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Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors

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Four varieties of *Jatropha curcas* which originated from Nicaragua (Cape Verde and Nicaragua toxic varieties cultivated in Managua), Nigeria (a wild variety from Ife; toxicity unknown) and Mexico (a wild non-toxic variety collected from Papantla) were studied. The average seed weight was 0.69, 0.86, 0.53 and 0.65 g for Cape Verde, Nicaragua, Ife-Nigeria and non-toxic Mexico varieties, respectively. The kernel to shell ratio in seeds was relatively similar (62.7:37.3 for both Cape Verde and Nicaraguan, 60:40 for Ife-Nigerian and 63.5:36.5 for non-toxic Mexican). The shell of the seeds composed mainly of fiber (>83% neutral detergent fiber) and very little crude protein (CP <6%). The kernels were rich in CP (22.2–27.7%) and lipid (53.9–58.5%). The meal (defatted kernels) had a CP content of 57.3, 61.9, 56.1 and 64.4% for Cape Verde, Nicaragua, Ife-Nigeria and non-toxic Mexico varieties, respectively, and about 90% of this CP was true protein. The pepsin insoluble nitrogen was from 5.5 to 7%. The amino acid composition of meals from Cape Verde, Nicaragua and non-toxic Mexico varieties was similar. The levels of essential amino acids except lysine were higher than that for the FAO reference protein. The meal from the toxic variety (Cape Verde) did not have any anti-fermentative activity on rumen microbes. The estimated digestible organic matter (DOM) and metabolizable energy (ME) for the shells were low (26.2–27.1% and 2.4–2.8 MJ kg^{-1}), whereas these values for jatropha meals were 77.3–78.4% and 10.7–10.9 MJ kg^{-1}. For commercially available (heat-treated) soyabean meal, DOM and ME were 87.9% and 13.3 MJ kg^{-1}, respectively. The in-vitro rumen degradable nitrogen (% of total nitrogen) for meals from Cape Verde, Nicaragua, Ife-Nigeria and non-toxic Mexico varieties was 43.3, 37.7, 38.7 and 28.9, respectively, and for the soyabean meal it was 80.9%. Tannins, cyanogens, glucosinolates and amylase inhibitors were not detected in meals of any of the four varieties. A small amount of tannins were present in shells (2.0–2.9% as tannic acid equivalent). High levels of trypsin inhibitor activity (18.4–26.5 mg trypsin inhibited g^{-1}), lectin (51–102; inverse of the minimum amount in mg ml^{-1} of the assay which produced haemagglutination in presence of 10 mM Mn^{2+}) and phytate (7.2–10.1%) were observed in the meals. The concentrations of phorbol esters in kernels of Cape Verde, Nicaragua and Ife-Nigeria varieties were 2.70, 2.17 and 2.30 mg g^{-1}, whereas kernels of non-toxic Mexican had a very low level (0.11 mg g^{-1}) of phorbol esters.

INTRODUCTION

Jatropha curcas also commonly referred to as 'Physic nut', 'Purging nut', 'Piñoncillo', 'Habb-El-Meluk', 'Black vomit nut', 'American purging nut', 'Barbados purging nut', 'Big purge nut' is a member of the Euphorbiaceae family which grows in most of the tropics. The plant (a shrub or small tree) grows readily in swamp or shade and is quick growing (Ishii *et al.*, 1987), survives in poor stoney soils and is resistant to drought (Munch and Kiefer, 1989). It reaches a height of 3–5 m and has an annual seed yield of up to 5 t per hectare (Raina and Gaikwad, 1987; Heller, 1996). In the tropics,

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J. curcas is traditionally used for medicines and as hedges (Jones and Miller, 1992). Its use as green manure to rice grown on loamy acid soil was reported (Scherchan *et al.*, 1989). The seed weighs about 0.75 g, contains 30–32% protein and 60–66% lipid (Liberalino *et al.*, 1988) indicating good nutritional value. However, the seed and/or oil were found to be toxic to mice (Adam, 1974), rats (Liberalino *et al.*, 1988), calves, sheep and goats (Ahmed and Adam, 1979*a,b*), humans (Mampane *et al.*, 1987) and chickens (Samia *et al.*, 1992). Hence, its use as a food or feed source is presently limited.

