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Biofuels and Industrial Products from *Jatropha curcas*

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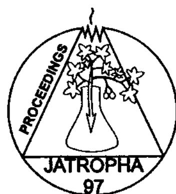

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5.2 Potential of *J. curcas* Seed Meal as a Protein Supplement to Livestock Feed, Constraints to its Utilisation and Possible Strategies to Overcome Constraints

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Abstract

Studies in our laboratory have shown that *J. curcas* seed meal (with 1 - 2 % residual oil) has 58 - 64 % crude protein (90 % of which is present in the form of true protein) and levels of essential amino acids except lysine are higher than for the FAO reference protein. However, the seed meal from the Cape Verde and Nicaragua varieties has been found to be highly toxic to fish, rats and chickens while seed meal from a Mexican variety was not. Although the growth rate of fish fed a diet containing *Jatropha* seed meal from the non-toxic variety for 7 days (50 % of fishmeal protein replaced by *Jatropha* meal) did not differ significantly from that of the control group, appearance of mucus in faeces was observed. The crude protein content and amino acid composition of this non-toxic variety were similar to those of the toxic Cape Verde or Nicaragua variety. In addition, in experiments with rats the protein efficiency ratio of the feed containing heat-treated seed meal from the non-toxic variety was found to be about 86 % of that obtained with casein. These observations suggest that both toxic and non-toxic varieties can be good protein sources for livestock. However, the seed meal from *Jatropha* varieties must be detoxified. The feeding of unheated meal from the non-toxic variety might have sub-clinical effects which could reduce the performance of animals when fed a diet containing this seed meal for a longer period of time. Some constraints identified by us which could restrict the optimum utilisation of seed cake from both the toxic and non-toxic variety are: very high levels of trypsin inhibitor activity (21 - 27 mg trypsin inhibited/g DM), lectin (51 - 102 when expressed as inverse of minimum concentration in mg of *Jatropha* meal per ml of the assay which produced hemagglutination) and phytate concentration (9 - 10 %). Saponins were also present at a level of 2.6 -3.4 % (as diosgenin equivalent). Phorbolesters were present in kernels of the toxic variety (2.2 - 2.7 mg/g) but virtually absent in the Mexican variety (0.11 mg/g). Tannins, cyanogens, amylase inhibitors and glucosinolates were not detected in any of the varieties. Trypsin inhibitors and lectins are heat labile and can therefore be destroyed by heat treatment. The unheated seed cakes from both the toxic and non-toxic variety were found to have low *in vitro* rumen degradable nitrogen ($IVRDN_{24h}$). The $IVRDN_{24h}$ after heat treatment increased almost 2-fold (38 to 65 %). Heat treatment for the Mexican variety and a combination of heat and chemical (NaOH and NaOCl) treatments or extraction with 80 - 90 % aqueous ethanol or methanol for toxic varieties hold promise for detoxification of *Jatropha* meals.

Introduction

J. curcas, a member of the Euphorbiaceae family, is a multipurpose tree of significant economic importance because of its several industrial and medicinal uses. *Jatropha* grows throughout most of the tropics. It survives on poor stony soils and is resistant to drought, reaches a height from 3 m to 8 m and can be used to reclaim land. The seed weighs approximately from

0.53 - 0.86 g and its kernel contains 22 - 27 % protein and 57 - 63 % lipid [2, 15, 18] indicating good nutritional value. However, the seed and oil have been found to be toxic to mice [1], rats [15], calves, sheep and goats [4], humans [19] and chickens [9, 10]. Hence, its use as a food or feed source is presently limited. The oil from these seeds can serve as fuel for diesel engines, indicating its potential as a renewable energy source [12]. The potential impact is immense for countries with no indigenous fossil fuel or for regions remote from a source of supply. Furthermore, use of biodiesel is friendly to the environment. The seeds can be transported without deterioration and at low cost due to its high specific mass. These features have generated interest in the *Jatropha* plant which is now becoming a cash crop in South and Central American countries.

In our laboratory, we have embarked on studies: i) to identify the toxic principle(s) in toxic varieties by comparison with the non-toxic Mexican variety, ii) to compare the nutritional value of the non-toxic and toxic varieties, iii) to detoxify and enhance the nutritive value of the seed meal from both the toxic and the non-toxic varieties in an effort to produce high quality livestock feed from a toxic, non-edible by-product, iii) to study performance of animals on diets containing *Jatropha* meals and iv) to promote the collection of non-toxic varieties of *Jatropha*, confirm their non-toxic nature with proper experimental model animals, compare their quality in terms of oil, organic matter digestibility, metabolizable energy, protein and amino acid composition with that of already existing toxic varieties and to propagate their cultivation in different climatic zones. Results on these aspects are presented.

Results and Discussion

Nutritional potential

Physical characteristics

The average fruit of a provenance from Ife, Nigeria weighed 2.1 g with a 71 : 29 (w/w) seed to husk ratio. Thus, the seeds formed a large proportion of the fruit. The seed weight of the 18 different seed provenances was 0.64 ± 0.10 g. The variation in weight of kernel as per cent of seed weight (53.9 - 64 %; 61.3 ± 3.1 % , mean ± sd) was not as large as for the seed mass [18]. The kernel forms a large proportion of the seed. It is noteworthy that the kernel to shell ratio for cultivated varieties (Cape Verde and Nicaragua) was the same (62.7 : 37.3) and that of the non-toxic provenance from Mexico was 63.5 : 36.5.

