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Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance

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Abstract. Unheated and heated (121 °C, 66% moisture; 15, 30 and 45 min) Jatropha meals of non-toxic provenance from Veracruz state in Mexico were evaluated using rats and fish. With rats, the weight gain was highest for the casein diet followed by heated (30 min; only this treatment was studied using rats) and unheated Jatropha meal containing diets. The protein efficiency ratio (PER) for unheated and heated Jatropha meal containing diets was 37 and 86%, respectively, of the casein diet. On the other hand, the body weight gain, PER and feed conversion ratio of fish were statistically similar for unheated and heated (15, 30 and 45 min) Jatropha meal containing diets fed for a period of 35 days. Although these parameters were statistically similar for the unheated and heated Jatropha meal containing diets, the body weight gain, PER and protein productive value were highest and the feed conversion ratio lowest with 15 min heated Jatropha meal, suggesting that the heat treatment for 15 min is optimal for the meal. Trypsin inhibitor and lectin activities decreased drastically (> 83 and 99%, respectively) after 30 and 45 min of heat treatment and after 15 min, the residual lectin activity was negligible and the residual trypsin inhibitor activity was 34%. These results, together with the nutritional parameters investigated, imply that Jatropha trypsin inhibitors and lectins do not have any adverse effects on carp at least up to 35 days of feeding. The nutritional value of Jatropha meal of the non-toxic provenance is high, and potential exists for its incorporation into the diets of monogastrics, fish and possibly humans.

Key words: Carp, Feed conversion ratio, Fish, *Jatropha curcas*, Lectins, Productive protein value, Protein efficiency ratio, Rats, Trypsin inhibitors

Introduction

Jatropha curcas, a multipurpose tree, is of Mexican and Central American origin. Presently, it is cultivated in large areas in many tropical countries. The genus name *Jatropha* is derived from the Greek *iatrós* (doctor) and *trophé*

(food) which implies medicinal uses. *J. curcas* is also commonly called physic nut or purging nut (English), Purgiernuß (German), purgueira (Portuguese), habel meluk (Arab), kanananaeranda, parvataranda (Sanskrit), bagbherenda, jangliarandi, safed arand, ratanjyoti (Hindi), pinoncillo (Mexico) and tempate (Costa Rica) [1]. *J. curcas* is a small tree or shrub (can reach a height up to 8 m) which is drought-resistant (requires a minimum of 250 mm rainfall per year) and survives in poor, stony soils. It can easily be propagated by cutting or seeding and it grows rapidly. The plant is cultivated mainly to produce oil. A seed yield of about 5 tons per year from a one hectare plantation has been achieved [2]; such yield can produce 2 tons of oil and 1 ton of protein-rich seed meal. Species of the genus *Jatropha* are known to be very toxic. Several cases of *J. curcas* nut poisoning in humans after accidental consumption of seeds have been recorded. Symptoms such as giddiness, emesis and diarrhea have been reported [3]. The oil is toxic but can serve as fuel for diesel engines, indicating its potential as a renewable energy source. The seed meal left after extraction of oil is presently used as a fertilizer and is not suitable for animal feed as it is toxic to fish, monogastrics and ruminants. The main toxic agent has been identified to be phorbol esters [3].

Recently, the nutritive potential and toxic characteristics of different provenances of *J. curcas* were investigated. A mixed sample of seeds obtained from different trees in the Papantla region of Veracruz state in Mexico was found to be non-toxic. Phorbol esters were present in minute amounts in the kernels of this mixed sample of seeds, but trypsin inhibitor, lectin and phytate were present in significant amounts, and their levels were similar to or higher than those in the toxic varieties [4]. In another part of Mexico (Quintana Roo state; approximately 1200 km southeast of Veracruz state), seeds of *J. curcas* are also consumed by humans after roasting as in Veracruz state. These seeds were also free of the main toxic agent, phorbol esters [5]. The nutritive value of meal of the non-toxic provenance, derived from the chemical composition, was similar to that of the toxic varieties and compared well with that of some conventional seed meals [4, 5]. The objective of the present investigation was to evaluate the nutritive quality of meal from seeds obtained from the Papantla region of Veracruz state in Mexico through feeding trials with rats and fish (carp, *Cyprinus carpio*). In addition, this work was also done to provide information on the possible physiological role of lectins and trypsin inhibitor in carp.