The oil from the kernels of *J. curcas* can serve as fuel for diesel engines (Ishii *et al.*, 1987; Munch and Kiefer, 1989; Ouedraogo *et al.*, 1991; Lutz, 1992), indicating its potential as a renewable energy source. The seeds can be transported without deterioration and at low cost due to its high specific weight. These features have generated interest in the jatropha plant which is now becoming a cash crop in South and Central American countries. The objective of this study was to investigate the nutritive potential and toxic characteristics of different varieties of *J. curcas*.

MATERIALS AND METHODS

Samples

Four varieties of *J. curcas* (Cape Verde, Nicaragua, Ife-Nigeria and Non-toxic Mexico) were used for the study. Two varieties (Cape Verde and Nicaragua) were cultivated varieties obtained from Managua in Nicaragua, while the other two (Ife-Nigeria and non-toxic Mexico) were wild growing varieties obtained from Ile-Ife in Nigeria and Papantla in Mexico. In our other study using rats and fish, we found that the meal (defatted kernels) of the seeds collected from Mexico is non-toxic whereas the meal from Cape Verde and Nicaragua cultivated varieties is toxic (Makkar, H.P.S. and Becker, K.; unpublished observations). The toxicity of seeds from Ife-Nigeria variety is not known.

The fruits (obtained for Ife-Nigeria only) were weighed, counted and the husk was separated from the seeds. The weights of fruits, husks and seeds, and the number of fruits were recorded. Ten handfuls of seeds were randomly taken from each of the four varieties. The weight of each handful and the number of seeds in it were used to calculate the average weight of seed in each handful. The overall average weight of seeds for the ten handfuls was recorded for each variety. Two kg of randomly collected seeds from each variety were cracked, the shells carefully removed and the weights of kernels and shells recorded. The husks and shells were milled through 1 mm screen. The kernels were ground using a coffee grinder and defatted in a Soxhlet-type extractor using petroleum benzene (boiling point, 40–60°C) several times until the evaporation of residual ether revealed $< 2\%$ lipid, resulting in meal (defatted kernels).

Heat treated soyabean meal was obtained from Soya Mainz GmbH and Co. KG, Dammweg 2, Mainz, Germany.

Chemical analyses

All Jatropha samples (husk, shell, kernel and meal) and a commercial soyabean meal were analyzed for dry matter (DM), organic matter (OM), crude protein (CP; N×6.25), lipid (L) and ash using the AOAC (1980) procedure. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest *et al.* (1991). Gross energy (GE, MJ kg^{-1} DM) was determined using a bomb calorimeter.

Determination of buffer-soluble nitrogen, non-protein nitrogen and pepsin insoluble nitrogen

Samples (5 g) of jatropha meals were homogenizied in 100 ml of phosphate buffer (0.05 M, pH 7.0) using an ultra-turrax at 10 000 rpm for 20 min (4×5 min) and then filtered. Ten ml of the filtrate was mixed with 10 ml of 20% trichloroacetic acid (TCA), refrigerated overnight and centrifuged (3000×***g***, 10 min) to collect the supernatant. Aliquots (10 ml) of the supernatant were analysed for non-protein nitrogen using the Kjeldahl analysis. Total soluble nitrogen was determined (using Kjeldahl analysis) using 10 ml aliquots of the filtrate after homogenization. Results were expressed as g CP per 100 g DM. Pepsin insoluble nitrogen was determined as described in Makkar and Becker (1997).

In-vitro rumen degradable nitrogen and fermentation studies

In-vitro rumen degradable nitrogen was determined according to the method of Raab *et al.* (1983) as described in Aderibigbe *et al.* (1997).

