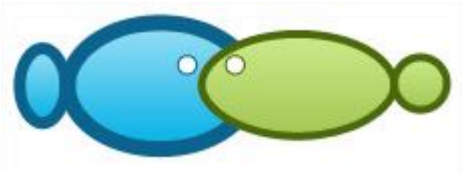

Jatropha curcas kernel meal as a replacement for fishmeal in practical Nile tilapia, *Oreochromis niloticus* feeds

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Abstract. *Jatropha curcas* is an upcoming oil-seed plant with increasing cultivation area each year throughout the tropics. After de-oiling the seeds, a protein-rich meal (JKM) is left behind, which has a similar amino acid composition and content compared to fishmeal. To test JKM as an alternative protein source, a mixed diet was formulated in which 25% of the total dietary protein was derived from fishmeal and the rest from soybean meal, rice bran and wheat meal (control). Three further isonitrogenous and isoenergetic diets replacing 30%, 70% or 100%, respectively, of the fishmeal protein with JKM were produced and fed to juvenile Nile tilapia (*Oreochromis niloticus*) for 8 weeks. There were no significant differences in growth parameters between all treatments containing JKM, however, regression analysis revealed a significant negative correlation of JKM content to final weight and specific growth rate. The 70% and 100% replacement levels showed higher body lipid and significantly lower ash content. JKM is a promising alternative protein source in aquaculture diets for tilapia, though slower growth in this experiment suggests the need for further research to improve the nutritional value of JKM.

Key Words: fish meal substitution, plant protein, tropical biofuel crops, essential amino acids, cichlids.

Introduction. Average global fish consumption has been increasing steadily from around 9.9 kg capita^{-1} (live weight basis) in the 1960s to 18.4 kg capita^{-1} in 2009 (Muir 2013). In developing regions of the world, these values are lower with 9.1 kg capita^{-1} in Africa and 9.9 kg capita^{-1} in Latin America and the Caribbean (Muir 2013), often because supply is lacking due to low production, which in turn is due to low quality feeds. The quality of fish feeds is largely determined by the amount and type of protein included. Fishmeal is considered to be the most suitable protein source for most fish species, however, fishmeal regardless of whether deriving from reduction fisheries or processing of residues from food processing is a finite resource (Tacon & Metian 2008). Therefore, the search of alternative high-quality protein sources for the aqua feed industry is the key to increase high-quality feeds in order to increase fish production in aquaculture to meet the increased demand (Tacon & Metian 2015). The utilization of by-products from other agricultural activities is advantageous, since it does not require additional space.

Jatropha curcas is a tropical oilseed plant of increasing interest to the biofuel industry. After oil extraction, the press cake or *J. curcas* Kernel Meal (JKM) remains. It is high in protein and has a similar amino acid composition to fishmeal, with the exception of lysine, which is lower as in most plant-derived proteins (Makkar & Becker 2009). However, JKM also contains anti-nutrients and toxins such as trypsin inhibitor and phorbol esters (Makkar & Becker 2009). Only recently, a method for detoxifying phorbol esters from the press cake has been patented (Makkar & Becker 2011). Ultimately, the market price of JKM will depend on the feasibility of detoxifying large amounts of press cake, which again will determine the practicability of JKM as a feed ingredient.

To date, most experiments have been conducted with JKM as the sole protein source replacing fishmeal as the sole control protein source (e.g. Kumar et al 2010; Krome et al 2014). In tilapia farming, fishmeal levels in feeds have already been

significantly reduced (Hardy 2010) and it is therefore of greater value to investigate replacement of fishmeal by JKM in feeds with mixed protein sources. The present experiment investigated the extent to which it is possible to replace fishmeal protein with JKM in a practical diet containing 25% fishmeal protein and the remaining protein originating from soybean meal, rice bran and wheat meal.