Chemical composition

The chemical composition of kernel and shell of *Jatropha* varieties is shown in Tab. 1. *Jatropha* kernel is composed mainly of lipid and protein, with very little moisture and ash. There were varietal differences in the crude protein (CP) content of the kernels (22 - 27 %). A seven-year-old seed had a similar kernel : shell ratio (63 : 37) and similar contents of CP (25.6 %), lipid (57 %) and ash (3.4 %) in the kernel as those observed for fresh seeds. The low moisture content of the kernel (< 6 %) and shell (< 10 %) could be partly responsible for the non-deterioration of seeds over a long period. The presence of antinutritional factors / toxins is also likely to increase the shelf-life of the seeds. The shell of *Jatropha* seed is composed mainly of fibre (> 83 % neutral detergent fibre (NDF)). The high acid detergent lignin, ADL (> 45 %) and very low protein (< 5 %) contents in the shell indicate its poor nutritional value. However, the shell can be a good source of fuel as it has high gross energy.

Tab. 1: Chemical composition of kernel and shell of *J. curcas* varieties

	Variety					
	Cape Verde		Nicaragua		Non-toxic Mexico	
	Kernel	Shell	Kernel	Shell	Kernel	Shell
Dry matter (%)	96.6	90.3	96.9	90.4	94.2	89.8
Constituents (% in DM)						
Crude protein	22.2	4.3	25.6	4.5	27.2	4.4
Lipid	57.8	0.7	56.8	1.4	58.4	0.5
Ash	3.6	6.0	3.6	6.1	4.3	2.8
Neutral detergent fibre	3.8[a]	83.9	3.5[a]	85.8	3.8[a]	89.4
Acid detergent fibre	3.0[a]	74.6	3.0[a]	75.6	2.4[a]	78.3
Acid detergent lignin	0.2[a]	45.1	0.1[a]	47.5	0.0[a]	45.6
Gross energy (MJ/kg)	30.7	19.3	30.5	19.5	31.1	19.5

[a] Calculated from values obtained for fat free samples since high lipid content interferred with fiber determination even after alpha amylase treatment.

The chemical composition of meals of the three varieties of *Jatropha* and soybean meal are shown in Tab. 2. The CP contents of meals varied from 56.4 % in the Cape Verde to 63.8 % in the non-toxic Mexican variety, which are both higher than that of commercial soybean meal (Tab. 2). The higher ash content of *Jatropha* meal than soybean meal (10 % vs. 6.4 %) suggests that *Jatropha* meal contains more minerals compared to soybean meal. The gross energy content of degreased *Jatropha* meals was similar to that of soybean meal. These data show that *Jatropha* meal contains very good nutrient profile, comparable to soybean meal but with a higher CP than soybean meal.

Tab. 2: Chemical composition (% in DM) of meals of *J. curcas* varieties and soybean meal

	Variety			
	Cape Verde	Nicaragua	Non-toxic Mexico	Soybean[a]
Crude protein	56.4	61.2	63.8	45.7
Lipid	1.5	1.2	1.0	1.8
Ash	9.6	10.4	9.8	6.4
Neutral detergent fibre	9.0	8.1	9.1	17.2
Acid detergent fibre	7.0	6.8	5.7	12.2
Acid detergent lignin	0.4	0.3	0.1	0.0
Gross energy (MJ/kg)	18.2	18.3	18.0	19.4

[a] Source, Soya Mainz GmbH and Co KG, Dammweg 2, Mainz, Germany.

Buffer-soluble nitrogen (BSN) and non-protein nitrogen (NPN)

The BSN and buffer soluble NPN in different varieties ranged from 7.4 - 8.0 g CP/100 g DM and 4.7 - 5.0 g CP/100 g DM respectively. The NPN represented about 62 - 64 % of the BSN. Only 7.8 - 9.0 % of the total nitrogen in the *Jatropha* meals was as NPN suggesting the presence of a high level (ca. 90 %) of true protein, which is comparable to that for soybean, sunflower and rapeseed meals. The NPN content of degreased seed meals of jojoba, soybean, sunflower and rapeseed were 21 - 30 %, 2.9 - 7.8 %, 5.0 % and 6.9 %, respectively [29].

total N) suggest that *Jatropha* meals have a significant amount of rumen bypass protein which will be available to animals post-rumen for production purposes. The $IVRDN_{24h}$ for soybean meal (80.9 %) was much higher than for *Jatropha* meals.

Tab. 4: Digestible organic matter (DOM), metabolizable energy (ME) and 24 h in vitro rumen degradable nitrogen (IVRDN24h) of meal of *J. curcas* varieties and soybean meal

	Variety			
	Cape Verde	Nicaragua	Non-toxic Mexico	Soybean meal
DOM (%)	78.0	78.0	77.3	87.9
ME (MJ/kg DM)	10.9	10.7	10.7	13.3
$INRDN_{24h}$ (%)	43.3	37.7	28.9	80.9

Toxic / antinutritional components

Antimicrobial components in *Jatropha* meal

The effect of a different mixture of meal (Cape Verde variety) and hay on 24 h gas production was studied using an *in vitro* rumen fermentation system containing rumen microbes [20]. Gas production for the *Jatropha* meal was similar to that for the hay sample, and the gas production for the mixtures (meal plus hay) was almost additive of the gas production from individual components (meal and hay), indicating that the presence of *Jatropha* meal had no adverse effect on the fermentation of hay. The *Jatropha* meal seems to be free of anti-fermentative factor(s).