The meal from Cape Verde variety was incubated for 24 h with or without various amounts of hay in an in-vitro rumen fermentation system (Menke *et al.*, 1979) to study the presence of anti-fermentative factor in the meal. Triplicate samples (200 mg each) of jaropha husk (Ife-Nigeria) and shell and meals from each variety were also subjected to 24 h in-vitro fermentation for calculation of digestible organic matter (DOM) and metabolizable energy (ME) according to Menke *et al.* (1979).

Determination of toxic/antinutritional components

The meal samples were analysed for tannins, trypsin and amylase inhibitors, saponins, and phytate as

described in Aderibigbe *et al.* (1997). Cyanogens and glucosinolates in jatropha meals were quantified as described in Makkar and Becker (1997) and lectin was quantified using a haemagglutination test using cattle erythrocytes (Gordon and Marquardt, 1974) in the presence of 10 mM Mn^{2+}. For soyabean meal, lectin was determined without the addition of Mn^{2+}.

Quantification of phorbol esters

Five seeds of each variety were weighed, ground with a small amount of sand using a pestle and mortar and then 20 ml dichloromethane was added. The mixture was again ground for about 5 min with the mortar. The material was allowed to settle and the liquid phase was filtered. The residue on the filter paper and in the pestle were pooled using about 20 ml dichloromethane and then ground for about 5 min using the mortar. The liquid phase was again collected. This extraction procedure was repeated three more times and the filtrate from all five extractions were pooled. The residue (sand plus kernels) was subjected to ultrasonic waves (105 W) for 3 min in the presence of about 50 ml dicholoromethane. It was then filtered and this filtrate was pooled with the pooled filtrates from the previous five extractions. The filtrate was dried under vacuum at 40°C. The dried residue was dissolved in 5 ml tetrahydrofuran, passed through a 0.2 μm glass filter and injected (20 μl) into the HPLC.

HPLC conditions for quantification of phorbol esters

The HPLC equipment used consisted of a Merck Hitachi L-7100 HPLC pump, an L.7450 photo diode array detector, an L-7200 autosampler, a D-7000 interphase module and an LC organiser. The analytical column was reverse phase C18 (LiChrospher 100, endcapped 5 μm) 250×4 mm I.D. (Lichrocart) protected with a guard column containing the material as in the main column. Three solvents were used: (A) 1.75 ml o-phosphoric acid (85%) in 1 l distilled water, (B) Acetonitrile and (C) tetrahhydrofuran. Solvent A was filtered before use and solvents B and C were of HPLC and analytical grade and used with filtration. All solvents were degassed by ultrasonication and by application of vacuum. The gradient used was: start with 60% A and 40% B, decrease A to 50% and increase B to 50% in the next 10 min, decrease A to 25% and increase B to 75% in the next 30 min, increase B to 100% in the next 15 min. Then the column is washed with C by increasing C to 100% in the next 15 min and then the column is adjusted to the starting conditions (A 60% and B 40%). Separation was performed at room temperature (ca. 22°C) and the flow rate was 1.3 ml min^{-1}. Phorbol esters (4 in number) appeared between 41 and 48 min (Adolf *et al.*, 1984; Hirota *et al.*, 1988). The peaks were integrated at 280 nm and the results are expressed as equivalent to phorbol-12-myristate 13-acetate (obtained from Sigma), which appeared between 52 and 53 min.

Amino acid analysis

The samples were hydrolysed with 6 M HCl at 100°C for 24 h under vacuum and amino acids were analysed using an amino acid analyser (Bassler and Buchholz, 1993).

All analyses were conducted at least in duplicate by taking a representative sample of each variety. The values reported are averages of two values. The individual values did not deviate from the mean by more than 5%.

RESULTS AND DISCUSSION

Jatropha samples

The physical characteristics of the four varieties of jatropha are shown in Table 1. The average fruit weight of Ife-Nigeria variety was 2.1 g with seed to husk ratio (w/w) of 71:30. Thus, the seeds formed a large proportion of the fruit. The average seed weight ranged from 0.53 g in Ife-Nigeria to 0.86 g in Nicaragua variety, indicating varietal difference. Liberalino *et al.* (1988) reported that *J. curcas* seed (variety was not mentioned) weighed about 0.75 g. The kernel to shell ratio ranged from 60:40 in Ife-Nigeria to 63.5:36.5 in the non-toxic Mexico variety, which could be due to varietal difference. The kernel formed 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).