Material and Method

Experimental diets. JKM was detoxified according to the procedures described by Makkar & Becker (2011). Four isonitrogenous (32% crude protein), isoenergetic diets were formulated with detoxified JKM replacing 30%, 70% and 100% of the 25% fishmeal protein in the control diet. Essential amino acids were added to ensure feeds met requirements (NRC 2011). The nutrient composition of detoxified JKM is shown in Table 1. Pre-experimental in vitro trials had shown 2000 U kg^{-1} phytase (Ronozyme, DSM) to have a maximum effect on JKM-phytate hydrolosis, which is why this concentration was chosen for all diets. Yttrium oxide was added as a digestibility marker.

Table 1

Nutrient (g kg^{-1} DM) and amino acid (g kg^{-1} protein) composition of dietary ingredients

Nutrients	*JKM*	*FM*	*SBM*	*Rice bran*	*Wheat meal*	
Dry matter	947.2	928.4	900.0	910.0	888.7	
Crude protein	598.9	698.0	535.0	154.0	126.0	
Crude lipid	1.2	105.0	33.0	209.0	21.0	
Crude ash	139.3	141.0	81.1	127.5	21.6	
Crude fiber	79.0	0.0	70.0	122.0	111.4	
NFE[1)]	128.8	0.0	282.9	387.5	720.0	
Essential aminoacids	*JKM[2)]*	*FM[2)]*	*SBM[3)]*	*Rice bran[3)]*	*Wheat meal[3)]*	*ΔFM [4)]*
Arginine	104.7	54.1	74.2	54.1	73.5	50.6
Histidine	34.6	27.1	26.8	18.5	33.3	7.5
Isoleucine	40.5	34.9	53.6	32.5	43.6	5.5
Leucine	72.7	63.8	78.4	64.3	78.6	8.9
Lysine	34.2	62.7	46.2	34.4	49.6	-28.5
Phenylalanine	46.1	33.4	55.7	35.7	47.0	12.7
Methionine	20.0	24.5	14.4	13.4	16.2	-4.5
Threonine	37.2	35.3	41.2	28.7	39.3	2.0
Tryptophane	11.4	7.5	14.4	13.4	21.4	3.8
Valine	46.2	44.9	55.7	41.4	59.0	1.3

1) NFE calculated as: 100 - (CP+CL+CA+CF+H_2O); 2) Data determined by Rodehutscord and colleagues at the University of Hohenheim, Germany; 3) Data from NRC (2011). International feed number: SBM: 5-04-612; Rice bran: 4-03-930; Wheat meal: 4-05-199; 4) Difference to fishmeal, calculated as content in JKM minus content in FM; DM = dry matter; FM = fish meal; SBM = soybean meal.

The composition of experimental diets is shown in Table 2. Dietary ingredients were weighed and mixed in a mixer. Distilled water was added to form a paste, which was then passed through a meat mincer and the resulting strands crumbled to pieces of palatable size. The resulting pellets were dried at 45°C for 48 hours in a well-ventilated, heated chamber.

Table 2

Composition and proximate analysis of experimental diets (g kg^{-1} FM)

Ingredient (g kg^{-1})	*Control*	*30%*	*70%*	*100%*
Fish meal	123.0	86.1	36.9	0.0
Wheatmeal	227.2	216.0	204.5	194.2
Soybean meal	365.0	365.0	365.0	365.0
Rice bran	200.0	200.0	200.0	200.0
JKM	0.0	43.0	98.0	140.0
Fishoil	38.0	42.0	46.0	50.0
Vitamin premix[a]	20.0	20.0	20.0	20.0
Mineral premix[b]	20.0	20.0	20.0	20.0
Lysine[c]	1.2	2.2	3.5	4.5
Methionine	2.2	2.4	2.7	2.9
Threonine	1.3	1.3	1.4	1.4
Histidine	1.8	1.8	1.7	1.7
Yttrium oxide	0.3	0.3	0.3	0.3
Phytase (U kg^{-1})	2000	2000	2000	2000
Proximate analysis				
Dry matter	949.3	949.1	959.1	956.0
Crudeprotein	317.1	314.2	308.6	313.8
Lipid	100.7	104.3	101.1	101.7
Ash	91.9	92.2	91.4	89.1
Gross energy (kJ g^{-1})	18.8	19.0	18.8	18.9
Protein: energy (mg kJ^{-1})	16.9	16.5	16.4	16.6