Phorbolesters

Phorbolesters were present in high concentrations in kernels of toxic seeds whereas a very low amount of phorbolesters was observed in those of the non-toxic variety (Tab. 5). The oil from the non-toxic variety is expected to have traces of phorbolesters (see below). Therefore, long term toxicological studies on feeding diets containing oil from this non-toxic *Jatropha* variety must be conducted on rats or other laboratory animals before it can be recommended for human consumption. Phorbolesters have been found to be responsible for purgative, skin-irritant effects and tumor promotion since they stimulate protein kinase C which is involved in signal transduction and developmental processes of most cells and tissues [3, 13]. Ingestion of plants from the Euphorbiaceae and Thymelaeaceae families that biosynthesise diterpene esters of the phorbol type cause severe symptoms of toxicity in livestock [14]. The results of the present study suggest that toxicity of *Jatropha* seeds could be attributed to phorbolesters present in toxic varieties. After roasting, the seeds of the non-toxic variety are consumed as peanuts by humans in Mexico without any apparent adverse effects, suggesting that the body's defence system can detoxify the low amounts of phorbolesters present in the non-toxic variety. However, systematic epidemiological studies need to be conducted to establish relationship, if any, between occurrence of cancer and consumption of these seeds, which might reveal the role of consumption of these low levels of phorbolesters in causing cancer.

Phorbolesters were also determined in two seed samples, designated as NC 7 and NC 9, collected from Quintana Roo in Mexico. The seeds of both NC 7 and NC 9 are consumed by both humans and chickens but NC 9 is consumed very frequently and NC 7 less so. Phorbolesters in NC 7 and NC 9 were 0.093 and 0.03 mg/g kernel. These observations indicate that acceptance of *Jatropha* seeds as food or feed is affected by the content of phorbolesters. The higher the

phorbolesters, the lower the acceptance of *Jatropha* seeds. The immature seeds (greenish yellow stage) of NC 9 had a higher level of phorbolesters (0.15 mg/g kernel) suggesting higher toxicity of immature seeds as compared to mature seeds. It may be noted that the content of phorbolesters in the non-toxic variety from Mexico was 0.11 mg/g kernel. The contents of phorbolesters appear to vary from provenance to provenance, and therefore there is a need to promote the collection of non-toxic varieties of *Jatropha*.

The concentration of phorbolesters in degreased meal was 1.81 mg/g DM. The level of phorbolesters in kernels was 2.7 mg/g DM and the oil content in kernels 58 %. Using these values, it can be calculated that about 72 % of the total phorbolesters get extracted with the oil using petroleum ether (bp 40 - 60°C) and the remaining 28 % is still present in the degreased meal which is almost free of oil (lipid content was < 0.8 %). This shows that a significant amount of phorbolesters are present in the degreased meal. This is an important observation as it does not support the generally accepted view that all phorbolesters are extracted with the oil fraction. The presence of this low amount of phorbolesters in *Jatropha* meal could have a significant effect on the non-acceptance of a diet containing *Jatropha* meal by animals or in causing toxicity. The phorbolester content of oil from the toxic and the non-toxic variety was found to be 2.49 and 0.27 mg/ml respectively.

Tab. 5: Some antinutritional / toxic factors in meal of *J. curcas* varieties and soybean meal

	Variety			
	Cape Verde	Nicaragua	Non-toxic Mexico	Soybean meal[a]
Phorbolesters[b] (mg/g kernel*)	2.70	2.17	0.11	---
Lectin [1(mg meal/ml assay which produced hemagglutination)]**	102	102	51	0.32
Trypsin inhibitor activity (mg trypsin inhibited/g meal)**	21.3	21.1	26.5	3.9
Phytate (% in meal)**	9.4	10.1	8.9	1.5
Saponins (% diosgenin equivalent)**	2.6	2.0	3.4	4.7

* DM content of kernel 96.6 %; ** on dry matter basis; [a] heat treated; source: Soya Mainz GmbH and Co KG, Dammweg 2, Mainz, Germany; [b] equivalent to phorbol-12-myristate-13-acetate

Lectins

Lectin activity was higher in *Jatropha* meals than in soybean meal. Soybean meal used in the present study was heat treated which could have lowered lectin activity. It would be interesting to compare the lectin values of *Jatropha* meal with unheated soybean meal. Amongst the *Jatropha* meals, lectin activity was lower in the non-toxic Mexican variety compared to other meals. The lectin assay used is based on hemagglutination of serially diluted extracts with two-fold increments of the sample and the sensitivity of this assay is ± 1 dilution. This implies that values of 51 and 102 obtained using the hemagglutination method (Tab. 5) are separated by only one dilution and therefore not much different from each other. Similar results were observed using a latex agglutination method [6]. Toxicity of *Jatropha* seeds is generally attributed to the presence

of lectin in these seeds [8, 21, 27]. But almost similar lectin values in the non-toxic Mexican and toxic (Cape Verde and Nicaragua) variety do not support this assumption. Lectins of *Jatropha* do not seem to be responsible for 'short-term' toxicity but may enhance toxic effects in combination with other toxins like phorbolesters.

Trypsin inhibitors

The trypsin inhibitor activity (TIA) of *Jatropha* meals ranged between 21.1 mg/g in the Nicaraguan to 26.5 mg/g in the non-toxic variety (Tab. 5), indicating varietal differences. These were higher than the TIA of 3.9 mg/g in soybean meal because the latter had been heat treated. Smith et al. [26] reported TIA (using the same method as in the present investigation) of 18.6 to 30 mg/g for raw soybean meals. It is known that consumption of unheated soybean meal produces adverse effects in monogastrics [11, 28]. It is clear from the above observations that TIA in *Jatropha* meal is high and is of the same order as in raw soybean meal, which could cause adverse physiological effects in monogastrics.