Chemical composition

The chemical composition of kernel, shell and husk of *J. curcas* varieties are shown in Table 2. *J. curcas* kernel is composed mainly of lipid and protein, with very little moisture and ash. There were varietal difference in CP content in the kernels (22.2–27.2%). A seven-year-old seed contained similar kernel: shell ratio (63:37), CP (25.6%), lipid (57%) and ash (3.4%) to those observed for fresh samples. The low moisture content of the shell (< 10%) and kernel (< 6%) could be partly responsible for the non deterioration of seeds over a long period. Presence of antinutritional factors/toxins is also likely to increase the shelf-life of the seeds. The shell of *J. curcas* seed is composed mainly of fiber (> 83% NDF and > 74% ADF) and lignin (> 45%) with very little protein (< 6%), indicating poor nutritional value, but can be a good source of fuel beacuse of its high gross energy.

The chemical composition of meals of the four varieties of *J. curcas* and soyabean meal are shown in Table 3. The CP contents of meals varied from 55.7% in Ife-Nigeria to 63.8% in the non-toxic Mexico variety. When corrected for fat content, CP contents of the meals ranged from 56.1% in Ife-Nigeria to 64.4% in the non-toxic Mexico variety. These values were higher

than the values obtained by Panigrahi *et al.* (1984) who reported 48 and 44% CP for oil-free *J. curcas* and soyabean meals, respectively. Commercial soyabean meal contained lower CP than *J. curcas* meals (Table 3). The *J. curcas* meals contained about 10% ash which was higher than that in soyabean meal (6.4%). The fiber (< 10% NDF and < 7.0% ADF) contents of *J. curcas* meals were lower than those in soyabean meal (17.2% NDF and 12.2% ADF). The gross energy content of defatted jatropha meals was similar to that of the soyabean meal. These indicate that *J. curcas* meals contain a very good nutrient profile, comparable to soyabean meal but with a higher CP than soyabean meal.

Buffer-soluble nitrogen, non-protein nitrogen and pepsin insoluble nitrogen

Table 4 shows the buffer-soluble nitrogen, non-protein nitrogen and pepsin insoluble nitrogen (as CP) in *J. curcas* meals. The buffer-soluble nitrogen and buffer soluble non-protein nitrogen were 7.4–8.0 g and 4.7–5.0 g CP/100 g DM respectively. The non-protein nitrogen

Table 1. Physical characteristics of four *Jatropha curcas* varieties

Item	Variety			
	Cape Verde[a]	Nicaragua[a]	Ife-Nigeria[b]	Non-toxic Mexico[c]
Average fruit wt (g)	–	–	2.1	–
Seed, % of fruit	–	–	71.1	–
Husk, % of fruit	–	–	28.9	–
Average seed wt (g)	0.69	0.86	0.53	0.65
Kernel, % of seed	62.7	62.7	60.0	63.5
Shell, % of seed	37.3	37.3	40.0	36.5

[a]Cultivated varieties from Nicaragua.
[b]Wild growing variety from Ile-Ife, Nigeria.
[c]Wild growing non-toxic variety from Mexico.

Table 2. Chemical composition of kernel, shell and husk of *Jatropha curcas* varieties

Item	Variety								
	Cape Verde		Nicaragua		Ife-Nigeria			Non-toxic Mexico	
	Kernel	Shell	Kernel	Shell	Kernel	Shell	Husk	Kernel	Shell
Dry matter (DM, %)	96.6	90.3	96.9	90.4	95.7	91.9	91.3	94.2	89.8
Analysis, % in DM:									
Crude protein	22.2	4.3	25.6	4.5	27.7	5.8	6.3	27.2	4.4
Lipid	57.8	0.7	56.8	1.4	53.9	0.8	1.1	58.5	0.5
Ash	3.6	6.0	3.6	6.1	5.0	4.6	15.4	4.3	2.8
Neutral detergent fiber	3.8[a]	83.9	3.5[a]	85.8	4.1	89.6	65.9	3.8	89.4
Acid detergent fiber	3.0[a]	74.6	3.0[a]	75.6	2.6	79.8	61.3	2.4	78.3
Acid detergent lignin	0.2[a]	45.1	0.1[a]	47.5	0.00	47.4	14.4	0.00	45.6
Gross energy (MJ kg^{-1})	30.7	19.3	30.5	19.5	29.7	19.5	15.6	31.1	19.5