Materials and methods

The non-toxic seeds (5 kg) were collected in 1996 from the Papantla region of Veracruz state in Mexico. They were kept in a dark place at room temperature

(approx. 20 °C) until use. Just before the experiment, the seeds were shelled to obtain the kernels. For both the rat and fish experiments, Jatropha meal was prepared by extracting the oil of kernels of the non-toxic seeds in a Soxhlet-type extractor using petroleum ether (40–60 °C). The Jatropha meal was autoclaved at 121 °C for 15, 30 and 45 min at 66% moisture and then lyophilized (Lyovac GT2; Finn-Aqua Santasaio-Sohlberg GmbH, Germany).

Experiments on rats

The experiment procedure and diets were essentially according to the standard method [6]. In brief, 7 Sprague-Dawley male rats, each weighing approximately 70 g, were used per group, with a preliminary period of 4 days and an experimental period of 7 days. The rats were housed individually in plastic cages (length, breadth and height = 50 cm, 30 cm and 22 cm) and provided with tap water and feed *ad libitum*. Body weight and feed intake were measured. Fecal and urine outputs were not measured. The PER was calculated as weight gained over a period of 7 days divided by protein consumed. Casein, unheated Jatropha and heated Jatropha diets were formulated to contain 10% protein from casein or unheated or heated (121 °C, 66% moisture, 30 min) Jatropha meal. The crude protein content of Jatropha meal was 58%.

Experiments on fish

A recirculating system containing a set of aquaria, each with a capacity of approximately 45 liters maintained at 23±1 °C, was used under a photoperiod of 12 hour light: 12 hour dark. Rates of water flow were adjusted to maintain oxygen saturation above 90%. One aquarium containing 5 carp (*Cyprinus carpio* L.) was used per group. The fish were weighed individually on the first day of the experiment and each was marked by 'fin cutting' (a cut at the top, lower, front-right or front-left fin for identification; the fin of 1 fish per group was not cut). The carp were obtained from Bundes Forschungs Anstalt, Ahrensburg, Germany.

A total of 25 fish was divided into 5 groups. The groups fed diets containing unheated Jatropha meal and meal heated for 15, 30 and 45 min were designated as unheated Jatropha, Jatropha 15, 30 and 45 min, respectively. The fifth group was the control group (Table 1). In the control group, fishmeal (powdered; 65% crude protein, Wurttembergische Zentral Genossenschaft, Germany) contributed a major proportion of protein to the control diet (Table 1). In the unheated and heated Jatropha diets, 50% of the fishmeal was replaced by the unheated or heated Jatropha meal. The diets were fed at a level of 16 g/0.8 kg/day divided into 7 portions using an automatic feeder. All aliquots of feeds were immediately consumed by fish. The aquaria were

Table 1. Ingredients and chemical composition of Jatropha meal diets fed to carp

	Control diet	Unheated	Heated (15 min)	Heated (30 min)	Heated (45 min)
Ingredients (%)					
Fishmeal	50	25	25	25	25
Jatropha meal	–	23	23	23	23
Wheat meal	42	42	42	42	42
Soy oil	4	6	6	6	6
Standard vitamin mixture[a]	2	2	2	2	2
Standard mineral mixture[b]	2	2	2	2	2
Chemical composition (% in DM)					
Crude protein	35.7	35.3	35.6	35.6	35.5
Lipid	9.4	9.2	8.9	9.0	9.3
Neutral detergent fiber	4	4.2	4.0	3.9	4.3
Ash	10	9.5	9.0	9.1	9.4
Gross energy (MJ/kg DM)	17.7	17.3	17.8	17.1	17.6

[a]Vitamin mixture (ingredient/g): A(IU) 500; B1, B2, B6 & B12 (μg) 5 each; C (mg) 10; D3 (IU) 50; E (mg) 2.5; K3 (mg) 1; Inositol (mg) 25; Pantothenic acid (mg) 10; Cholinchloride (mg) 100; Niacin (mg) 25; Folic acid (mg) 1; Biotin (μg) 250.
[b]Mineral mixture (mg/g): $CaCO_3$ 336; KH_2PO_4 502; $MgSO_4.7H_2O$ 162.
Source of vitamin and mineral mixture: Alma Pharm. Kempten, Germany.

cleaned every day by syphoning out the debri. Fish were weighed individually once a week by transfering them to a small amount of water tared on an analytical balance and the feed offered was accordingly adjusted every week. After 35 days, the fish were sacrificed and analyzed for crude protein content, fat, energy and ash.

