2007). Petro China invested in JCL plantations from 2007 to 2008 and added about 31,333 ha (470,000 mu) of JCL plantation to this prefecture. The JCL area in Qianjin township was mostly planted in 2007, yet further planting was discontinued in 2008 because of an insufficient supply of water and finance. In Zongfa township JCL was planted in 2006.

The large-scale plantation of JCL in Yunnan province started in 2006. Petro China invested in a larger area and also biodiesel companies such as Yunnan Shenyu New Energy Co. and other foreign and Chinese afforestation companies. Some of these plantation projects made use of the clean development mechanism under the Kyoto protocol (Yunnan Provincial Department of Forestry, survey 2009). At present, the growth of *Jatropha*

plantations has slowed down. Yunnan Province has downsized its plan of developing a total area of 666,667 ha (10,000,000 mu) by 2015 to a total area of 400,000 ha (6,000,000 mu) by 2020.

Actors

The National Development and Reform Commission (NDRC) initiated a large-scale JCL plantation in southwest China. NDRC entrusted two national energy companies and the State Forestry Administration (SFA) to ensure the development of the plantation.
Most of the JCL seeds used for planting the large-scale plantation were collected from the natural vegetation in Yunnan and Sichuan provinces (Panzhihua Forestry Bureau, survey 2008 and 2009). Actors involved in this stage include individual households and private entrepreneurs. Both groups mainly collect JCL seeds along the roads where JCL is distributed. The collectors sell the seeds either directly to nurseries or to middlemen, who then provide it to larger seeds supplying companies. Besides these two actors, there is another group of official breeders who collect seeds and propagate JCL in own nurseries. Seeds collectors in Renhe district sold seeds to Guizhou and Sichuan provinces to both state and private nurseries (survey, 2008). The peak demand for JCL seed and seedlings was in 2007 and 2008 (survey 2009).

Actors involved in JCL seedlings production include two national energy companies (Petro China and Sinopec), state and local forestry bureaus, private nurseries (SOHO) who won regulatory approval from the local forestry bureau, private energy companies (Yunnan Shenyu New Energy Co. and China Grand Forestry), and private foreign companies working in afforestation and reforestation projects under the Clean Development Mechanism. Individual household involve in seedling production as hired labor in nurseries working on seedbed preparation, planting, weeding, caring, and harvesting (survey 2008-2009). Not all plantations are well-maintained, but the pilot or demonstration sites usually area.

After a production period of five to six months for the seedlings, local forestry bureaus purchase the seedlings from the nurseries. Other actors involved in this stage include private energy companies, private afforestation companies, and individual households who participated in the restoration program on waste and sloping lands (survey, 2009). Actors involved in the establishment of plantations are mostly the same as in the production of seedlings.

JCL plantations established since 2006 are still idle as the trees are not mature enough to give a harvest (survey, 2008 and 2009). There are therefore still no actors working in seed harvesting or seed processing. Although some government officials reported that plantations established in 2006 and 2007 have started to bear fruits, only about 10 percent of the total plantation area actually gives a yield (survey, 2009). Actors that can be expected to engage in harvesting and processing include the local forestry bureaus while individual farm households will be the main supplier of labor to harvest the seeds.

According to the Collective Forestland Right System Reform policy implemented in 2009, the local authorities would allocate a quota of JCL plantations to individual farm households for caring and harvesting (Han, 2009). It is unclear at the moment whether this allocation of Jaropha lands to farmers has actually taken place. Additionally, the forest authority plans to provide a small financial incentive to individual households for caring and harvesting. In line with this policy, individual households could harvest seeds and sell them to the national oil companies that invested in the plantations (see Han 2009 for details).

At the seed processing stage, the state oil companies are likely to play a significant role. Petro China selected the Nanchong refinery, which already existed before the investment in JCL, and prepared it to use the existing equipment and technology to produce biodiesel from JCL. The company planned to invest 170,000,000 RMB into this refinery. In spite of these preparations and future plans, the development of the biodiesel refinery came to a stop in 2009 (personal communication from Petro China, survey, 2009). Biodiesel from JCL, possibly blended with petroleum-based fuels, can be distributed through existing channels. Other actors that are likely to enter the JCL value chain at this stage include companies specialized in the processing of glycerin, such as the cosmetics and pharmaceutical industries.

In the Chinese livestock industry, several large-scale livestock producers operate large-scale hog and poultry production units, and produce their own feed (various media sources). These companies could potentially enter the JCL value chain at the feed production segment. The main actors in this group include the large meat processing groups in China such as Shineway Group, Shuanghui Group, and feed and additive processing companies such as A&Z Food Additives Co., Ltd. (various media sources). In addition to the livestock companies, firms producing bio-pesticides could also enter to the JCL value chain by using the toxins extracted during the seed meal detoxification.

Governance structure

The JCL production chain described above shows the functional linkages among the various actors. Based on this current structure of the JCL industry, we can identify a group of state-owned oil companies—including SINOPEC and CNPC—as the key actors dominating the JCL value chain. This section analyzes the type of governance that is being formed by these main actors. We focus on the roles of the leading actors and the strategies they use to secure their positions in the value chain.

Forms of governance

For an area of 0.15 ha (1 mu) Petro China invests on average 200 RMB through a national forestry bureau, and SFA invests another 200 RMB for producing and planting of seedlings. In the area of Panzhihua prefecture alone, Petro China is reported to have invested over 100,000,000 RMB in JCL plantations (survey, 2008). This investment was mostly made in 2007-2008.

The leading companies structure the JCL production process by signing a contract with the local government. The companies ensure the budget for establishing a JCL plantation according to the signed contract via collaborative accounts. Through the institutional mechanism between subordinate levels of the SFA, local forestry bureaus are in charge of the implementation of the plantations. The local forestry bureau then assigns its officials to do this. The local forestry bureaus also included private companies (SOHO) to develop additional JCL nurseries and plantations. In this cluster, the relationship between SOHO and the forest authority has become more complex. The local forestry bureau designated locations and areas for the plantations of SOHO. In addition, the local authorities impose their own regulations on SOHO for production activities (survey, 2008-2009). SOHO in turn involves individuals to work in the nurseries and plantations but prefers to contract people from far away villages rather than people from the vicinity of its farms.

For maintaining the plantations, the leading companies extend their governance into the chain, using the same mechanism mentioned in the plantation establishment stage, but based on the Collective Forestland Right System Reform policy. Following this policy, the local forestry bureaus allocate a quota of the forest land to the JCL plantations and to individual farm households for caring and harvesting and for selling the seeds to the oil companies. This policy is expecting to be fully implemented by the end of year 2009 (Han, 2009).

The leading companies further develop their relationship with the SFA by initiating a collaborative demonstration project for JCL biodiesel production near the plantations. The demonstration project includes the development of genetic seed sources, nurseries for seed propagation, fine (or high) yielding plantations, and biodiesel production facilities using advanced technologies. However, the construction for this pilot project has come to a halt as half of the allocated investment by the government was delayed (survey, 2009). The management structure formed around the JCL value chain by the oil companies has therefore come to a stop at this stage.

If the seeds production activities and the development of biodiesel refineries could be resumed, then the JCL refinery would be under the control of the leading companies. This refinery would eventually be integrated into the conventional biodiesel industry. To add value to the seed meal, the leading companies are likely to expand relationships to several large-scale animal feed processors. Such coordination between the companies cannot be observed at present.

Along the JCL value chain, we observed that the national energy companies, as the key actors, presently pursue to control the capital- and technology-intensive operations rather than to concentrate on distribution and marketing channels. This reflects a necessity to bring down the cost of the production through an increase in production capacity, which is typical for the biodiesel industry. The roles of the oil companies as described reflect the attributes of a producer-driven value chain rather than a buyer-driven value chain.

Organization structure of the key actors

National energy companies, at present, have integrated into both upstream and downstream industries related to petroleum. This includes petrochemicals, chemical markets for fibers, fertilizers, and other chemical products; storage and pipeline transportation of crude oil and natural gas. The companies therefore control the largest conventional oil refineries and distributors in China. The investments made by the national energy companies in JCL seeds plantations are by far the largest advance into the JCL industry in China.

The current structure including the leading companies related to JCL industry is illustrated in Figure 7. In spite of the abrupt stop of the development of the JCL industry in 2009, the dotted lines indicate anticipated links that the energy companies could use to integrate into the current structure of the biodiesel and livestock industries once JCL production activities are being resumed.