[a]Calculated from values obtained for fat free samples since high lipid content interfered with fiber determination.

Table 3. Chemical composition (% in DM) of meals of *Jatropha curcas* varieties and soyabean meal

Item	Variety				
	Cape Verde	Nicaragua	Ife-Nigeria	Non-toxic Mexico	Soyabean meal[a]
Crude protein	56.4 (57.3)	61.2 (61.9)	55.7 (56.1)	63.8 (64.4)	45.7 (46.5)
Lipid	1.5	1.2	0.8	1.0	1.8
Ash	9.6	10.4	9.6	9.8	6.4
Neutral detergent fiber	9.0	8.1	8.9	9.1	17.2
Acid detergent fiber	7.0	6.8	5.6	5.7	12.2
Acid detergent lignin	0.4	0.3	0.1	0.1	0.0
Gross energy (MJ kg^{-1})	18.2	18.3	17.8	18.0	19.4

[a]Source, Soya Mainz GMbH and Co KG, Dammweg 2, Mainz, Germany.
Values in parentheses are the CP values of lipid-free meal.

represented about 62–64% of the buffer-soluble nitrogen. Only 7.8–9.0% of the total nitrogen in jatropha meals was as non-protein nitrogen, suggesting the presence of a high level (ca. 90%) of true protein. The non-protein nitrogen content (as % of total CP) of defatted seed meals of jojoba, soyabean, sunflower and rapeseed were 21–30%, 2.9–7.8%, 5.0% and 6.9%, respectively (Wolf *et al.*, 1994). The pepsin digestible nitrogen in jatropha meal was very high (93–95%; Table 4), suggesting high availabilty of proteins to animals.

Amino acid composition

The amino acid composition of meals from the non-toxic, Cape Verde and Nicaragua varieties was similar. The levels of essential amino acids except lysine were higher than that for the FAO reference protein (Table 5). This table also shows amino acid composition of castor bean meal (castor bean also belongs to Euphorbiaceae family) and soyabean meal. The levels of essential amino acids, except isoleucine, in the jatropha meals were higher or similar when compared to the castor bean meal. A comparison between the amino acid composition of jatropha meal (Table 5) and of soya beans (Bau *et al.*, 1994; Sarkar and Peace, 1994) revealed an almost similar pattern for all essential amino acids except lysine and sulphur-amino acids; lysine and sulphur-amino acid levels were lower and higher, respectively in jatropha meals. Compared with casein, the levels of essential amino acids, except sulphur-amino acids, were lower in jatropha meals; the sum of methionine and cystine in jatropha meals was higher than that in casein (Sarkar and Peace, 1994).

In-vitro digestible organic matter (DOM), metabolizable energy (ME) and rumen degradable nitrogen

Table 6 shows the DOM and ME of the shell and husk. The DOM and ME were higher for the husk than for the shell, which could be attributed to lower NDF and ADL contents in the husk (Table 2). The DOM, ME and 24 h in-vitro rumen degradable nitrogen ($IVRDN_{24}$) of *J. curcas* meals and soyabean meal are shown in Table 7. The DOM and ME values were high

Table 4. Buffer-soluble nitrogen, non-protein nitrogen and pepsin insoluble nitrogen as crude protein (g CP/100 g DM) in meals of *Jatropha curcas* varieties