The growth performance of fish was monitored in terms of feed conversion ratio (FCR; g feed consumed/g live wt gain), protein efficiency ratio (PER; g live wt gain/g protein consumed), percent productive protein value (PPV; g body protein gain$*$100/g protein consumed) and percent energy retained (body energy gained$*$100/energy consumed). Using the system described above, it was possible to measure the growth of each fish in a chamber. For calculation of FCR and PER, it was assumed that the feed offered in each chamber was consumed in equal parts by all fish. For determination of PPV and energy retained, the protein and gross energy contents were determined on the combined carcasses of the five fish in a group; the small weight of fish ($<$ approximately 15 g) did not allow determination of body composition of individual fish.

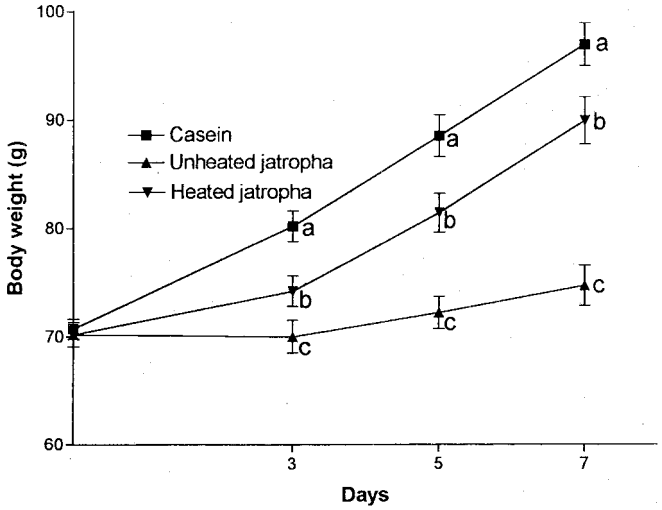

Figure 1. Body weight gain of rats fed diets containing casein, unheated and heated (121 °C, 66% moisture, 30 min) Jatropha meals. The values represented are mean ± s.e (means with different letters differ at $p < 0.05$).

The following assay procedures were used: dry matter (DM), nitrogen (N; Kjeldahl digestion), lipid (Soxhlet extraction), neutral detergent fiber (NDF), gross energy (bomb calorimetry) and ash [7]. Crude protein (CP) was calculated by multiplying N by 6.25. Lectin was determined using the haemagglutination method as described by Aregheore et al. [8] and trypsin inhibitor activity by the method of Smith et al. [9] as described by Aderibigbe et al. [10].

The significance of differences between means was compared using Least Significant Difference test after ANOVA for one-way classified data. A level of $p < 0.05$ was chosen as the minimum for significance. Values expressed are mean ± s.e.

Results and discussion

Rats

Figure 1 data show changes in body weight of rats fed the three diets over a period of 7 days. The growth rate was highest with the casein diet followed by diets containing heat-treated and unheated Jatropha meals. A similar pattern was observed for PER. The PER of the diet containing unheated Jatropha meal was 1.29 ± 0.28 but 3.52 ± 0.48 for the casein group. The heat treatment increased the PER to 3.02 ± 0.31. At the end of the experiment, the average

Table 2. Lectin and trypsin inhibitor activity of unheated Jatropha meal and Jatropha meal heated for different lengths of time (15, 30 and 45 min) at 121 °C and 66% moisture

	Unheated Jatropha meal	Heated Jatropha meal (15 min)	Heated Jatropha meal (30 min)	Heated Jatropha meal (45 min)
Lectin[a]	51	1.2	0.21	nd
Trypsin inhibitor activity[b]	24.8	8.3	4.2	1.3

nd, not detected.
[a] as reciprocal of the minimum quantity (in mg) of the meal per ml of the assay which produced haemagglutination.
[b] as mg trypsin inhibited per g.

body weight of the rats fed unheated and heated Jatropha meal containing diets was 23 and 7% lower than those fed the casein diet. Feed intake of the diet containing heated Jatropha meal did not differ significantly compared to the casein diet, but the intake of unheated Jatropha meal diet was 21% lower than noted in the animals fed the casein diet. The reduced growth of rats eating the diet containing heat-treated Jatropha meal could not be due to lower feed intake but to lower protein utilization. However, for rats consuming the diet containing unheated Jatropha meal, the reduced body weight gain was due to both reduced intake and poor protein utilization. The higher feed intake and protein utilization in animals fed heated Jatropha could be due to inactivation of trypsin inhibitor and lectin by heat processing (121 °C for 30 min and 66% moisture) used in the treatment of the Jatropha meal (Table 2). The better utilization of protein could also be due to unfolding of proteins under the conditions of the heat treatment making them more susceptible to proteolytic hydrolysis [10] and, hence, leading to better utilization.