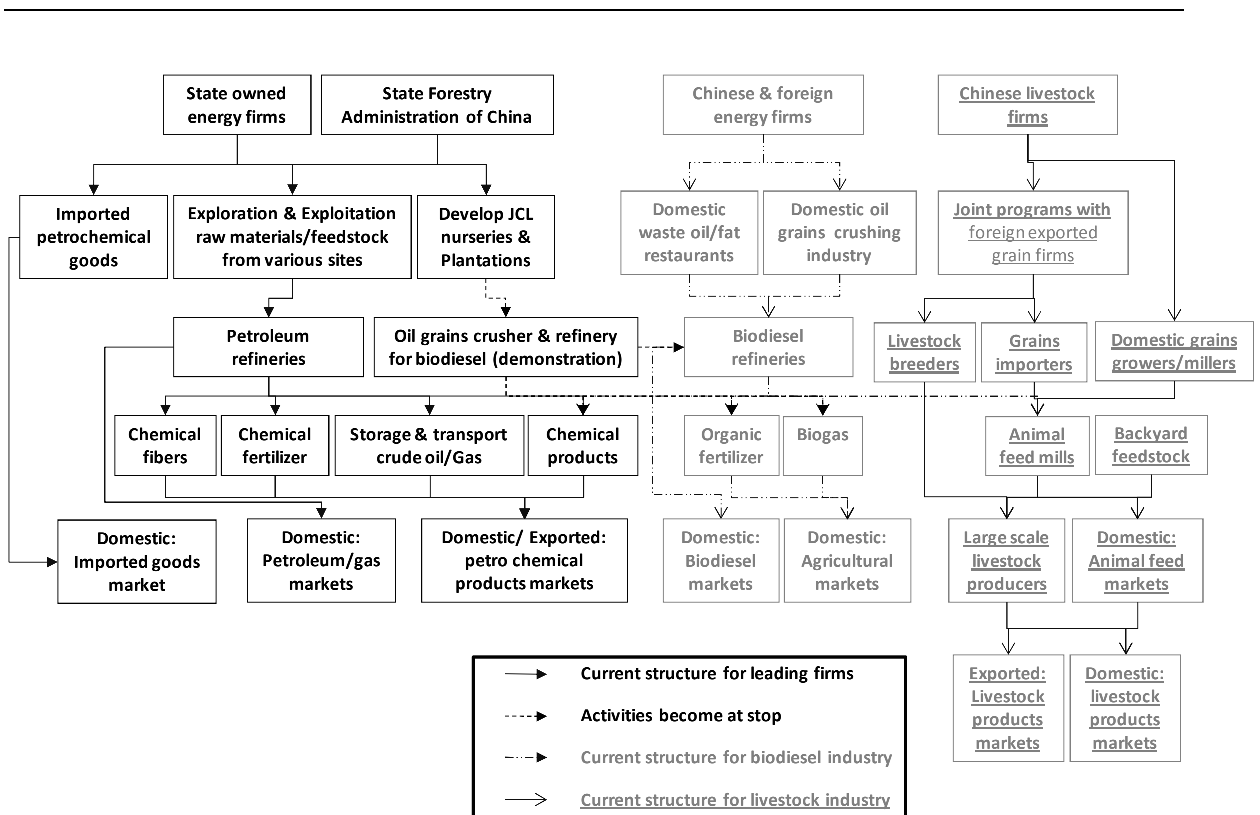

Figure 7: Structure of and links between actors involved in JCL value chain in China
Source: Survey 2008-2009 and various media sources

The structure of the biodiesel industry in China at present is shaped by numerous small-scale operators rather than a few large biodiesel operators. Most of these processors have a production capacity of less than 100,000 ton per annum, and thus do not need a license for biodiesel production from NDRC. The actors involved in this industry are therefore largely unregulated (Laan et al., 2009). Supply of inputs for producing biodiesel mainly comes from domestic waste oil and animal fats. A few operators use vegetable oil seeds to produce biodiesel (Laan et al., 2009; Weyerhaeuser et al., 2007).

The right-hand-side of the Figure 7 shows the present structure of the feed industry. The industry is dominated by several large-scale livestock producers, who have been moving upstream (feed and animal breeding industries) and downstream in the chain (animal products markets) (Allan, 2008). The supply of animal feed is mainly obtained from imports of high protein grains (American Soybean Association, 2009). The leading livestock processors have developed cooperation with grain exporting companies to improve their animal breeding activities, and to secure supplies to their feed processing mills (various media sources). In addition, feed processors reduced risk of variable raw material supplies by allowing individual households and private companies to participate in feedstock production through agricultural contracts, and by involving vegetable oil mills

to supply large quantities of oil cake. Another characteristic of the feed industry is that the leading firms tend to locate their animal feed mills close to their head quarters to ensure sufficient technological support.

In the current structure described above, national energy firms, by far, have not integrated into the biodiesel value chain. Once JCL production resumes, one could anticipate more integration of oil firms into the conventional structure of the biodiesel industry as well as into the animal feed industry.

Strategies that influence the prominent positions of leading firms

The above description of the structure and governance in the value chain points to several strategies of the leading companies (in JCL, biodiesel, and livestock industries) to control the value chain and strengthen their positions. These strategies can be summarized as follows.

1. Key actors make an investment into liquid fuels from inedible JCL seeds. The exploration and exploitation of this new raw material gives these companies a competitive advantage over other companies using raw materials from agriculture.

2. The key actors extend their value chain into upstream and downstream industries. These movements can help the actors to become closer to market demand, thereby enhancing the quality of feed processing, raising sales, and also through the integration of subsequent chains to improve the companies' ability to deal with price fluctuations and enhance profitability. For example, in the JCL industry, national energy companies are developing large-scale grain processing in order to control production in the feedstock sector. This is similar to the livestock industry, where some feed companies extended their chain into the downstream breeding industry (various media sources).

3. The key actors seek cooperation from the various parties along the value chain to secure their raw material supplies. For example, energy companies set up demonstration sites with the help of the SFA. In addition, joint investments by energy firms and government can help the government to improve environmental quality and stimulate development of rural areas. Similarly, a leading livestock processor developed a technical collaboration with an U.S. company that is supplying soybeans to feed mills. The collaboration between this feed supplier and feed processor focused on improving the industry and helping it to modernize its animal production technology, to improve nutritional standards and to promote the use of soybean in pig feeds.

4. Another strategy that the leading companies can employ is the use of contractual agreement with various parties. Contracts usually include advanced prices and specifications of the raw materials and the goods produced. Through such an agreement, the companies can control the price and quality of materials. In addition, the companies can guarantee the supply of raw materials to their customers.
5. Leading companies secure input supplies engaging in international markets to obtain raw materials.
6. The leading companies invest into research and development to enhance the productivity and production efficiency throughout the value chain. National energy companies can get involved in improved production activities related to JCL (such as the development of high yielding varieties and methods of oil extraction). The firms expand the scope of research related these aspects towards several research institutions.

In spite of these strategies that – to certain extend – have already been employed by the leading companies to strengthen their positions in the chain, we note that the leading companies apparently do not attempt to influence government policy in favor of JCL production. On the contrary, the central government is trying to shape the regulatory environment in favor to the JCL production, influencing and anticipating the participation of the companies.

Institutional framework

There are three main objectives for the development of JCL industry in China: (1) to substitute biodiesel for petrol oil in the transportation sector; (2) to reduce the regional disparity through the expansion of a productive land and reduction of non-cultivated marginal land; and (3) to reduce income equality between urban and rural habitants through the development of agro-industries. In line with these objectives, there are various institutional factors affecting the development of the JCL industry, as described in the following.

Domestic agreements to develop JCL plantations

The SFA and state energy companies agreed to establish JCL plantations. The parties jointly estimated that an investment of 400 RMB would be needed per 0.15 ha (1 mu) of plantation. The two parties agreed to share of this cost equally. The investment by the national energy company was channeled to the local forestry bureau for implementation.

The local forestry bureau had to ensure that the development of large-scale plantations would take place in areas not used for food crops.

During the period plantation development between 2006 and 2008, SOHO advanced the investment and later received about 158 RMB per 0.15 ha of plantation. This amount is lower than the cost originally specified in the agreement (survey, 2008). As a consequence, this caused the disruption of production activities in 2009 (survey, 2009). The participating companies have no measures at their disposal to claim their advanced investments. Implementing actors from SOHO gathered in front of the forestry bureau as an informal way to remind the bureau about the promised investment (survey, 2009). After the plantations were established, caring of plantations was only practiced at the demonstration and pilot sites.

Government policies

The second factor influencing the development of the JCL industry includes rules, regulations, taxes, duties, subsidies and other support provided for development of the JCL industry. In line with the guidance provided by NDRC to achieve the renewable energy target, state authorities developed policies to ensure reaching the target. The following policies have been legislated and come into effect in China:

1. Policies related to land use and land allocation are perhaps the most critical affecting JCL development. The government has stressed the use of non-productive areas (wasteland) for environmental protection, and that it should yield economic production. To achieve this, various schemes were introduced. This includes the land reclamation program, the Collective Forestland Right System Reform policy, and the Farmland Redistribution policy. We observed different definitions of wasteland used by various authorities (survey, 2008-2009).

   A second set of policies that contribute to the development of the JCL industry relates to the development of the renewable energy industry. Schemes in this group includes: (1) the grain for green policy, (2) mandatory distributions of blended ethanol 10 percent into convention petroleum diesel in ten provinces of China, (3) implementation of the Polluter Pays Principle for a large-scale energy firms through the CDM, and (4) the involvement of SOHO to implement the JCL plantations.

   During the seed production stage, more specific regulations include rules that the local forestry bureaus imposed on SOHO for seedlings production and planting of JCL (survey, 2008-2009). Another scheme is the Collective Forestland Right System

Reform policy in which quotas of land under the JCL plantations will be allocated to individual households. In addition to allocating the land, the government promised to provide a subsidy of 80 RMB per household and year to be assigned to those farm households maintaining the trees and selling seeds to the oil companies (survey, 2008).