[a] Vitamin premix for fish supplied by Altromin Spezialfutter GmbH. Composition (mg kg^{-1} premix, unless otherwise stated): Vitamin A: 500,000 I.U. kg^{-1}; Vitamin D3: 50,000 I.U. kg^{-1}; Vitamin E: 2,500; Vitamin K3 (as Menadione): 1,000; Vitamin B1: 5,000; Vitamin B2: 5,000; Vitamin B6: 5,000; Vitamin B12: 5; Nicotinic acid: 25,000; Pantothenic acid: 10,000; Folic acid: 1,000; Biotin: 250; Choline chloride: 100,000; Inositol: 25,000; Vitamin C: 20,125.

[b] Mineral premix for fish supplied by Altromin Spezialfutter GmbH. Composition (mg kg^{-1} premix): Calcium: 122,160; Phosphorus: 83,670; Magnesium: 14,960; Sodium: 18,180; Potassium: 210,250; Sulfur: 15,460; Chlorine: 29,720; Iron: 1,220; Manganese: 1,000; Zinc: 1,630; Copper: 155; Iodine: 120.

[c] supplied as lysine hydrochlorid

Experimental design. The feeding trial was carried out from April to June 2012 at the aquaculture lab of Thuenen Institute of Fisheries Ecology, Ahrensburg, Germany. Sixteen (16) tanks with 40 L net volume (4 diets and n = 4 replicates) stocked with four (4) all-male tilapia (initial weight: 4.94±0.29 g, obtained from Kirschauer Aquakulturen, Schirgiswalde-Kirschau, Germany) were set up in a randomized pattern and connected to a recirculation system. Aerated water at 1.5 L min^{-1} at 26°C was supplied to each tank. Water quality parameters (DO level, pH, nitrite, ammonia) were measured weekly and values were below critical levels for tilapia. Photoperiod was 14:10 hours light:dark regime. Feed was supplied at five times the basal requirement, by hand, divided into three feeds per day (08:00, 13:00, and 17:00) for 8 weeks. Amount of feed to meet the basal requirement was calculated according to the following formula:

$$f = (a/1000)^{0.8} * b$$

where, f = amount of feed (g); a = fish weight (g); b = metabolic exponent (g $kg^{-0.8}$). The metabolic exponent is a constant estimating the maintenance ration dependent on fish species and water temperature and was calculated to be 2.83 at 26°C according to Richter et al (2002). Every two weeks, fish were weighed individually and the feed amount was adjusted for the subsequent two weeks. Faeces were collected with a siphon during the last two weeks of the experiment until enough material was obtained for yttrium and nutrient analysis. After collection, the faeces water mix was centrifuged at 3000 g for 5 minutes and the supernatant discarded before storage at -20°C, subsequent freeze-drying and homogenization prior to analysis.

Pre- and post-experimental activities. Before the experiment, 8 fish were sacrificed with ethylene-glycol-monophenylether and stored at -20°C in polyethylene bags. After 8

weeks, all experimental fish were sacrificed in the same manner. For further processing, fish were autoclaved and demineralised water was added. Subsequently, fish were homogenized using an ultra turrax blending device and transferred to a pre-weighed plastic container. The homogenate was frozen and then freeze-dried. After samples were completely dry, they were reweighed and again homogenised in a laboratory grinder (Retsch GM 200) to obtain a fine powder on which protein, lipid, ash and dry matter content analyses were conducted.

Performance parameter calculation and equations. Performance was expressed as specific growth rate (SGR), feed conversion ratio (FCR), body mass gain (BMG), metabolic growth rate (MGR), protein efficiency ratio (PER), protein productive value (PPV), lipid productive value (LPV) and energy productive value (EPV) and were calculated according to the following equations:

$$SGR\ (\%\ d^{-1}) = [(\ln \text{final body mass in g}) - (\ln \text{initial body mass in g})/\text{number of trial days}] \times 100;$$

$$FCR = \text{dry feed fed (g)}/\text{body mass gain (g)};$$

$$BMG = \text{final body mass (g)} - \text{initial body mass (g)};$$

$$MGR\ (g * kg^{0.8}\ d^{-1}) = (\text{body mass gain in g})/[\{(\text{initial body mass in g}/1000)^{0.8} + (\text{final body mass in g}/1000)^{0.8}\}/2]/\text{number of trial days};$$