Fish

The changes in body weight of fish fed the five diets are shown in Figure 2. Unlike rats, the growth rates of fish fed diets containing heated Jatropha meal were lower than for the unheated Jatropha meal group; the growth rate decreased with increase in time of heat treatment, although the mean body weight did not differ between the groups fed diets containing 15, 30 or 45 min heat treated meals at both 21 and 28 days. However, at 35-d the body weights of the fish fed the 45 min heat treated Jatropha meal containing diet were significantly lower than those of the 15 and 30 min heated Jatropha groups. After 30 and 45 min of heating Jatropha meal, lectin and trypsin inhibitor activities were reduced drastically (Table 2). After 15 min of heating, the residual lectin activity was negligible and the residual trypsin inhibitor activity

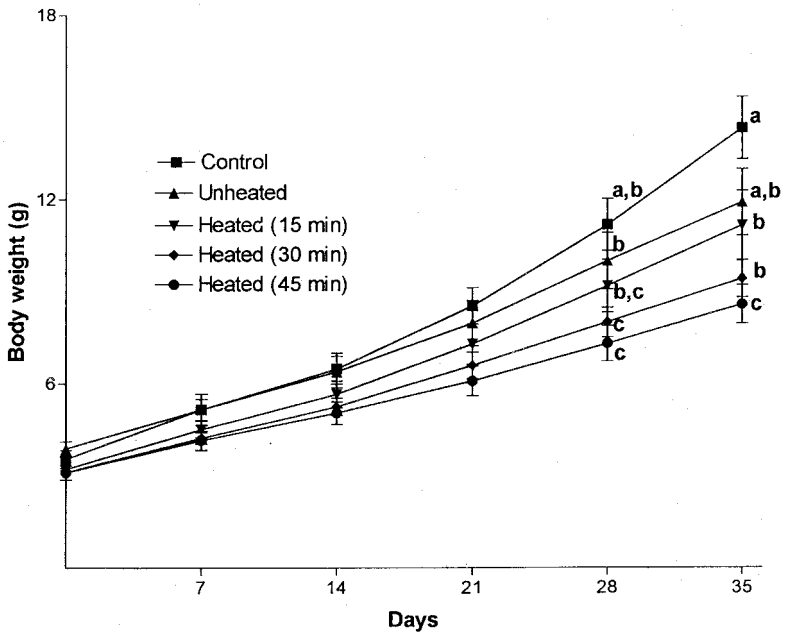

Figure 2. Body weight gain of fish (carp) fed control, unheated and heated Jatropha meal containing diets. The values represented are mean ± s.e (means with different letters differed at $p < 0.05$; the significance of difference for means at 21-d was similar to that at 28-d, and at 14-d both control and unheated were significantly different from 30 min heated only; rest were statistically similar).

was 34%. Also of note was the lower but statistically similar body weights of the unheated Jatropha meal fed group compared to the control, suggesting no physiological relevance of heat-labile factors such as lectins and trypsin inhibitors present in Jatropha meal and of heat-stable factors such as antigenic proteins, if any, for carp.

Trypsin inhibitor activity in Jatropha meal is of the same order of magnitude as in soybean meal [4]. Trypsin inhibitors and lectins are known to decrease the weight gain performance of animals [11]. It appears that carp are not sensitive to the effects of Jatropha lectins and trypsin inhibitors. The larvae of common carp also do not appear to be susceptible to purified trypsin inhibitor [12], while many other fish species are considered to be sensitive to trypsin inhibitors [13–17]. The studies by Abel et al. [18] and Viola et al. [19] suggested that carp are sensitive to trypsin inhibitors. However, in those studies, the fish were of higher body weight than those used in the present study or in that by Escaffre et al. [12]. Carp are stomach-less fish and digest protein only by tryptic-chymotryptic digestion. It is tempting to speculate that in the early stages of growth carp might have some mechanisms to counteract