A third set of polices evolved from concerns about food security. As the price of food grains spiked between 2005 and 2007, securing agricultural products, especially grains has become a top priority for the Chinese government. Several measures have been introduced, including: (1) direct and indirect financial incentives to food grain producers, (2) the establishment of more demonstration sites to enhance the productivity of arable lands, (3) the expansion of the grain stocks (national reserves), (4) the banning of the use of food grains for ethanol production since 2006, and (5) the expansion of livestock production to stimulate the development of agro-industries, especially in rural areas.

2. Taxes and duties were also introduced into the JCL sector. SOHO engaging in JCL nurseries and plantations are obliged to pay a tax of 6-7 percent of the total bidding price offered to the local forestry bureaus (survey, 2009). Tax exemptions, however, were implemented for pilot projects and demonstration projects employing new production technologies. With the objective of securing food supplies, various other taxes and tariffs were introduced on the import and export of food grains.

3. Another scheme affecting the development of JCL industries involves quotas, subsidies, and other forms of support. In line with the policies of the Collective Forestland Right System Reform and the Farmland Redistribution, both farmland and forest land will be evenly distributed to farm households. Although both policies have not yet bee implemented, they would give farmers more freedom in making their own land use decisions (survey, 2009).

The government further supports the JCL sector through various research for state enterprises engaged in the JCL industry. Several research institutes are assigned to conducting research on biodiesel production from non-food grains and its possible applications for the agro-industry and other related industries. Research also aims to improve oil yields and production technologies.

In spite of much support to research, we observed a lack of cooperation and technical advice from research institutions to the JCL sector. For example, in the biodiesel industry, most of biodiesel refineries are advancing their own technologies regardless

of the inputs they use (survey, 2009). Authorities did not verify production technologies related to biodiesel production (survey, 2009). There is furthermore a lack of knowledge among the operators involved in the JCL chain about the oil yield from plantations and the extraction and detoxification of seed meal for animal feed production. This lack of knowledge discourages nurseries, afforestation companies, and oil refineries from entering the JCL value chain (Shenyu New Energy Co. Ltd., 2009, personal communication).

4. Financial support for biodiesel is the most prominent regulation mentioned by most participants. The local government promotes the development of JCL industry giving financial support to any private entrepreneur who claims to be participating in the development of the JCL production chain (such as in developing germplasm, nurseries, plantations and refineries).

Quality control

In the segment of seed production, observed measures include:

(1) A quality standard that refers to the certification for high (or fine) oil varieties provided by the state authorities (survey, 2008).

(2) A set of criteria from the local forestry authorities imposed on SOHO to produce JCL seedlings that meet their standards before the seedlings will be purchased (survey, 2008). The quality control system also extends to the plantations. In line with this measure, SOHO plantations will only be accepted if the survival rate of the seedlings is above 90 percent after 12 months of planting (survey, 2009).

Currently, quality control measures related to JCL seed processing into biodiesel industry do not exist. Most of the current biodiesel refineries are small or medium-sized processors who produce biodiesel from waste cooking oil, not from JCL, and each of the biodiesel operators develop their own technologies (survey, 2009). At present, there are no commitments by the biodiesel producers that warrantee the quality their produce.

Costs and benefits of the JCL production chain

The objective of this chapter is to analyze the cost structure of the JCL seed production activities, including the anticipated activities for processing JCL seeds into biodiesel and animal feed. The analysis from this chapter provides an understanding about the cost structure that adds utility or value to the products and co-products along the value chain. The results can be use to improve the firms' cost structure and profitability and thus help strengthen the competitiveness of the production chain.

The chapter is organized as follows. The first section provides data on production costs and benefits for the seeds production sector, including seed collection, seedling production, and seed plantations. For the projects which existed in China during the course of this research, general information for each project is given prior the analysis. The section is followed by the analysis of the seed processing sector.

Seed collection from households and small-scale companies

Case 1: Maoshengyuan Seedling Base

Maoshengyuan Seedling Base is a small-scale, privately run company. It collects JCL seeds from various sources and supplies it to seed nurseries. The company did not purchase the land it uses, infrastructure or other machinery, except one truck. The price of JCL seeds the firm purchased from individual households has increased gradually since 2003 when it commenced operation. In 2003 the price of JCL seeds was 4 RMB/kg air-dried seeds while the price in 2009 was 8-10 RMB/kg. The company collects JCL seeds from different locations and households several times a year, usually between July and August. The firm secures its seed supply by arranging verbal contracts with households and paper-based contracts with other small seed collecting companies. The company sells the seeds to other nurseries at a price of 4-6 RMB/kg air-dried seeds. Usually, the buyers provide their own transportation for picking up the seeds. The information about the firm and the additional assumptions for this segment of the value chain are presented in the table below.

Table 3: Summary and additional assumptions made for seed collection

Company name:	Maoshengyuan Seedling Base
Operational condition:	Own operation
Location of the operator:	Renhe District, Panzhihua, Sichuan
Starting year of seed collection:	2003
Area operated in 2009:	Along the Jinsha river basin; 30,000 mu in Sichuan; 270,000 mu in Yunnan
Production period:	July-September, every year
Annual yield, expected:	2,250 kg, air dried seeds/ha (150 kg/mu)
Production method:	The company arranges own transportation and drives around the area along the Jinsha river basin to purchase JCL seeds from individual households in the area. These individual households collect JCL seeds from the forests of the Jinsha river basin. The Jinsha river is in the upper reach of the Yangtze river and flows through the provinces of Qinghai, Sichuan and Yunnan. People collect JCL seeds by using a pole to knock the fruits down from the tree. After collection, they remove the fruit shells manually at home.
Method to secure raw materials:	Verbal agreements and paper contracts
Potential supplier for raw materials:	(1) Individual households in Renhe district, Panzhihua, Sichuan province (2) Other seeds operators
Potential buyers:	(1) Energy companies in Guizhou province (2) Forestry bureaus in Sichuan and Guizhou provinces
Transport of seed:	Buyers arrange their own transport
Additional assumptions	
Lifespan of the project:	10 yr
Operating area per year:	416.67 ha/yr (25% of productive JCL forest area in Panzhihua)
Seeds collected, air dried:	938 t/yr

Distance for collecting seeds:	100 km/round-trips, 8 trips / yr
Operating staff:	none
Financial support:	not available

Based on these data and assumptions, the cost and benefits seed collection are presented in the table below.

Table 4: Costs and benefits of seed collection

Costs	PPP$
(1) Capital cost, total	
Land and auxiliary facilities	90,116
Equipment	-
(2) Operation cost, annual	901
(3) Production cost, annual	-
Renting of land, annual	-
Land preparation, total	-
Feedstock (JCL seeds, air dried)	1,907,703
Labor use	-
Energy (electricity, coal, gas, diesel)	
Other inputs and utilities	-
Transport of collected seeds (diesel, handling)	5,582
Annual maintenance & repairs [(a)]	2,703
Depreciations of the fixed assets [(a)]	2,854
Annual insurance of capital [(a)]	451
Duties and indirect taxes [(a)]	12,264
Total production cost	*1,931,557*
Production cost per ton of collected seed	*2,060*
Total cost	***2,022,575***

Benefits	
(1) Annual revenues	
Seed, air dried	1,226,381
Fruit shells, air dried wt.	-
(2) Annual non operating income	
Total benefits	***1,226,381***
Net benefits, before tax	***(705,177)***
Break-even for JCL seed (PPP$/ton)	n.a

Financial indicators

Cash flow (without financing)	negative every year
Opportunity cost	12%
Net Present Value of the project	(4,069,957)
Tax on property (1%)	90
Tax on income (6%)	73,583
Cash flow, after taxes	negative every year
IRR, after tax	n.a [b]
B/C at 12%	-47
N/K	0.00
Employment, annual average (man-day/yr)	188

Notes:

[a] indirect cost

[b] all value is negative

Table 5: Sensitivity of the net present value of seed collection to changes in production costs

Production cost	Current value	Level I (-1.5%)	Level II (+3.0%)
Distance for collecting feedstock km, round trip	100.0	0.004%	-0.01%
Labor cost (RMB/man-day)	60.0	0.01%	-0.01%
Price of diesel (RMB/l)	5.1	0.005%	-0.01%
Price of feedstock (RMB/kg)	7.0	3.46%	-7.26%
Price of seeds sold (RMB/kg)	4.5	-2.25%	4.50%
Income taxes (%)	6.0	0.14%	-0.31%

Nurseries for JCL seedling production

Company 1: Huajun Nursery Agriculture and Forestry Company

Huajun nursery is owned by a private entrepreneur who obtained official approval to produce seedlings for the local forestry bureau. This nursery produced seedlings for the local forestry bureau between 2007 and 2008. The nursery participated in the value chain through a bid organized by the Panzhihua forestry bureau. The company is obliged to pay a tax of about 6 percent from the bidding price with which it won the contract. This bidding price includes the cost of purchasing seed, planting, maintenance, and harvesting. The nursery was obliged to sell a certain amount of seedlings to the forestry bureaus. The nursery purchased the land it uses and also made other investments since it commenced operation in 1996. During the investment period, the company did not use any machinery. The procedure for establishing the nursery started from site selection, design of the plots, clearing the land and fertilizing the soil. The irrigation infrastructure was later installed. It takes about six months to raise JCL seedlings. The nursery arranges its own harvest, and sells the seedling to different forestry bureaus at a price between 0.18 to 0.20 RMB per seedling. In 2009 it did not receive any orders for JCL seedlings but the nursery continues to produce other types of seedlings.