Phytate

The phytate content of *Jatropha* meals varied between 8.9 % in the non-toxic to 10.1 % in the Nicaraguan variety (Tab. 5). These values were extremely high especially when compared with 1.5 % in soybean meal. In addition, soybean meal is considered to have high a phytate content. These indicate that phytate constitutes a major heat-resistant antinutritive component in *Jatropha* meals, the consumption of which can decrease the bioavailability of minerals, particularly of Ca^{2+} and Zn^{2+}. Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interacting with enzymes such as trypsin and pepsin [23].

Saponins

The saponin content (as diosgenin equivalent) for soybean meal was higher than for *Jatropha* meals (Tab. 5). Saponins from some plants produce adverse and from others beneficial effects [16, 22]. Saponins from soybean are relatively innocuous [16]. Saponins do not appear to play an important role in eliciting toxicity as the saponin content of the non-toxic variety was higher than for the toxic varieties.

Amylase inhibitor, cyanogens and glucosinolates

These were not detected (results not shown) in any of the samples investigated.

Tannins

A negligible amount of total phenols was found in *Jatropha* meals (0.29 to 0.36 %) and husk (0.19 %). Tannins and condensed tannins were not present in the meals and husk. *Jatropha* shell contained 2.8 to 4.4 % total phenols and 2.0 to 2.9 % tannins.

Effect of heat treatments on nutritive value and antinutritional factors in meal

The effect of several heat treatments (moist: 100° C, 66 % moisture; 130 and 160°C, initial moisture 80 %, dry: 130 and 160°C, combination of moist and dry heating: 100°C, 66 % moisture, 60 min followed by dry heating at 160°C) on nutritive value and deleterious components of partially degreased (PD, about 23 % oil residual) and degreased (D, about 1.5 % residual oil) *Jatropha* meals was studied in our laboratory. Moist heating (66 % moisture) at 100°C for 60 min increased $IVRDN_{24h}$ of PD and D meals to 66.5 and 67.4 % respectively, decreased TIA to about 5 mg trypsin inhibited/g meal but had no effect on lectin activity while moist heating (80 % initial moisture) at 130°C for 30 min increased $IVRDN_{24h}$ of PD and D meals to 55.2 and 73.3 %,

respectively, decreased TIA to about 4 mg trypsin inhibited/g sample and completely inactivated lectin activity (as measured by hemagglutination and latex agglutination assays). Dry heating at 160°C for 120 min decreased $IVRDN_{24h}$ to 23.7 % for PD meal and to 38.6 % for D meal, due to generation of Maillard products. The TIA of these treated meals was about 4 mg trypsin inhibited/g sample. Presence of oil was found to decrease the effect of heat in increasing $IVRDN_{24h}$ and inactivating trypsin inhibitors. The levels of phytate and saponins did not decrease by any of the treatments studied. The level of phorbolesters decreased by only approximately 5 %. The pepsin insoluble nitrogen in the moist heated samples was only 4 to 6 % of the total nitrogen and that in the dry heated samples was from 8 to 15 %. The calculated DOM, ME and $IVRDN_{24h}$ for D *Jatropha* meal following heat treatment (80 % initial moisture, 130°C, 30 min) were: 82.9 %, 11.8 MJ/kg and 73.3 % respectively which were lower by 5 % units, 1.5 MJ/kg and 7.6 % units respectively compared to soybean meal [2]. Heat treatments increase the nutritive value of *Jatropha* meal by increasing the DOM, ME and $IVRDN_{24h}$, and by inactivating TIA and lectin.

In vivo studies

Untreated Jatropha meal and oil from the Cape Verde toxic variety

Fish

A diet containing untreated *Jatropha* meal (50 % of the diet) was fed to fish (carp, *Cyprinus carpio* L and tilapia, *Oreochromis niloticus*). Mortality was not observed, although various undesirable symptoms such as loss of balance, nervousness, striking against wall of the chambers, which are typical effects arising from adverse action on the nervous system, were observed. In addition, white spots on skin and heavy peeling of skin were also observed.

A 5 % (w/v) extract of *Jatropha* meal (residual oil 1 %) from the toxic variety (Cape Verde) was prepared in distilled water and after filtration, 280 ml of this extract was added at 1500 h to a chamber containing 20 litres water (continuously flushed with air) which killed all five fish of average body mass of 3.5 g overnight. Up to 1600 h the fish consumed feed but on the next day at 0900 h all fish were found dead with hemorrhagic spots near anus and near the lower gills of all the fish. An extract of *Jatropha* meal from the non-toxic variety prepared in the manner mentioned above for the toxic meal did not have any such effect. These results show the presence of water soluble toxin(s) in the *Jatropha* meal besides oil soluble toxin(s) (see below). Saponins are soluble in water but almost the similar levels of saponins in the toxic and non-toxic variety (Tab. 5) and similar nature of saponins (both varieties showed six Libermann-Burchard reagent positive spots; Rf of 0.11, 0.20, 0.32, 0.39, 0.46 and 0.50 in $CHCl_3 : H_2O : MeOH :: 60 : 18 : 55$) suggested that the death of fish cannot be attributed to saponins. Hemorrhagic spots could be due to lectins but again the lectin activity of the toxic variety was slightly higher than that of the non-toxic variety. Further studies should be directed towards identification of the water-soluble toxin present in *Jatropha* meal and the possible role of lectins in inducing death in fish. It may be of interest that the extract of the toxic variety prepared in the manner described above had a brownish tinge whereas that of the non-toxic variety was white. Similarly, the residue left after filtration of extract from the toxic *Jatropha* meal also had a brownish tinge. A brownish colour is generally attributed to the presence of tannins but tannins were not present in *Jatropha* meal. The addition of *Jatropha* meal extract to water in chambers with fish (carp) could possibly be used as a bioassay for distinguishing a toxic from a non-toxic variety and could also be of immense use in identifying water-soluble toxin(s) present in the toxic *Jatropha* kernels. Further studies are required on these lines. It may also be noted that although the fish which were exposed to the extract from the non-toxic variety for 18 h did not die, their feed intake was lower for about

two days and a substantial feed refusal was observed. This observation suggested that the extract from the non-toxic variety also has a water-soluble toxin(s), at a level lower compared to in the extract from the toxic variety. Longer duration of exposure of the extract from the non-toxic variety to fish or an extract of a concentration > 5 % might kill fish.