$$PER = \text{fresh body mass gain (g)}/\text{crude protein fed (g)};$$

$$PPV\ (\%) = [(\text{final fish body protein in g} - \text{initial fish protein in g})/\text{total protein consumed in g}] \times 100;$$

$$LPV\ (\%) = [(\text{final fish body lipid in g} - \text{initial fish body lipid in g})/\text{total crude lipid consumed in g}] \times 100;$$

$$EPV\ (\%) = [(\text{final fish body energy in kJ} - \text{initial fish body energy in kJ})/\text{total energy consumed in kJ}] \times 100$$

Statistics. One-way analysis of variance (ANOVA) and linear regression were used to analyse treatments. The significance level was set to 5% ($p = 0.05$). Tukey HSD test was applied as post-hoc test and percentages were arcsine transformed before analysis. Statistics were conducted with Statistica 8 software. Values are expressed as means± standard deviation.

Results and Discussion. Fish grew well over the experimental period, feed palatability was good and all feed was consumed at all times. Due to there being only four fish per aquarium, combat behaviour was observed leading to mortalities between 6.25 and 31.25% dependent on treatment. When a fish died, it was weighed and feeding rate of the respective fish tank was adjusted immediately. There was no connection between mortality and the extent of fishmeal replacement of the different treatments ($p = 0.73$).

Figure 1 shows the weight development of fish over the experimental period. BMG of the control group was highest and there was a significant negative correlation between SGR of the control diet and treatments 30%, 70% and 100% ($R^2 = 0.29$; $p = 0.031$, Table 3). FCR for the control diet was lower, but no significant difference or correlation could be observed ($R^2 = 0.08$, $p = 0.28$). The same counts for nutrient productive values (Table 3). Crude lipid content of fish fed all JKM-based diets was higher, with the differences of 70% and 100% being significant compared to the control diet. Vice versa, ash content was significantly lower for these treatments. There was a significant, negative correlation of dry matter digestibility from the control diet to the treatment diets.

The results show that it is possible to substitute 100% of fishmeal protein in a mixed fishmeal and plant-based diet with JKM with only limited effects on growth. However, the significant negative correlation of JKM inclusion in terms of SGR shows that JKM does have a negative impact on growth. In previous studies, we saw no difference between diets containing phytase and a control diet without phytase with both diets containing additional phosphate (Krome et al 2013). Spinelli et al (1983) observed reduced amino acid availability in diets containing phytic acid. However, Riche et al (2001), showed that there was reduced amino acid availability of soybean meal based diets in tilapia caused by phytase pre-treatment and attributed this to the removal of phytates. Altogether, it is unlikely that the reduced growth observed in the experiments by Kumar et al (2011) and in the present JKM treatments arose from reduced mineral or amino acid availability caused by phytate. Other antinutritional factors, such as oxalate or left-over trypsin inhibitor or phorbol esters (Francis et al 2001; Makkar & Becker 2009) are more likely to have been the cause.