Case 2: Forestry Technology Extension Central Station, Panzhihua Forestry Bureau

This nursery belongs to the Panzhihua forestry bureau. The nursery has produced different types of seedlings according to the policies of the bureau. The nursery is managed by the forestry bureau and already had a good infrastructure (irrigation, flat land) before starting

with JCL seedling production. There was therefore no need to invest in the land for producing JCL seedlings. The nursery was designated as a forestry scientific research base for the JCL program. The nursery produced JCL seedlings between 2007 and 2008 and allocated about 80 percent of its arable area (4 of 5 mu) for this purpose.

To establish the nursery, first the land has to be prepared, followed by installation of irrigation and improvements to the soil fertility. It took about six months to produce seedlings. The forestry bureau used the seedlings and transported them to their plantations. To produce seedlings, the authorities do not have to pay any taxes.

The table below lists information and assumptions made for these two nursery projects.

Table 6: Summary and assumptions made for the nurseries

Case:	I	II
Company name:	Huajun Nursery Agriculture and Forestry Company	Forestry Technology Extension Central Station, Panzhihua Forestry Bureau
Production condition:	Official permission granted from forestry bureau	Own operation by the forestry bureau
Location of the operator:	Ala Farm, Renhe District, Panzhihua, Sichuan	No. 470, Middle Panzhihua Road, Panzhihua, Sichuan Province
Production period for JCL:	2007 and 2008, other seedlings in 2009	2008
Planting space (mxm):	0.1 x 0.1	0.2 x 0.2
Density (stem/ha):	525,000	225,000
Annual expected yield (stems/ha):	525,000	225,000
Propagation method:	Sexual propagation (seeds), 6 months Double raising (2 seeds/poly bag)	Sexual propagation (seeds), 5 months Double raising (2 seeds/poly bag)
Method to secure raw materials:	Oral agreements (purchased from afforestation div. forestry bureaus of Sichuan and Yunnan)	Collected seeds from natural forest in certain areas by their own staff
Potential	(1) Forestry bureau of Yuanmou	Produced for their own plantations

supplier for raw materials:	county, Yunnan (2) Forestry bureau of Huili county, Sichuan	
Potential buyers:	(1) Renhe Forestry Bureau, Panzhihua, Sichuan (2) Individual households using the sloping land (in the sloping land conservation program) in Renhe district, Panzhihua, Sichuan province	
Transportation seedlings:	Buyers arrange their own transport	Forestry bureaus arrange transport
Additional assumptions for the company		
Project period:	5 yr	5 yr
Operating area:	1.40 ha/yr	0.27 ha/yr
Annual seedling produced:	735,000 seedlings/yr	60,000 seedlings/yr
Distance: feedstock:	100 km/round-trip	100 km/round-trip
Distance: seedlings:	-	50 km/round-trip
Operating staff:	1 person	1 person
Financial support:	None	None

Table 7: Costs and benefits of JCL nurseries

Cost	Case I (PPP$)	Case II (PPP$)
(1) Capital cost, total		
Land and auxiliary facilities	989	349
Equipments	-	-
(2) Operation cost, annual	1,033	1,021
(3) Production cost, annual		
Renting the land, annual	-	-
Land preparation, total	3,885	4,699
At planting stage (direct cost)		
Feedstock (JCL seeds, air dried)	1,221	100
Labor	29	1,863
Other inputs and utilities	145	28
Transportation seeds (diesel, handling)	25	25
At maintaining stage (direct cost)		
Labor	1,047	1,047
Other inputs and utilities	53,965	4,257
At harvesting stage (direct cost)		
Labor	801,312	65,442
Other inputs and utilities	1.2	0.2
Transportation seedlings (diesel, handling)	-	47
Annual maintenance & repaired [(a)]	11	10
Depreciations of the fixed assets [(a)]	12	11
Annual insurance for the capitals [(a)]	-	-
Duties and indirect taxes [(a)]	406	-
Total production cost	*858,175*	*72,830*
Production cost per seedling	*1.17*	*1.21*
Total cost	**864,082**	**78,899**

Benefits		

(1) Annual revenues		
Seedlings	40,596	3,314
(2) Annual non operating income	-	-
Total benefits	40,596	3,314
Net benefits, before tax	(817,579)	(69,516)
Breakeven price for seedling ($/seedling)	n.a.	n.a.

Financial indicators		
Cash flow (without financial)	(819,587)	(71,546)
Opportunity cost	12%	12%
Net Present Value of the project	(2,955,265)	(258,776)
Tax, properties (1%)	1	-
Tax, income (7%)	2,436	
Cash flow, after taxed	(822,024)	(71,546)
IRR, after tax	n.a [b]	n.a [b]
B/C at 12%	(640)	(63)
N/K	n.a	n.a
Employment, annual average (man-day/yr)	46,050	3,807

Notes:

[a] indirect cost

[b] all value is negative

Table 8: Sensitivity of the net present value of nurseries to changes in production costs

Production cost	Current value	Level I (-1.5%)		Level II (+3.0%)	
		Case I	Case II	Case I	Case II
Germination rate (%)	83.0	0.00%	0.00%	0.00%	0.00%
Labor, general (RMB/man-day)	60.00	1.46%	1.43%	-2.93%	-2.87%
Price of diesel (RMB/l)	5.09	0.00%	0.00%	0.00%	0.00%
Price of seeds purchased (RMB/kg)	4.50	0.00%	-0.07%	0.00%	0.15%
Price of seedling sold (RMB/seedling)	0.19	-0.07%	-0.07%	0.15%	0.15%
FYM price (RMB/kg)	1.90	0.00%	0.00%	0.00%	0.00%
Income tax (%)	6.0	0.00%	0.00%	0.00%	0.00%

JCL plantations

Case 1: Huajun Nursery Agriculture and Forestry Company

The Huajun Nursery Company, described above, also participated in the JCL plantation program. The company entered this segment of the chain by participating in a bidding process organized by the local forestry bureau. The company won a bid and obtained the official permission for executing plantations for the Panzhihua forestry bureau. Under this agreement, the company does not need to purchase land but purchase seedlings from the forestry bureau. Certain areas in the barren hills belonging to the forest category in the land use planning were designated by the forestry bureau for establishing JCL plantations. The company establishing JCL plantations for four sites in 2008, all located in Renhe district. The plantations have an average size of 78 ha (1,175 mu).

To establish a plantation, the company hired labor for clearing the land, weeding and preparing holes for the seedlings. The company then invited officers from the forestry bureau to inspect the conditions. After approval, the company planted seedlings under the supervision of forestry officials. The forestry bureau supplied seedlings to the company at a price of 0.2 RMB/seedling. Right after the planting, the company organized a second inspection. The company had to re-plant several times to meet the targets regarding the survival rate and density, as set by the forestry bureau. The third round of inspection from the authority was organized six months after planting. At this stage, the survival rate of the

seedlings should be close to 90 percent. After twelve months, the company had to organize a last inspection by the forestry bureau, after which the plantations were transferred to the forestry bureau.

Case 2: Forestry Technology Extension Central Station, Panzhihua Forestry Bureau

The Panzhihua forestry bureau itself also set up one plantation in 2008, covering an area of 800 mu (53 ha). The authority did not need to purchase the land or invest in infrastructure. There was no need for machinery during land preparation.

The plantation started at site selection followed by land clearing, preparation of planting holes, and the transplanting of seedlings. After the planting stage, the plantation should have been managed but this was not implemented because of a lack of budget. Issues related to seed yield and the handlings of seeds are the main concerns for officials at present.

Case 3: Yunnan Shenyu New Energy Co., Ltd, Kunming, Yunnan province

Yunnan Shenyu New Energy developed an area of about 275,000 mu (18,333 ha) of JCL in Shuangbai county between 2006 and 2008. The company has its own nurseries near the plantation sites. During the first four years of the project, the company cooperated with a forestry university through a research and training program for JCL.

The JCL production area was allocated to the company free of charge by the Shuangbai county government. The local government designated barren hills of 213,000 mu (14,200 ha) to the firm to develop JCL plantations over a period of 50 years. In addition, the company rented collective waste hills of 133,000 mu (8,867 ha) at a special fee of 8 RMB/mu/year over a period of 50 years (usually, the rented fee of waste hills is 100 RMB/mu per year). The firm used improved varieties and planted the seedling at a density between 1.5x1.5m and 2.0x2.5m. The firm applied fertilizers and insecticides every year to maintain the plantation.

Case 4: Petro China, Yunnan province

Petro China developed a demonstration plot in cooperation with the Yunnan provincial government. Through this cooperation, the firm developed four plantations located in Yuanmou county, Yuanyang county, Shuangbai county, and Shuangjiang county. The one in Shuangjiang county was developed in 2007 and is the smallest of the four. The company received all land free of charge.