In our another study, oil from the toxic variety was extracted five times with methanol (oil : methanol :: 1 : 2), methanol was removed under vacuum at room temperature and the residual fraction (oil-like) was mixed to a standard fish feed to give phorbolesters content of 2.5 mg/g DM feed. This feed was fed, to five fish starved for 24 h with an average body mass of 8 g, at a level of 5-time maintenance, split into 7 portions per day. The fish consumed all feed on the first day and approximately one hour after consumption of the first two portions of the feed, mucus in the form of a fine tubing as if the intestinal cell lining has come off was observed. These tubings seemed to be filled with feed in the form of a slurry, suggesting membrane irritating and purgative effects toxin(s). Thereafter, rejection of feed started. The chamber was cleaned every day to remove mucus and the rejected feed. Release of mucus in the form of a thin tubing continued for about 5 days, thereafter release of mucus stopped probably due to complete rejection of feed. Fish were sluggish and had a tendency to remain together. Symptoms such as nervousness and striking against the chamber wall were absent. After 7 days of feeding period, the average body mass of fish in the control and treated group was 10.8 g (gain of 35 %) and 7.3 g (loss of 9 %) respectively. These results show the presence of a methanol-soluble toxin(s) in oil.

In order to unequivocally decide the role of phorbolesters in causing toxicity, we collected phorbolesters after separation from the HPLC column. The phorbolesters were present in acetonitrile and water mixture. Acetonitrile was removed at room temperature, in dark, by application of vacuum and the remaining solution was mixed in ground standard fish feed. The feed was pelleted, lyophilised and fed to fish (carp) with an average body mass of 5.0 g, at a level of 5-time maintenance for 7 days, split into 7 portions a day. The concentration of phorbolesters in the fish feed was 2 mg/g. The typical symptoms of *Jatropha* toxicity in fish (production of mucus in the form of intestinal tubing, rejection of feed and loss in body mass) were observed.

Other animals

Jatropha seed has been shown to be highly toxic to mice at a level of 40 to 50 % in diets; the mice died within 3 - 16 days of dosing, with mortality rates of 87 and 67 %, respectively [1]. Even at lower concentration in the diet (37 %), Liberalino et al. [15] reported 100 % mortality within 2 - 3 days in rats. A very low concentration of *Jatropha* seeds (0.5 %) in the diet of Brown Hissex chicks produced no lethal effects [9]. Mourgue et al. [21] and Stripe et al. [27] reported that the toxicity of *Jatropha* is due to the presence of toxalbumin curcin. *Jatropha* also has toxic effects on ruminants. Goats which received *Jatropha* seeds at levels of 1 g/kg LM/day to 10 g/kg LM/day died within 2 - 9 days of dosing (drenching in water). In animals, the feeding of *Jatropha* seeds led to damage and necrosis of liver, kidney, heart, lungs, gastrointestinal tract, blood vessels, nervous system and bone-marrow [4, 5, 9, 10].

In our studies on fish fed a diet containing up to 50 % *Jatropha* meal from toxic varieties, mortality was not observed in fish, although various undesirable symptoms were observed. This difference in the results observed in our study and those obtained by other workers could be attributed to the absence of *Jatropha* oil in the diet of fish; we used *Jatropha* meal containing about 1 % residual oil whereas other workers used whole kernel, which contained 58 - 60 % of the oil. The *Jatropha* oil seems to have a toxin (probably phorbolesters) which causes mortality. A major portion of phorbolesters is known to be extracted with the oil fraction. Hirota et al. [13] reported that in addition to tumour promotion, phorbolesters bring about a wide range of biochemical and cellular effects, alter cell morphology, serve as lymphocyte mitogens and induce

platellet aggregation. Phorbolesters bind to a receptor site that is represented by a protein kinase C, an enzyme that plays an important role in signal transduction for several hormones. In our *Jatropha* meal preparation, phorbolesters were present (1.78 mg/g meal or 0.89 mg/g fish diet). However this level appears to be low to produce mortality in fish or fish could be lesser susceptible to phorbolesters than other animals. Future experiments should be directed to resolve these questions.