Item	Variety			
	Cape Verde	Nicaragua	Ife-Nigeria	Non-toxic Mexico
Buffer-soluble nitrogen	7.4	7.7	8.0	7.9
Non-protein nitrogen	4.7	4.8	5.0	5.0
Pepsin insoluble nitrogen	4.0	4.3	3.3	3.5

Table 5. Amino acid composition of *Jatropha curcas* Cape Verde, Nicaragua and non-toxic Mexican varieties, FAO Reference protein and soyabean

Amino acids	Amino acid composition* (g 16 g^{-1} N)					
	Cape Verde	Nicaragua	Non-toxic Mexico	Castor bean[a]	FAO Reference protein[b]	Soyabean[c]
Lysine	4.28	3.74	3.40	3.86	5.80	6.08
Leucine	6.94	7.03	7.50	4.48	6.60	7.72
Isoleucine	4.53	4.46	4.85	6.27	2.80	4.62
Methionine	1.91	1.56	1.76	1.65	2.5	1.22
Cystine	2.24	1.76	1.58	1.42		1.70
Phenylalanine	4.34	4.52	4.89	4.04	6.30	4.84
Tyrosine	2.99	2.79	3.78	2.65		3.39
Valine	5.19	5.24	5.30	5.53	3.50	4.59
Histidine	3.30	3.20	3.08	2.19	1.90	2.50
Threonine	3.96	3.71	3.59	3.35	3.40	3.76
Serine	4.80	4.88	4.82	5.60	–	5.67
Glutamic acid	14.68	15.4	15.91	19.48	–	16.90
Aspartic acid	9.49	9.73	9.92	9.60	–	11.30
Proline	4.96	5.27	3.80	4.02	–	4.86
Glycine	4.92	4.66	4.61	4.22	–	4.01
Alanine	5.21	5.04	4.94	4.31	–	4.23
Arginine	11.8	13.2	12.90	12.21	–	7.13
Tryptophan	1.31	1.23	–	–	1.10	1.24

[a]Rhee (1987).
[b]Data from FAO/WHO/UNO (1985) and FAO/WHO (1990); taken from Zarkadas *et al.* (1995).
[c]Bau *et al.* (1994).

Table 6. Digestible organic matter and metabolizable energy of the shell and husk of *Jatropha curcas* varieties

Item	Variety				
	Cape Verde	Nicaragua	Ife-Nigeria		Non-toxic Mexico
	Shell	Shell	Shell	Husk	Shell
Digestible organic matter (%)	26.5	26.8	26.2	54.4	27.1
Metabolizable energy (MJ kg^{-1} DM)	2.4	2.5	2.4	5.8	2.8

Table 7. Digestible organic matter, metabolizable energy and 24 h in-vitro rumen degradable nitrogen of meal of *Jatropha curcas* varieties and soyabean meal

Item	Variety				
	Cape Verde	Nicaragua	Ife-Nigeria	Non-toxic Mexico	Soyabean meal
Digestible organic matter (%)	78.0	78.0	78.4	77.3	87.9
Metabolizable energy (MJ kg^{-1} DM)	10.9	10.7	10.8	10.7	13.3
24 h in-vitro rumen degradable nitrogen (% of total nitrogen)	43.3	37.7	38.7	28.9	80.9

(about 78% and 10.8 MJ kg^{-1}, respectively) and similar for the different jatropha meals. These values were lower than that for soyabean, but were comparable with that for cottonseed, rapeseed and sunflower meals (Makkar and Becker, 1997). The $IVRDN_{24}$ for the jatropha meals was low, ranging from 28.9% in the non-toxic Mexico to 43.3 in Cape Verde variety. The $IVRDN_{24}$ for soyabean meal (80.9%) was much higher than for jatropha meals. These observations indicate that jatropha meal proteins are not easily degraded by reticulo-rumen microorganisms. This observation together with low values of pepsin insoluble nitrogen (5.5–7% of the 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.