For the plantation in Shuangjiang county, the firm concurrently developed it as a demonstration plantation and undertook extension activities for seedling production in 2007. The total operating area of this plantation is 240 mu (16 ha). The company

propagated seedlings using vegetative and sexual methods and developed improved variety seeds in addition. The firm expects an annual seed yield of 250 kg of air-dried seed p. mu, i.e. 3,750 kg of air-dried seeds per hectare.

Table 9: Summary and assumptions for the JCL plantations

Case:	I	II	III
Company name:	Huajun Nursery Agriculture and Forestry Company	Forestry Technology Extend Central Station, Panzhihua Forestry Bureau	Yunnan Shenyu New Energy Co., Ltd
Investor:	SINOPEC	Panzhihua Forestry Bureau	Yunnan Shenyu New Energy Co., Ltd
Production condition:	Official granted permission from forestry bureau	Own operation	Own operation
Location of the operator:	Ala Farm, Renhe District, Panzhihua, Sichuan	No. 470,Middle Panzhihua Road, Panzhihua, Sichuan Province	Kunming, Yunnan Province
Started year to execute JCL plantations:	2008	2008	2006
Production period:	2008 (1 year)	2008 (1 year)	2006 to 2008 (3 years)
Planting space (mxm):	2.0 x 3.0	2.0 x 3.0	between 1.5 x 1.5 & 2.0 x 2.5
Density (stem/ha):	1,667	1,667	2,000
Annual yield, expected from year 5 onwards (air dried seeds kg/ha):	3,250.00	3,250.00	3,250.00
Propagation method:	Sexual propagation (seedlings 6 months old)	Sexual propagation (seedlings 6 months old)	Sexual propagation (seedlings 6 months old)
Method to secure raw materials:	Paper based contract with the forestry	Produced their own seedlings	Produced their own seedlings

	bureaus (as a bidder)		
Potential supplier for raw materials:	(1) Forestry Bureau of Renhe district, Panzhihua city, Sichuan Province	(1) Own nurseries (Forestry bureau)	(1) Own nurseries (Yunnan Shenyu New Energy Co., Ltd)
Potential buyers:	(1) SINOPEC	(1) SINOPEC	(1) Yunnan Shenyu New Energy Co., Ltd (own use)
Handling seeds, air dried:	n.a	n.a	n.a
Additional assumptions			
Period of operation:	1 yr	50 yr	50 yr
Operating area:	313.33 ha/yr	53.33 ha/yr	23,067 ha/yr
Expected seeds production:	972.73 t/yr	165.57 t/yr	71,574.73 t/yr
Distance for collecting feedstock (seedlings)	-	50 km/round-trip	-
Distance for transporting seeds:	n.a.	n.a.	n.a.
Operating staff:	1 person	1 person	1 person
Financial support:	None	None	None
Bidding price:	400 RMB/mu		

Table 10: Costs and benefits of JCL plantations

Cost (PPP$)	**Case I**	**Case II**	**Case III**
(1) Capital cost, total			
Land and auxiliary facilities	-	-	-
Equipments	-	-	-
(2) Operation cost, annual	5,116	2,417	60,375
(3) Production cost, annual			
Renting the land, annual			3,711,628
Land preparation, total	820,164	465,310	60,675,406
At planting stage (direct cost)			
Feedstock (seedlings)	64,367	10,956	105,300,029
Labor	1,696,008	288,682	46,321,318
Other inputs and utilities	-	-	18,842,248
Transportation seeds (diesel, handling)	-	99	296
At maintaining stage (direct cost)			
Labor	327,907	543,628	234,246,628
Other inputs and utilities	9,275	9,177	165,310
At harvesting stage (direct cost)			
Labor	-	395	155,962
Other inputs and utilities	-	2	2
Transportation seedlings (diesel, handling)	-	-	-
Annual maintenance & repaired [(a)]	-	-	-
Depreciations of the fixed assets [(a)]	-	-	-
Annual insurance for the capitals [(a)]	-	-	-
Duties and indirect taxes [(a)]	364	1,227	446,641
Total production cost	*2,097,921*	*854,166*	*405,478,433*
Production cost per stem of seedling planted	*4*	-	-
Production cost per ton seeds, air	-	*5,159*	*5,665*

dried			
Total cost	**2,923,202**	**1,321,893**	**469,925,841**
Benefits			
(1) Annual revenues			
Seeds, air dried	**-**	96,262	41,613,217
Pruned branches	-	26,434	3,050,887
Price offered from the bidding	36,434		
(2) Annual non operating income			3,414,698 [(b)]
Total benefits	***36,434***	***122,696***	***48,078,801***
Net benefits, before tax	**(2,061,487)**	**(731,470)**	**(357,399,632)**
Breakeven price for seedling ($/seedling)	n.a		
Financial indicators			
Cash flow (without financial)	negative	4 yr negative	5 yr negative
Opportunity cost	12%	12%	12%
Net Present Value of the company	(2,577,471)	(1,120,698)	(474,082,257)
Tax, properties (1%)			
Tax, income (7%)	2,550	8,589	3,126,487
Cash flow, after taxed	1 yr negative	4 yr negative	5 yr negative
IRR, after tax	n.a [(c)]	12%	12%
B/C at 12%	n.a	n.a [(c)]	n.a [(c)]
N/K	n.a	-13	-184
Employment, annual average (man-day/yr)			
(1) At planting stage	39,584	39,495	17,074,679
(2) At maintaining stage	18,801	31,169	13,430,141
(3) At harvesting stage	-	23	8,942

[(a)] indirect cost

[(b)] indirect subsidy from renting the land

[(c)] all value are negative

Table 11: Sensitivity of the net present value of JCL plantations to changes in production costs

Production cost	Current value	Level I (-1.50%)			Level II (+3.0%)		
		Case I	Case II	Case III	Case I	Case II	Case III
Survival rate of seedlings (%)	94.0	-0.021%	0.000%	-0.010%	0.063%	0.001%	0.030%
Labor, general (RMB/man-day)	60.00	1.051%	1.800%	1.779%	-2.101%	-3.601%	-3.557%
Price of diesel (RMB/l)	5.09	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
Price of urea fertilizer (RMB/kg)	2.20	0.000%	-0.072%	0.000%	0.000%	0.169%	0.000%
Price of P_2O_5 fertilizer (RMB/kg)	0.90	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
Price of K_2O fertilizer (RMB/kg)	3.96	0.000%	0.000%	0.001%	0.000%	0.000%	-0.002%
Price of compounded fertilizer (NPK, RMB/kg)	4.00	0.000%	0.000%	0.002%	0.000%	0.000%	-0.003%
Price of FYM (RMB/kg)	1.90	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
Price of pesticides (RMB/kg)	16.00	0.000%	0.000%	0.001%	0.000%	0.000%	-0.002%
Price of seedling purchased (RMB/seedling)	0.20	0.000%	0.001%	0.016%	0.000%	-0.001%	-0.032%
Price of seed sell, air dried (RMB/kg)	2.00	0.000%	17.430%	-0.289%	0.000%	0.580%	0.579%
Price of pruned branches	0.50	0.000	0.000	-	0.000%	0.000%	0.057%

sell (RMB/kg)		%	%	0.028 %			
Income tax (%)	7.0	0.001 %	0.027 %	0.023 %	- 0.003%	- 0.054%	- 0.045%

The processing of JCL seeds

During the course of this research, seed processing plants for JCL did not exist at a commercial scale. The estimation of costs and benefits for seed processing is therefore based on several assumptions. One assumption we make is that seed processing can be done at a large scale as based on the expected amounts of available seeds from plantations and estimated yields for the Yunnan Shenyu New Energy Co. in Kunming, Yunnan province (see the profile of this company in section above). We also assumed the supply of JCL seeds to be continuous over the period of one year. The capacity of the JCL biodiesel production plant should therefore be realistic.

The estimation of costs and benefits for seed processing considers the construction of a biodiesel production facility. The equipment cost for biodiesel production is derived from Haas et al. (2006) for a project similar in size. However, the capital cost estimated in the study of Haas et al. is based on supercritical transesterification which has the great advantage of eliminating pre-treatment capital costs. Most biodiesel plants in China, however, use pre-treatment steps to reduce the free fatty acids and the water contents of the input feed stream. An additional 25 percent capital cost was therefore assumed to capture the pre-treatment before the start of chemical processing. For the transesterification process, we assumed cost based on an alkali catalyzed process as this process is regarded as the most economical among available options.

The estimation was based on the use of JCL seeds as feedstock, and on the assumption that the biodiesel production facility and the detoxification facility were integrated into a single JCL seed crushing and processing plant. The calculations for this designed processing plant assume an additional unit for detoxification extraction of crude protein from seed meal.

The detoxification process is expensive at a small scale while there is currently no detoxification at a commercial scale. It was therefore difficult to estimate the capital cost for detoxification. A medium-sized detoxification unit was therefore assumed in the cost estimation, which accounted for 45 percent of the capital cost of the biodiesel processing

unit. As data were not available, a thermodynamic model to estimate the mix of chemicals used in the detoxification process could not be used here.

The calculations for the processing plant assume raw JCL seeds as a starting material. We assumed the operation of the plant to be a continuous-process of vegetable oil transesterification, and ester and glycerol recovery. We further assumed that the processing of biodiesel requires catalyst and pre-treatment steps of the extracted JCL crude oil prior the main chemical reaction. The table below shows additional assumptions.