Jatropha meal from a non-toxic variety

Rats

The protein efficiency ratio (PER) of a diet containing *Jatropha* meal from the non-toxic variety was 1.29 ± 0.28 whereas for the casein group it was 3.52 ± 0.48. Heat treatment (66 % moisture, 121°C for 30 min) increased the PER to 3.02 ± 0.31 (86 % of the casein containing diet). The body mass of rats fed diets containing unheated and heated *Jatropha* meal were 23 and 7 % lower than those on casein diet. Feed intake of the diet containing heated *Jatropha* meal did not differ significantly from that of the casein group, but the intake of untreated meal was 21 % lower than for the control. The reduction in growth rate of rats in the heat-treated *Jatropha* group cannot be attributed to lower feed intake but to reduced protein utilisation whereas for rats on unheated *Jatropha* meal, the lower growth rate is attributed both to lower feed intake (21 %) and to lower protein utilisation (63 %). Better performance of rats on the heat-treated *Jatropha* meal appears to be due to higher protein degradability and inactivation of trypsin inhibitors and lectins by heating. The PER can possibly be increased by supplementation of lysine (the only limiting essential amino acid in *Jatropha* meal; see Tab. 3) to the diet containing *Jatropha* meal. The PER observed for the heat treated *Jatropha* meal was much higher than that obtained for the untreated and treated (detoxified) castor bean meals (untreated 0.88; detoxified 0.75 to 1.04; Rhee et al. [24]). These observations suggest that the protein quality of heat-treated *Jatropha* meal from the non-toxic variety is quite high, and as the protein content and amino acid composition of the toxic and non-toxic *Jatropha* meals are almost similar, we can expect the protein quality of the toxic *Jatropha* meal after detoxification to be as high as that of the meal from the non-toxic variety.

Fish

A diet containing unheated *Jatropha* meal was fed to fish (carp) for 7 days. Fifty per cent CP of the fishmeal in the standard diet was replaced by unheated *Jatropha* meal. Both the standard diet and the diet containing *Jatropha* meal had a CP content of 40 %. The diets were fed at a level of 3-time maintenance, split into 4 portions a day. The average body mass gain did not differ significantly between the two groups (10.1 ± 5.05 % and 12.3 ± 6.62 % in the treated and control groups respectively). Although, the diet containing *Jatropha* meal produced gain in body mass comparable to the control group, there were signs of feed unpalatability, and mucus was observed in the faeces which indicates presence of some toxin(s) in unheated *Jatropha* meal from the non-toxic variety. The production of faecal mucus could be due to the presence of high lectin activity in the unheated *Jatropha* meal. Long duration feeding of unheated *Jatropha* meal from the non-toxic variety is likely to produce adverse effects on fish.

Feeding of *Jatropha* meal from the non-toxic variety did not produce any symptoms associated with feeding of *Jatropha* meal from the toxic variety (see above), nor was it toxic to rats.

Heat treated Jatropha meal from the Cape Verde toxic variety

The degreased meal was heat treated (moisture 66 %, 121°C for 30 min) to inactivate lectin and TIA. This heat-treated meal was used for feeding to rats, chickens and fish.

Rats

Rats did not consume a feed containing 16 % of the heat treated meal which contributed 10 % CP to the diet. The intake of food was only 0.65 ± 0.41 g (n = 7) per day for the first two days. On the third day, one portion of the diet containing treated toxic *Jatropha* meal was mixed with nine portions of the control diet (10 % CP; from casein only) and this diet continued for 7 days. None of the rats died. The rats lost body mass from 129.7 ± 12.6 g to 98.6 ± 8.2 g. The average intake of this feed was 5.4 ± 0.46 g per day. The intake and body mass gain of rats on control diet were 81.9 g and 26.2 g per animal in 7 days of feeding.

Chickens

Sixteen post-hatched male broilers were divided into two groups of eight each. The chickens in the control diet (10 % CP; from casein only) increased in body mass from 47.6 ± 2.8 g to 62.1 ± 7.3 g in 9 days. The other group was fed, for the first four days, a diet containing one portion of the diet containing heat-treated toxic *Jatropha* meal (16 % of the heat-treated meal which contributed 10 % CP) and nine portions of the control diet. In four days, average gain in body mass per day was 1.9 ± 0.87 g which was comparable to that of the control group for the same period (1.8 ± 0.95 g). The control group consumed 20.4 g feed per day and the other group 19.0 g of feed per day during these four days. On the fifth day, these chickens were fed a diet containing three parts of the diet containing treated toxic *Jatropha* meal and seven parts of the control diet. On the ninth day, one of the eight chickens died. The remaining seven chickens had an average mass of 55.1 ± 8.0 g which was lower than the average mass of these chickens at the time they were shifted to this diet (on the fifth day, average mass was 57.6 ± 4.7 g). On the tenth day, another chicken died. These two chickens and another two chickens from this group and the from control group were opened for internal examination of the organs. No apparent signs of toxicity could be observed.

Fish

A diet containing the heat treated *Jatropha* meal (standard fish feed and the treated *Jatropha* meal were mixed 1 : 1, pelleted and dried using a lyophilizer giving a CP content of 48 %) was fed to fish (carp). This diet and the standard fish feed were manually fed at an amount equivalent to 5-time maintenance split into three portions a day, for a duration of 7 days (phase I). Then all fish were shifted to the standard diet for a duration of three weeks. During this period, fish were fed 5-time maintenance split into seven portions a day, using an automatic feeder (phase II).

On the first day of phase I, fish on the treated *Jatropha* diet had difficulty in maintaining their balance in the water column for about 2 h after consumption of the first portion of the feed. This abnormal behaviour disappeared subsequently and was not observed thereafter, suggesting adaptation to some deleterious factor present in the treated *Jatropha* meal. Mucus was observed from the second day onwards in chambers with treated *Jatropha* meal diet. The fish consumed this diet fully for the first two days and thereafter rejection of feed started which increased with each day of feeding. The fish lost body mass. During 7 days of feeding, average metabolic growth rate (AMGR) per fish was -3.44 g/kg^0.8/day. On the other hand this value for the standard group was 5.32. Symptoms such as loss of balance, nervousness and striking against wall of the chambers, which are typical effects arising from adverse action on the nervous system and were observed on feeding untreated *Jatropha* meal, were not observed with the present treatment.

In addition, white spots on skin and heavy peeling of the skin observed on feeding untreated *Jatropha* meal were also absent in the present experiment.