Table 3. Weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) of carp fed the control, unheated and heated Jatropha meal containing diets

	Control diet	Unheated	Heated (15 min)	Heated (30 min)	Heated (45 min)
Initial wt (g)	3.6 ± 0.29 a	3.9 ± 0.23 a	3.2 ± 0.15 a	3.1 ± 0.12 a	3.1 ± 0.23 a
Final wt (g)	14.3 ± 1.0 a	11.9 ± 1.08 a,b	11.2 ± 1.14 b	9.4 ± 0.61 b	8.6 ± 0.63 c
Weight gain (%)	303 ± 28 a	201 ± 14 b,c	243 ± 23 b	201 ± 13 b,c	178 ± 14 c
FCR	1.0 ± 0.09 a	1.34 ± 0.19 a,b	1.24 ± 0.16 a,b	1.40 ± 0.14 b	1.56 ± 0.16 b
PER	2.91 ± 0.24 a	2.23 ± 0.24 a,b	2.42 ± 0.31 a,b	2.08 ± 0.17 b	1.88 ± 0.18 b
PPV (%)	39.5	30.5	33.0	28.4	26.9
Energy retained (%)	30.8	24.6	25.5	21.4	22.7

Values are mean ± s.e. (n = 5).
Means with different following letters in a row differ at $p < 0.05$.

Table 4. Body composition of fish (carp) fed the control, unheated and heated Jatropha meal containing diets for 35 days and at the start of the experiment

	After 35 days					Start of the experiment
	Control diet	Unheated	Heated (15 min)	Heated (30 min)	Heated (45 min)	
Crude protein (% in DM)	62.8	62.6	64.3	64.4	61.8	61.8
Fat (% in DM)	25.7	25.4	25.5	25.2	27.4	22.5
Ash (% in DM)	9.5	9.7	8.3	8.3	9.1	12.8
Gross energy (MJ/kg DM)	23.9	23.9	24.3	23.2	24.6	21.9

Each analysis was conducted in duplicate from one pooled sample.

higher levels of trypsin inhibitors compared to later stages of development. The occurrence of this mechanism could be essential for survival of carp at early stages of growth. It would be worthwhile to test this hypothesis. Little is known about the role of lectins in fish nutrition, which also needs to be further investigated.

The heat treatment of Jatropha meal had adverse effects on the weight gain performance of carp. A similar conclusion can be made from the FCR, PER and PPV (Figure 2, Table 3). The lower PER and PPV of the 30 and 45 min heated Jatropha groups compared to those of the unheated group imply loss of amino acids and their lower availability due to Maillard reaction products and/or heat-induced changes in the structure of Jatropha proteins which are less susceptible to digestion by fish trypsin. The energy retained was also

lower in the 30 and 45 min heated Jatropha groups (Table 3). Heat treatment has been shown to increase protein digestibility of Jatropha protein by rumen proteases [10]. Among the various time intervals of heat treatment studied, the best utilization of Jatropha protein in carp appears to be achieved with heat treatment for 15 min (Table 3). The body compositions of control, unheated and heated (15, 30 and 45 min at 121 °C) Jatropha groups after 35 days of feeding were almost similar (Table 4). There was a tendency for lower crude protein and higher fat contents in the carcasses of the 45 min heated Jatropha group (Table 4). A statistical analysis of these samples was not possible since the composition was determined from a pooled sample of all five fish per group.

A good amino acid composition of Jatropha meal [4] and the results from the present study show that the biological value of Jatropha meal protein is high. For rats, the availability of proteins from the unheated Jatropha meal was low, but heat processing increased the availability. On the other hand, for fish (carp), the availability of protein from the unheated Jatropha meal was higher. Heat treatment of Jatropha meal decreased the weight gain performance of carp. The potential exists for incorporation of Jatropha seed meal in the diets of monogastrics, fish and possibly humans. In addition, oil from the non-toxic Jatropha seeds could be a potential edible oil for humans. Supplementation of Jatropha meal with lysine, the only deficient amino acid, is likely to increase the nutritive value of the meal. This study also shows that different animal species can behave completely differently to a diet and that results obtained using one animal species should be extrapolated to another species with great caution.

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