Table 12: Assumptions made in calculating costs and benefits of the seed processing plant

Main assumptions made for the designed seeds processing project		
(1) Project period	30	yr
(2) Oil content (fine variety)	42%	
(3) Production process is continuous		
a. Seeds pre-treatment, yield good seeds	95%	good seeds left
b. De-hulling seeds	5%	of selected seeds
c. Cold press, oil recovery rate	90%	
d. Filtered crude oil, recovery rate (sell)	87%	
e. Biodiesel process is base catalyzed		
f. Seed meal preparation (for crude protein), using solvent		
g. Recycled solvent for de-fatting seed meal, recovery rate	80%	
h. Glycerin (80% w/w) is a co-products sold to an industry (sell)		
i. Obtaining crude protein and deactivated anti nutrition (sell)		
(4) Annual operating capacity	125,000	t biodiesel/yr
(5) Annual operating hours	8,000	hrs/yr
(6) Labor per shift	2.00	persons/shift
(7) Number of shift	3.00	shift/day
(8) Storage capacity for feedstock and goods, Storage is empty at the beginning and at the end of the year	25	days/shipping period

(9) Transport capacity for feedstock and good produced (loading)	10.0	t/load
a. The company collect seeds from plantations, distance	100	km, round trip
b. The company distribute biodiesel to distributors, distance	250	Km, round trip
c. A chemical industry arrange a transport for glycerin		
d. A feed processor arrange a transport for crude protein		
(10)		
(11)		

Table 13: Cost-Benefits analysis for the designed seeds processing plant

Cost (PPP$)	**With detoxification unit**	**Without detoxification unit**
(1) Capital cost, total		
Land and auxiliary facilities	21,087,107	21,087,107
Equipments at seeds treatment unit	633,983	633,983
Equipments at oil extraction unit	1,391,261	1,391,261
Equipments at biodiesel processing unit	37,684,076	37,684,076
Equipments at detoxification unit	30,581,038	-
(2) Operation cost, annual		
Operation cost at a preparation & oil extraction unit	1,017	1,017
Operation cost at biodiesel unit	1,017	1,017
Operation cost at detoxification unit	1,017	-
Operation cost at all units	234,632	234,632
(3) Production cost, annual		
Renting the land, annual	-	-
Land preparation, total	9,118	9,118
<u>Seeds pre-treatment unit</u>		
Feedstock (JCL seeds)	157,806,494	157,806,494

Labor (together with the oil extraction unit)	-	-
Other inputs and utilities	32,080	32,080
Transportation for collecting seeds (diesel, handling)	7,627,314	7,627,314
Annual maintenance & repaired [(a)]	19,019	19,019
Depreciations of the fixed assets [(a)]	20,076	20,076
Annual insurance for the capitals [(a)]	921	921
Duties and indirect taxes [(a)]	62,465	62,465
At oil extraction unit		
Feedstock (treated seeds)	-	-
Labor	116,279	116,279
Other inputs and utilities	782,289	782,289
Transportation (diesel, handling)	-	-
Annual maintenance & repaired [(a)]	41,738	41,738
Depreciations of the fixed assets [(a)]	44,057	44,057
Annual insurance for the capitals [(a)]	2,022	2,022
Duties and indirect taxes [(a)]	-	-
Biodiesel processing unit		
Feedstock (JCL crude oil)	-	-
Labor	116,279	116,279
Other inputs and utilities	20,600,095	15,382,603
Transportation for biodiesel (diesel, handling)	2,978,527	2,224,140
Annual maintenance & repaired [(a)]	1,130,522	1,130,522
Depreciations of the fixed assets [(a)]	1,193,329	1,193,329
Annual insurance for the capitals [(a)]	54,773	54,773
Duties and indirect taxes [(a)]	99,624	99,624
Detoxification unit		
Feedstock (JCL seed meal)	-	-
Labor	116,279	-
Other inputs and utilities	405,826,350	-
Transportation (diesel, handling)	-	-

Annual maintenance & repaired [a]	917,431	-
Depreciations of the fixed assets [a]	968,400	-
Annual insurance for the capitals [a]	44,449	-
Duties and indirect taxes [a]	-	-
Production cost per ton of crude oil filtered	*926*	*1,240*
Production cost per ton of biodiesel produced	*157*	*162*
Production cost per ton of crude protein produced	*1,925*	**-**
Total production cost	*600,664,086*	*186,778,041*
Total cost	**692,288,353**	**247,820,253**

Table 13 (continuation)		
Benefits (PPP$)	**With detoxification unit**	**Without detoxification unit**
(1.1) Annual revenues from conventional goods		
Hulls	6,246,507	6,246,507
Filtered cake	976,282	729,014
JCL biodiesel	9,732,422	7,267,442
Glycerin (80% w/w)	655,722	489,644
(1.2) Annual revenues from detoxification unit		
Crude protein	3,178,073	-
Phorbol Ester	-	-
(2) Annual non operating income		
Total benefits	20,789,006	14,732,607
Net benefits, before tax	**(579,816,064)**	**(172,001,366)**
Breakeven ($/ton)	n.a	n.a

Financial indicators		
Cash flow (without financial)	Negative every year	Negative every year
Opportunity cost	12%	12%
Net Present Value of the project	**($4,414,310,362.29)**	**(1,340,849,365)**
Tax, properties (1%)	57,899	32,480
Tax, income (7%)	1,017,975	594,027
Cash flow, after tax		
IRR, after tax	**-37.10**	**- 0.86**
B/C at 12%	n.a (b)	n.a (b)
N/K		
Employment, annual average (man-day/yr)		

At an oil extraction unit	92,477	92,477
At biodiesel processing unit	35,485	27,006
At a detoxification unit	2,001	

Notes:

[a] indirect cost

[b] all value is negative

Table 14: Sensitivity of the net present value of JCL processing to changes in production costs

Production cost	Current value	Cash flow after taxed		Production cost p. ton of protein	
		Level I (-1.5%)	Level II (+3.0%)	Level I (-1.5%)	Level II (+3.0%)
Labor, wage (RMB/man-day)	200.00	-0.02%	0.04%	0.00%	0.00%
Price of diesel (RMB/l)	5.09	0.00%	0.01%	0.00%	0.00%
Price of coal (RMB/t)	350.00	-0.01%	0.02%	0.00%	0.00%
Price of electricity (RMB/kWh)	0.49	0.00%	0.00%	0.00%	0.00%
Price of water (RMB/m3)	0.11	0.00%	0.00%	0.00%	0.00%
Price of solvent (RMB/l)	6.00	-1.04%	2.08%	1.51%	-2.90%
Price of seed purchased (RMB/kg)	1.20	-0.45%	0.90%	0.00%	0.00%
Price of hulls sell (RMB/kg)	1.00	0.01%	-0.03%	0.00%	0.00%
Price of filtered cake (RMB/kg)	0.125	0.00%	-0.01%	0.00%	0.00%
Price of biodiesel (RMB/kg)	0.20	0.02%	-0.05%	0.00%	0.00%
Price of crude protein (RMB/kg)	0.05	0.01%	-0.01%	0.00%	0.00%
Oil content (%)	42.0	2.40%	-2.29%	0.01%	-0.01%
Annual production capacity (t/yr)	125,000.00	-1.48%	2.95%	-0.01%	0.01%
Solvent recovery rate (%)	80.0	3.46%	-6.93%	-4.74%	11.05%
Good seeds after pre-treatment (%)	95.0	0.30%	-0.86%	0.00%	0.00%
De-hulling of the seeds (%)	5.0	-0.10%	0.21%	0.00%	0.00%

Discussion

Financial viability of JCL production

The analysis showed that at present prices all actors participating in the JCL production chain receive negative returns. Figure 8 gives an overview of the production situation of these actors, including actors that are expected to enter the chain in the future.