In phase II when the fish fed *Jatropha* meal were shifted to the standard diet, all fish showed almost similar metabolic growth during the first two weeks. The fish which were previously on the diets containing *Jatropha* meal had a better growth rate compared to those on the standard diet in the third week of the recovery phase (AMGR for previous *Jatropha* group and the standard group were 18.0 and 14.7 g/kg^0.8/day respectively), suggesting a compensatory growth. Furthermore, it is evident from these results that the changes produced in fish in 7 days of feeding the diet containing treated *Jatropha* meal (phase I) were reversible in nature.

The meal used in the above feeding studies for rats and chickens was free of TIA and lectin (as judged by hem- and latex-agglutination assays), but contained phorbolesters at a level of 1.66 mg/g. As the feed used for feeding rats and chickens containing 16 % meal, phorbolesters were present in the feed at a level of 0.27 mg/g. Phorbolesters level as low as 0.27 mg/g diet appears to have a dramatic effect on intake of feed by rats and chickens. On the other hand, the fish feed contained phorbolesters at a level of 0.83 mg/g which was consumed completely by fish in the first 2 days and partially in the next 5 days of feeding. Intake of a feed containing phorbolesters appears to be affected to a lesser extent in fish by phorbolesters as compared to in rats and chickens. The appearance of mucus in fish in the treated group is a typical symptom of consumption of a feed containing a lectin-rich diet. But the lectin was absent as determined by the two methods mentioned above. It is possible that the intestine of fish (carp) is much more sensitive to lectin than trypsinized erythrocytes and ovalbumin adsorbed latex used in hem- and latex-agglutination assays. The heat treatment used (moisture 66 %, 121°C for 30 min) might not be sufficient to inactivate lectins thus preventing adverse effects on fish intestine. Another possibility that phorbolesters also damage intestinal membranes and lead to mucus production seems to be more likely.

Chemical and heat treated Jatropha meal from toxic variety

Fish

In another study we used a combination of chemical treatments (NaOH and NaOCl) with and without heat treatment for detoxification. Heat treatment (moisture 66 %, 121° C for 30 min) in presence of both NaOH (2 %) and NaOCl (0.5 % active chlorine) was most effective. This treatment decreased phytate, saponins, phorbolesters, trypsin inhibitors and lectin by 18, 13, 75, 99 and 100 % respectively. Fifty per cent CP of the fishmeal in the standard fish diet was replaced by the treated *Jatropha* meal. The CP content of the control and the treated *Jatropha* meal diets was 40 %. The diets were fed at a level of 3-time maintenance, split into 4 portions a day to fish (carp). After 7 days of feeding, the average mass gain of the control group was 12.3 ± 6.62 % whereas that of the group receiving *Jatropha* was -0.8 ± 2.69. Although there was a decrease in the level of phytate, saponins and phorbolesters and complete inactivation of trypsin inhibitors and lectin (as judged by hem- and latex-agglutination assays) in the treated toxic *Jatropha* diet, there was no growth in the fish fed this diet, meaning that the treatment did not completely detoxify the *Jatropha* meal. However, this treatment was effective in eliminating some adverse effects observed on feeding untreated *Jatropha* meal (see above). Mucus in the faeces was detected in the treated *Jatropha* group on the second day of feeding and increased with each day of feeding. The negative growth rate appears to be due to the loss of endogenous protein in the form of mucus. The loss / destruction of intestinal membrane integrity may also lead to inhibition of nutrient transport, absorption and hence their utilisation. These adverse effects are typical symptoms associated with consumption of lectin containing diets, although production of mucus by phorbolesters cannot be ruled out. It may be noted that the level of phorbolesters in the

diet was 0.13 mg/g. This experiment again supports the contention that: i) fish intestine might have higher sensitivity towards lectins (although lectin activity was found to be absent as judged by hem- and latex-agglutination methods, the lectin activity could still be sufficiently high to cause destruction of the fish intestine) and (or) ii) phorbolesters even at a level of 0.13 mg/g feed damage the fish intestine. The treatment used in this study was partially effective: there were no apparent signs of toxicity but had negative effects on growth. The treatment used did not inactivate growth inhibiting factor(s).

Solvent extracted Jatropha meal from toxic variety

We also attempted to remove / extract above mentioned possible toxic factors from *Jatropha* meal by various solvent treatments. The solvents used for extraction were aqueous solutions of ethanol (60 % ethanol, 80 % ethanol and 96 % ethanol) and 100 % dichloromethane. Trypsin inhibitors, lectins and phytate, present in high levels in *Jatropha* meal, could not be extracted using any of the treatments studied. Amongst various extraction conditions used, 80 % aqueous ethanol (1 : 5, w/v) seems to be promising as it removed both saponins and phorbolesters by about 88 and 93 % respectively after 4 extractions. Dicholoromethane (100 %) was also very effective in extracting phorbolesters but not saponins. Aqueous ethanol (80 %) treatment is also expected to extract toxic amino acid(s) and / or toxic sugar(s), if any, from the meal. Fish are known to be highly sensitive to saponins and therefore 80 % aqueous ethanol treatment might provide a meal suitable not only for monogastrics and ruminants but also for fish. As lectin and trypsin inhibitors are heat labile, they can be inactivated by heat treatment. The physiological role of the lectin values obtained by hem- and latex-agglutination assays for fish nutrition needs further investigations. *In vivo* studies on fish and rats using diets containing *Jatropha* meal after extraction with 92 % aqueous ethanol have shown encouraging results. None of the fish or mice died or showed any apparent adverse effects when fed a diet containing the treated *Jatropha* meal from a toxic variety, which provided about 10 % CP in the diet (N. Foidl; personal communications).