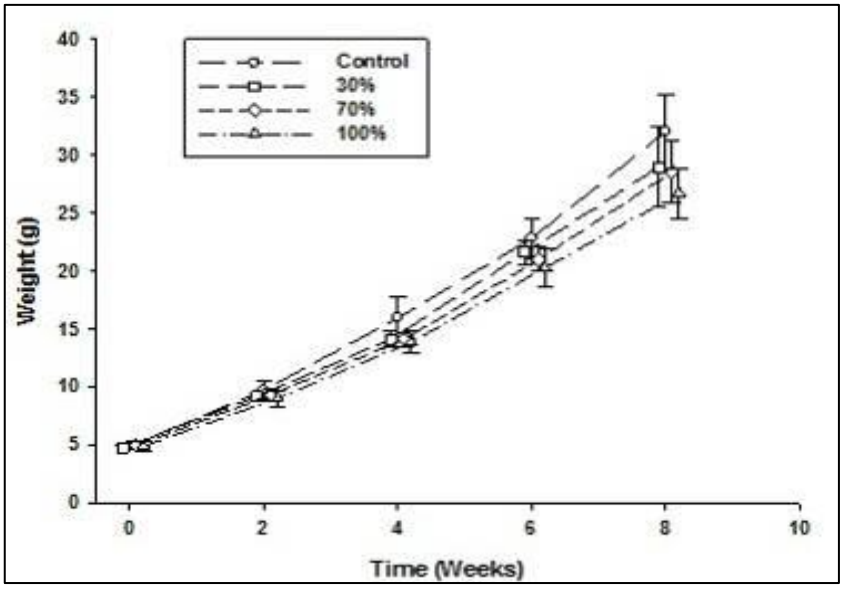

Figure 1. Fish body weight development of experimental fish fed different experimental diets. Error bars represent standard deviation of the mean.

Table 3

Growth performance parameters and nutrient utilization of fish fed experimental diets for 8 weeks

Treatment	*Control*		*30%*		*70%*		*100%*		R^2	*P*
IW	4.96	±0.28	4.83	±0.22	5.01	±0.30	4.96	±0.42		
FW	32.0	±3.23	29.0	±3.50	28.6	±2.65	26.7	±2.15	0.33	0.020
SGR	3.32	±0.27	3.19	±0.26	3.10	±0.17	3.01	±0.07	0.29	0.031
FCR	1.00	±0.20	1.02	±0.15	1.03	±0.09	1.10	±0.06	0.08	0.280
PER	3.23	±0.57	3.18	±0.42	3.16	±0.27	2.89	±0.15	0.11	0.222
PPV	49.3	±6.97	46.9	±7.61	45.8	±6.83	44.6	±2.51	0.09	0.265
LPV	45.9	±12.1	47.0	±3.81	59.1	±16.4	55.1	±8.21	0.16	0.127
EPV	26.3	±4.93	25.7	±3.93	27.2	±5.22	25.9	±1.45	0.00	0.970

IW, initial weight (g); FW, final weight; SGR, specific growth rate (% day^{-1}); FCR, feed conversation ratio; PER, protein efficiency ratio; PPV, protein productive value (%); LPV, lipid productive value (%); EPV, energy productive value (%). R^2: respective values are regressed to the dietary level of JKM.

Dependent on market price of JKM, it might be more economical to dispense with fishmeal entirely despite slightly slower growth shown in this experiment. The market price for JKM is currently unknown and difficult to estimate as the key step towards a useful product is the detoxification process to dispose of toxic phorbol esters and trypsin inhibitor. Studies to identify commercially viable large-scale processes to detoxify JKM

are under way. Furthermore, availability of press cake from *J. curcas* seeds will depend on the future of this plant as an oilseed crop. This depends on the performance and oil yield of *J. curcas* seeds under commercial plantation conditions in arid or semi-arid areas and it is still under discussion whether *J. curcas* is suitable for the task (Openshaw 2000).

Conclusions. As *J. curcas* is likely to play an increasingly important role as an oil-seed crop, its significance as a feedstuff in diets for aquaculture will also increase. This study shows how detoxified JKM can be used in mixed diets for tilapia demonstrating a gradual decline in SGR with higher JKM inclusions, but still showing good growth rates of fish fed on 100% JKM. Further research on JKM as a feedstuff for tilapia should focus on more realistic and if possible long-term pond or net cage trials, possibly incorporating different feed additives to minimize the effects of anti-nutritional factors besides phytate.

Acknowledgements. The project was partly supported by funds of the Federal Ministry of Food, Agriculture and Consumer Protection (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support program. Further funding was granted by the University of Stirling, Stirling, UK. Thanks to the staff of the Thuenen Institute of Fisheries Ecology at Ahrensburg for support in fish and laboratory matters.

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Received: 13 April 2016. Accepted: 22 May 2016. Published online: 07 June 2016.
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How to cite this article:
Krome C., Jauncey K., Focken U., 2016 *Jatropha curcas* kernel meal as a replacement for fishmeal in practical Nile tilapia, *Oreochromis niloticus* feeds. AACL Bioflux 9(3):590-596.