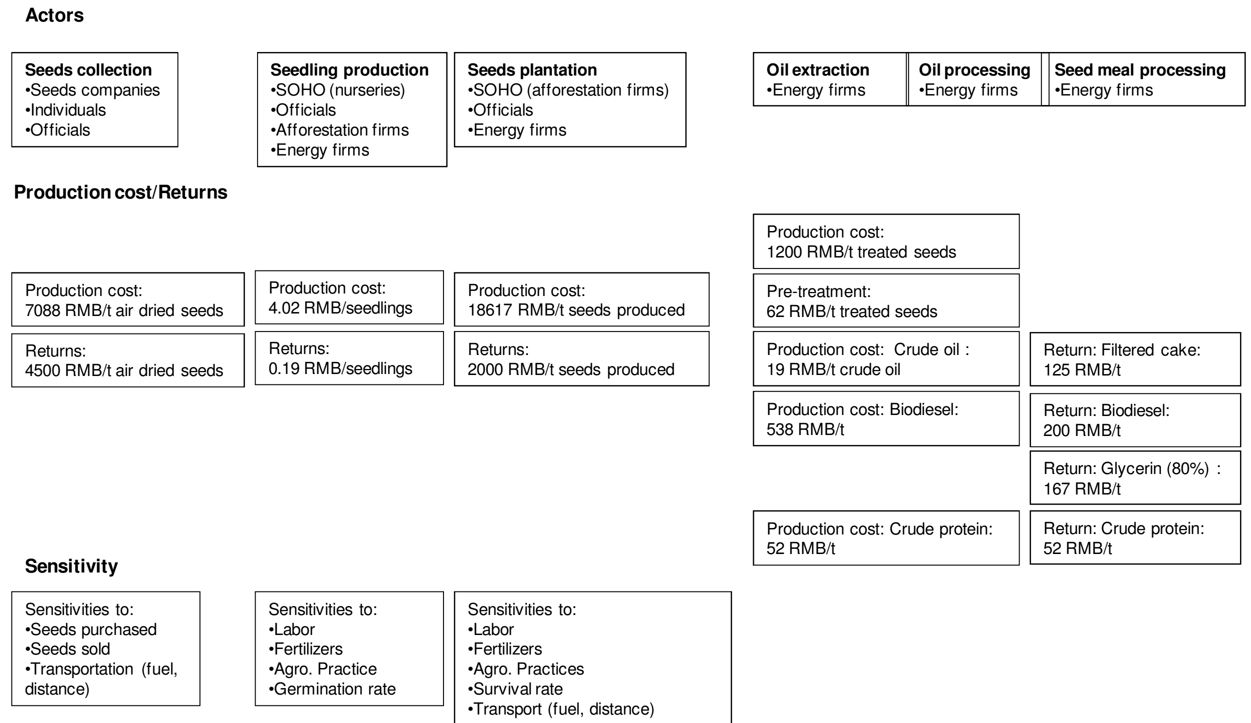

Figure 8: Production structure of the actors participating in the JCL production chain in China

Fixed capital cost

The capital cost for the seed production sector (seeds collection in table 4, seedling production in table 7, and the plantations in table 10) are relatively small compared to the seed processing sector. Existing companies enter the business of seed collection on a voluntary basis. Their investment in fixed capital is relatively small as no land or auxiliary facilities are needed.

At the nursery production stage actors include companies that voluntary participated such as private energy companies and entrusted actors such as SOHO and the forestry bureau. For private companies, capital cost form a significant part of the total production cost. This is because the location for establishing a nursery required water

sources for irrigation. For SOHO and state-owned nurseries, the share of capital cost is relatively small because they have produced other types of seedlings before, and new investments in facilities were therefore not needed.

Actors engaged in plantations include SOHO, forestry bureaus, and private energy companies. For these, the capital cost is a small share of the total production cost. This is because the land for the plantation was allocated to it by the local government. For private companies that voluntary participate in setting up JCL plantations, the price for land (rented over a period of 50 yrs) was subsidized by the local government in response to the national renewable energy policies of the central government. The capital cost of land was almost zero for SOHO, national energy companies, and the local forestry bureaus as land was provided for free. Exceptions are the national energy companies that developed demonstration sites; for these the capital cost was significant as they integrate the development of germplasm and breeding programs.

For seed processing plants, the cost of capital for the detoxification unit forms a significant part of the capital cost of the biodiesel processing plant, although the size of the production unit was assumed to be relatively small. Lacking more detailed data, the cost of the detoxification unit was assumed here to be 45 percent of the total equipment cost of the biodiesel processing unit. Additional capital costs for developing the detoxification unit include the cost of an additional storage unit for keeping seed meal and crude protein. The overall cost of the processing plant with a detoxification unit is therefore larger than that of a processing plant without detoxification facilities.

Operation cost

Operation cost is practically zero for actors engaged in seed collection. For actors in seedling production and plantation, such as SOHO, the operation cost results from organization and inspection by forestry authorities. For the forestry bureaus and private energy companies (especially those with demonstration sites), the inspection by authorities is not necessary, but there are operation costs related to research and training and overhead for the regular staff.

For the seed processing sector, the operation cost is a small share of the total production cost. Hiring regular staff, overheads, research and training contribute to this category. The assumption that the production plant will develop a moderately sized detoxification unit for seed meal does not significantly increase the operation cost as we

assumed that the production process is continuous and the staff is working in time shifts. However, this is based on the assumption that the seed processors do not need to develop additional training on top of their regular training program. The operation cost might increase if more intensive training is required for the staff to operate the detoxification unit.

Production cost

For the seed collectors, the cost of feedstock and transportation for collecting seeds is the most significant cost (see Table 4). For the nurseries, the cost of production factors like labor and fertilizers have a large share of the total production cost. However, the production cost varies from case to case because there are no standard procedures for producing seedlings. Although the forestry bureaus impose their regulation for participating SOHO, the regulation only concerns the quality of the seedling.

The production cost of nurseries run by private energy companies and the forestry bureaus appears to be higher than that of SOHO. This is because SOHO minimized their production cost by using very little fertilizers. On the other hand, the nurseries of the forestry bureaus and private energy companies applied more fertilizer and had higher costs. Production costs for the plantations mostly come from the hiring of labor (land preparation, planting, re-planting, caring, and fertilizing, – about 45 percent) and the purchase of seedling (about 10 percent). The cost of irrigation was zero for all cases analyzed in this study because planting was done during the rainy season to minimize the production cost. The difference in production cost for the plantations partly stems from different techniques of planting. Regulations imposed on SOHO concern the survival rate of the seedlings several months after planting.

For the seed processing segment of the value chain, the cost of air dried seeds contributes about 80 percent of the total production cost. In addition, the cost of energy, water, and labor makes a significant contribution to the total production cost for preparing crude oil for the biodiesel processing unit.

To process JCL seed meal according to the assumed technologies, the addition of a pre-treatment unit increases the direct production cost. This includes the cost of power, chemicals, and labor. The cost of this pre-treatment process accounts for about 90 percent of the total production cost for producing detoxified crude protein.

For the seed processing plant, revenues from byproducts such as crude protein appear to be an important to covering the direct manufacturing cost and the total production cost.

Benefits before and after tax

At the assumption that the biodiesel processing plant could work at a capacity of 120,500 tons/yr and that the detoxification unit is installed, revenues from the sale of the byproducts like crude protein from JCL seed meal would be about 15 percent of the total revenues, given selling it at the cost of processing the good (see table 13). The revenues from detoxified crude protein from seed meal therefore have a significant and positive impact on the net value of the total production of the biodiesel production plant. Together with the revenues from conventional byproduct like glycerin, detoxified crude protein could lead to a small reduction in total production cost for the biodiesel processing plant about 1 percent (Table 13).

In spite of this finding, business returns are negative under current prices. Tables 4, 7, 10 and 13 show that each stage of JCL seed production and processing has a negative net benefit each year (annual net benefits). If financial support was not available, cash flows of the plant would be negative every year after deducting income and property taxes. Comparing benefits for the processing plant without the detoxification unit, the benefits (after tax) are about 3.3 times higher than the annual benefits for processing plants without a detoxification unit; this is because the amount of glycerol and detoxified meal that were produced originate from a fixed amount of JCL seed. The cost of glycerol, detoxified crude protein, and the storage of these two byproducts are constant irrespective of their selling prices.

The analysis shows that the market value of these byproducts could influence the biodiesel production price in the contest of financial return at sale. As the price of the detoxified crude protein increases, a comparable amount could be subtracted from the biodiesel production cost, with no increase in the cost of biodiesel production cost. On the other hand, if the price of the detoxified crude protein decreases as to compete with other sources of protein for the animal feeding, the biodiesel cost does not necessary increase but the benefit of the biodiesel processing plant will diminish.

The high cost of preparing air-dried seeds and seed meal for the detoxification unit constrains the profit of the processing plant. The cost of preparing the seeds before oil extraction is 18 $/t seeds (excluded price of feedstock), processing JPPO into biodiesel costs another 156$/t biodiesel, and preparing the seed meal for detoxification costs 1,925 $/t crude protein.

Sensitivity analysis

We varied the price of the raw materials and good produced and analyzed the impact on the after-tax cash flow at each segment of the value chain. We found the profitability of the operators along the value chain to be sensitive to tax and the transportation. The effect of other factors was, however, larger. We note that the sensitivity of benefits to changes in tax is only relevant for SOHO and private companies but not for the state-run operators as these are exempted from taxes. Factors that influenced the profitability of most companies included changes in labor costs and variable inputs, such as prices of energy, fertilizers, and chemicals.

At the seed collection stage where seeds were collected from individual households, the benefits of the companies are most sensitive to the changes in the price of seeds purchased from individuals, and the selling price of seeds to nurseries.

For nurseries, the benefits are sensitive to the germination rate of the seed in the propagation. However, this effect is relatively small compared to the effects of price changes of seedlings sold to the forestry bureaus. Benefits of the nurseries also appeared to be sensitive to labor costs as the nursing of seedlings is labor intensive. The benefits of the companies are also affected by price change of fertilizers, though only for those nurseries that applied fertilizers.

The benefits of the plantations appeared to be most sensitive to the price of the harvested seeds. Other factors had a smaller effect on benefits, such as the survival rate of the seedlings, labor, price of fertilizers (if used), and price of chemicals and pesticides.

The benefits of plants processing JCL seeds were sensitive to changes in the price of the main production factors: energy (diesel and coal), water, labor, chemicals, and the air dried JCL seeds. The benefits of these plants were also sensitive to the choice of production, the assumed production efficiency, and the quality of the seeds as indicated by the percentage of de-hulled seeds, the percentage of good seeds, the average oil content of the seeds, the recovery rate of the products from each process, and the annual production capacity of the processing plant.