Conclusions

J. curcas meal contain high true protein, high energy and low fibre. The estimated digestible organic matter and metabolizable energy in the meals compare well with those in some conventional seed meals. These constituents in the meal from the non-toxic variety from Mexico were similar to those from the toxic varieties. The amino acid composition of meals from the non-toxic variety and the toxic varieties was also similar, and the levels of essential amino acids except lysine were comparable with that for the FAO reference protein. The non-toxic variety is of as good a quality as the toxic varieties. The meal contained significant levels of trypsin inhibitor, lectin and phytate, and their levels did not differ much between the non-toxic and the toxic varieties. The high levels of trypsin inhibitor, lectin and phytate might aggravate adverse effects but do not contribute to the 'short-term' toxicity. Very low levels of phorbolesters in the seeds of non-toxic variety (about 20 times lower compared to toxic varieties), together with the fact that seeds of this non-toxic variety are consumed by humans in Mexico suggest that one of the toxic principles in seeds from toxic varieties is phorbolesters. Phytate constitutes a major single antinutritive component of *Jatropha* meals which is not heat labile and can have adverse effects especially mediated by decreased bioavailability of minerals, whereas other antinutritional factors like trypsin inhibitors and lectins can be destroyed by heat treatments. The non-toxic variety of *Jatropha* from Mexico can be a suitable alternative to the toxic *Jatropha* varieties, and it is suggested to propagate its cultivation. This non-toxic variety of *Jatropha* could be a potential

source of oil for human consumption, and the seed meal / cake can be a good protein source for humans as well as livestock. As seed shells from both toxic and non-toxic varieties are high in gross energy, they make good fuel. The husk with a nutritional value comparable to cereal straws, can be utilised for animal feeding or used as mulch.

Although level of phorbolesters is quite low in *Jatropha* meal obtained from the toxic variety, this level appears to prevent rats and chickens from consuming diets containing *Jatropha* meal, whereas in fish phorbolesters do not seem to affect the intake to such an extent. The results from our various studies indicate that *Jatropha* meal from toxic varieties can be detoxified by removal of phorbolesters and inactivation of lectins. Extraction of phorbolesters using 80 - 90 % aqueous ethanol or methanol followed by inactivation of lectins by heat treatment (moisture 66 %, 121°C for 30 min) might hold potential for converting toxic *Jatropha* meal to a high quality protein source for livestock feed. Before incorporating the meal into fish feed, it might have to be subjected to drastic heat treatment as the fish intestine appears to be highly sensitive to *Jatropha* lectins. Other treatments, such as a combination of heat and chemical treatments (NaOH and NaOCl) also need further investigation as the phorbolester levels decreased by about 75 % on addition of NaOH and active chlorine in the form of NaOCl at 2 % and 0.5 % (w/w) respectively in *Jatropha* meal followed by heat treatment (moisture 66 %, 121°C for 30 min). All our detoxification studies were conducted using *Jatropha* meal produced by defatting in petroleum ether using a Soxhlet apparatus and therefore the residual oil content was < 1 %. *Jatropha* seed cakes obtained by other procedures such as pressing and extrusion are likely to have higher oil contents and hence higher phorbolesters. In addition, presence of oil protects lectins and trypsin inhibitors during heat treatment and heat treatment therefore is less effective in inactivating these antinutritional factors. These observations imply that detoxification of *Jatropha* seed cake obtained by pressing or extrusion would require different and more drastic measures as compared to solvent extracted *Jatropha* meal.

Future research areas of our group

1. The collection of non-toxic varieties of *Jatropha* based on observations by local people that the seeds are consumed by human and or animals, confirmation of their non-toxic nature with proper experimental model animals, comparison of their seed and oil yield, resistance of plants to diseases and quality of seed meal in terms of organic matter digestibility, metabolizable energy, protein and amino acid composition with that of already existing toxic varieties, propagation of their cultivation in different climatic zones using conventional and tissue culture approaches, study of nutrients and antinutrients in the seeds and leaves of non-toxic variety obtained from different climatic zones and evaluation of seed meal as a protein supplement to the diets of fish, chickens, rabbits and ruminants. Evaluation of oil from the non-toxic varieties for human consumption. Seeds of a non-toxic variety have already been distributed for planting in Nicaragua, India, Mexico and Zimbabwe.

2. The production performance of ruminants on diets containing heated and unheated *Jatropha* meals from the non-toxic variety. The heat treatment would inactivate lectins and trypsin inhibitors and increase extent of rumen protein degradation. Lectins and trypsin inhibitors are not considered to be of any physiological significance for ruminants as being proteins, they might be degraded by rumen microbes [this study would assume that toxic effects observed in ruminants on drenching ground seeds of toxic varieties were not due to lectins or trypsin inhibitors but to phorbolesters] and therefore feeding of unheated *Jatropha* meal from the non-toxic variety with

higher levels of rumen bypass protein should produce better response in lactating, wool producing and growing ruminants.

3. The acceptance of raw seeds by humans and animals and levels of lectins, trypsin inhibitors and phorbolesters in raw and roasted seeds as sold on the market.

4. Development of economically viable techniques for detoxification of solvent extracted *Jatropha* meal and press cake and oil from toxic varieties, and evaluation of the detoxified meal / cake as a protein supplement to the diets of fish, chickens, rabbits and ruminants and of the detoxified oil to the human diet.

A proposal

We are looking for research groups for participation in some of the above-mentioned areas. Readers interested in collaborating are requested to contact the authors.

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