For processing detoxified crude protein from seed meal, the cost of detoxified crude protein showed to be sensitive to changes in the price of energy and chemicals.

Competitiveness of JCL

Although the analysis showed that the detoxification of JCL seed meal for use in livestock industry is feasible at a commercial scale, there are factors that are likely to influence the

competitiveness of protein from JCL. First, many of the big Chinese livestock processors are merging with upstream and downstream industries in the value chain (such as animal breeding, feed processing, livestock raising, and meat processing). Second, large-scale livestock producers increase their productivity by using feeds with high protein contents (various media sources). Third, the main feedstock that is currently used for animal feed production by large-scale feed operators is soybean imported from the U.S.A. (American Soybean Association, 2009). Fourth, feed processing plants belong to large-scale feed processors and are operated near areas where there is large-scale livestock raising, as it is easier to provide technical services and advice for possible improvements for the feed production systems. Examples are the feed mills that belong to Sichuan New Hope Agribusiness Co., Ltd, Tongwei group, Tech-bank and Zhengbang Group (various media sources).

These ongoing structural changes in the Chinese livestock industry rather suggest that feed processors would prefer high quality of protein-based feed and a reliable and stable source of protein supply for their feed mills. The entrance of these actors into the JCL value chain might only be possible when the cost of detoxified crude protein is competitive with the currently used soy-based feed, while taking into account the availability of feedstock and a transportation costs.

Factors limiting the supply of JCL seed

Given the adjustments in the structure of the Chinese livestock industry, the crude protein from JCL seed meal would be able to compete with other types of crude protein if JCL seed yield could be produced in a large quantity. This issue points to some critical implications that link all actors participating in the JCL production chain.
First, the annual production of the crude protein from JCL is a function of processing choices (such as the percentage de-hulling of kernels and preparation of the seed meal). Second, the quantity of detoxified crude protein is fixed. The annual production dependents on the JCL seed supply, which at the moment is very limited.

Based on an expected seed yield of about 3.75 t/ha (from year five onwards, for a well-managed plantation), it would be possible to meet the seed demand of large-scale processing plants. The practices of private energy companies reflect that the seed yield much depends on JCL varieties and regular agronomic practices affecting the growth of the JCL trees. Yet most current plantations planted between 2006 and 2009 use varieties selected from natural forests rather than improved varieties and are not well-managed or

not managed at all. As a result the current seed yield in the 2-3 year old plantations is only about 1.35 t/ha (own survey, 2009). This situation creates doubts about the availability of seeds, which could severely limit the competitiveness of JCL-based protein as compared to other sources of protein feeds for the livestock industry.

Production cost

The cost of feedstock constitutes a significant portion of the total production cost for processing JCL seeds whereas the cost of labor and inputs, which are dependent on management practices, plays a significant role for the seed production sector. This highlights the need for creating additional value from the seed cake by using the byproducts from biodiesel production in the livestock industry.

Although the processing plant does not need to purchase seed meal as an input to process the crude protein (as it is a waste product from biodiesel production), the required selling price of crude protein is 1925$/t. This is largely because of the chemicals required for a pre-treatment of the seed meal and the energy to de-activate anti-nutritional factors. Furthermore, the cost of detoxification is sensitive to the cost of the solvents used to prepare the seed meal, the cost of additional pre-treatments and the possibility of recycling of solvents. The choice of production technology and technical efficiency are therefore play significant role to improve the competitiveness for JCL crude protein.

Technical aspects

Given the assumption about processing JCL seed meal, the sensitivity analysis showed that the cost of producing crude protein is sensitive to the choice of production method as well as the technical efficiency of the process. For example, the choice of the proportion of de-hulled seeds and the recovery rate of the solvent used in the pre-treatment process. In addition, the cost of production shows economies of scale. The factors mentioned above point to the need to develop and improve the production techniques, which might then strengthen the competitive of the JCL processors.

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Appendix

Appendix 1: General assumption for all projects

Item		
Opportunity cost (discount rate)	12%	
Salvage value of the fixed assets	5%	
Transport truck (small)	4.8	t/load
Transport truck (medium)	7	t/load
Transport truck (large)	10	t/load
Diesel consumption rate for land preparation	55	l/hr
Diesel consumption rate for irrigation pump	1.25	l/hr
Gasoline consumption rate for pick up	12	Km-l
1 Man-day	8	Working hours, equal to 1 shift
Inspection from the authority at a plantation	3	times for the first year of planting for SOHO
Inspection from the authority at a plantation	1	time per year for a voluntary participated firm

Appendix 2: Price assumptions for all projects

Investment		
Land price (wasteland)	15,000	RMB/ha
Rented land, general (wasteland; no access to irrigation)	1,500	RMB/ha/yr; for 50 yrs
Rented land, general (wasteland; could access to irrigation)	5,250	RMB/ha/yr; for 50 yrs
Rented land for JCL participants (wasteland; no access to irrigation)	1,200	RMB/ha/yr; for 50 yrs
Equipments for general operation/harvesting	3.8%	of the equipments cost

Equipments support technology, investigation	2.3%	of the equipments cost
Equipments for preparing seed meal	30%	of the equipment cost for biodiesel unit
Equipments for detoxification process	45%	of the equipment cost for biodiesel unit
Unexpected expenses/ contingencies (a fraction of basic equipments)	18%	of the equipments cost
Annual maintenance & repaired for fixed assets	3.0%	of the equipments cost
Insurance of the capitals	0.5%	of the equipments cost
Expenses of auxiliary facilities (power, irrigation, roads, signs, etc)	30%	of the equipments cost
Finance		
Short term loan-Interest rate	15%	Payment period <=12 months
Long term loan-interest rate	12%	Payment period 10 years
Power		
Labor wage for land survey and preparation	200	RMB/man-day
Labor wage for other works in seeds production sector	60	RMB/man-day
Labor wage for other works in seeds processing sector	200	RMB/man-day
Salary for fixed staff	3,500	RMB/month/person
Machine work	200	RMB/day
Other variable inputs		
Diesel	5.09	RMB /l
Gasoline	5.63	RMB /l
Coal	350	RMB/t
Electricity from authority	0.49	RMB /kWh
Water from authority	0.11	RMB /m3
Chemicals		
Solvent use for de-fatting seed meal	6.00	RMB /l
Sodium methylate, 25%(w/w) (a standardized base solution)	7.25	RMB /kg

Sodium hydroxide (base-catalyst for transesterification)	4.57	RMB /kg
Methanol (alcohol to make up triglyceride)	2.12	RMB /kg
Hydrochloric acid for product purification (neutralized the un-used base catalyst)	0.98	RMB /kg
Operation		
Inspection from authorities	15.00	RMB/ha
Publications and exchanges for an appraisal	70,000	RMB/total project
Research and training for plantation, during the first 4 years (only for private firm, not for a bidder & authority)	146.25	RMB/ha
Expenses on project management	1%	of the fixed asset cost (except land)
Feedstock and by products		
Wild seeds purchased from households	7.00	RMB/kg, air dried, wt.
Seeds sell to a nursery	4.50	RMB/kg, air dried, wt.
HY seeds used in a nursery	709.22	RMB/kg, air dried, wt.
Scions purchased for a nursery	0.25	RMB/scion
Seedling sell to forestry bureau	0.19	RMB/stem
Seedling sell to a plantation executor (bidder)	0.20	RMB/stem
Seedling produced by own nursery of Petro China	0.50	RMB/stem
Seeds harvested from plantations	1.20	RMB/kg, air dried, wt.
Pruned of small stems & branches	1.00	RMB/kg, air dried, wt.
Hulls, air dried wt.	1.00	RMB/kg, air dried, wt.
Seed meal (pressed kernel after oil is extracted)	1.50	RMB/kg, air dried, wt.
Filtered cake	0.125	RMB/kg
JPPO (filtered)	0.69	RMB/kg
JCL biodiesel	0.20	RMB/kg
Glycerin (80% w/w)	0.17	RMB/kg
Crude protein	0.05	RMB/kg
Others		
Duties and indirect taxes	1.0%	of revenues

Income tax	7.0%	of revenues
Properties tax	0.1%	of fixed asset cost, incl. land

Appendix 3: Production assumptions for a designed seed processing plant

Item	
Seeds selection	
Selected good seeds	0.95
De-hulling	0.05
Mechanical processing	
Oil contents in a good seed with shell	0.42
Seed meal	0.58
Oil recovery rate from cold press (screw press)	0.90
Oil recovery rate from cold press (efficient technology)	0.98
Oil recovery rate from filtering	0.87
Filtered cake	0.13
A transesterification & purification	
Transesterification efficiency	0.98
Biodiesel phase conversion rate from JPPO	0.85
Glycerol (80% w/w) recovered from purification	0.11
Detoxification	
Pre-treatment seed meal	
(1) De-fatted seed meal (with shells)	1
(2) Oil recovered from de-fatting seed meal	0.15
Obtaining protein (oil <1%)	
(1) Crude protein	0.85
(2) Crude oil	0.06
(3) Others and lost	0.70
Deactivate anti-nutritional factors	
(1) Crude protein, toxic freed	1.0
(2) Phorbol Ester	-
(3) Others and lost	-

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