Table 8. Effect of different mixtures of meal (Cape Verde) and hay on 24 h in-vitro rumen gas production

Sample	ml gas observed
Jatropha meal (mg)	
100	20.1
200	39.1
300	56.3
400	71.6
Hay (mg)	
100	19.5
200	41.0
Jatropha meal (100 mg)	41.0
plus hay (100 mg)	(39.6)
Jatropha meal (200 mg)	79.8
plus hay (200 mg)	(80.1)
Jatropha meal (300 mg)	94.7
plus hay (200 mg)	(97.3)
Jatropha meal (400 mg)	91.1
plus hay (100 mg)	(91.1)

Values in parenthesis are the expected gas values.

Toxic/antinutritional components

The effect of different mixtures of meal (Cape Verde variety) and hay on 24 h in-vitro gas production is shown in Table 8. Gas production for the jatropha meal was similar to that of the hay sample, and the gas production for the mixtures (meal plus hay) was almost summative 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).

Total phenol, tannins and condensed tannins concentration in meal, shell and husk of jatropha varieties are shown in Table 9. Negligible amount of total phenols (0.2 to 0.4%) was found in jatropha meals and husk (0.2%). 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. Amylase inhibitor activity, cyanogens and glucosinolates were not detected (results not shown) in any of the meal samples investigated. Table 10 shows trypsin inhibitor activity (TIA, mg trypsin inhibited per g), lectin (inverse of minimum amount of meal in mg ml^{-1} assay mixture which produced haemagglutination) and saponin (% as diosgenin equivalent) contents of jatropha and soyabean meals. TIA of jatropha meals ranged between 18.4 mg g^{-1} in Ife-Nigeria to 26.5 mg g^{-1} in the non-toxic Mexico variety, indicating varietal differences. These were higher than the TIA of 3.9 mg g^{-1} in heat-treated soyabean meal. Smith *et al.* (1980) reported TIA (using the same method as used in the present investigation) of 18.6 to 30 mg g^{-1} for raw soyabean meals. It is known that consumption of unheated soyabean meal produces adverse effects in monogastrics (White *et al.*, 1989; Hajos *et al.*, 1995).

Table 9. Total phenols, tannins and condensed tannins in meal, shell and husk of *Jatropha curcas* varieties (data are on dry matter basis)

Item	Variety								
	Cape Verde		Nicaragua		Ife-Nigeria			Non-toxic Mexico	
	Kernel	Shell	Kernel	Shell	Kernel	Shell	Husk	Kernal	Shell
Total phenols (% tannic acid equivalent)	0.36	3.0	0.29	2.8	0.31	3.1	0.18	0.22	4.4
Tannins (% tannic acid equivalent)	0.04	2.2	0.03	2.0	0.0	2.2	0.01	0.02	2.9
Condensed tannins (% leucocyanidin equivalent)	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd, non detectable.

Table 10. Trypsin inhibitor activity, saponin, lectin and phytate contents of meal of *Jatropha curcas* varieties and soyabean meal and phorbol ester content in kernel

Item	Variety				
	Cape Verde	Nicaragua	Ife-Nigeria	Non-toxic Mexico	Soyabean meal[a]
Trypsin inhibitor activity (TIA, mg g^{-1} meal)[b]	21.3	21.1	18.4	26.5	3.9
Lectin [1/(minimum amount of meal in mg ml^{-1} assay which produced haemagglutination)][b]	102	102	102	51.0	0.32
Saponin (% diosgenin equivalent in meal)[b]	2.6	2.0	2.0	3.4	4.7
Phytate (% in meal)[b]	9.4	10.1	7.2	8.9	1.5
Phorbol esters (mg g^{-1} kernel)[c]	2.70	2.17	2.30	0.11	–

[a]Heat treated; source: Soya Mainz GMbH and Co KG, Dammweg 2, Mainz, Germany.
[b]On dry matter basis.
[c]Equivalent to phorbol-12-myristate 13-acetate.

It is clear from these observations that trypsin inhibitor activity in *J. curcas* meal is high and is of the same order as in raw soyabean meal, which could cause adverse physiological effects in monogastrics. Lectin activity in jatropha meals was higher than in soyabean meal. Soyabean used in the present study was heat treated which could have lowered the lectin activity. It would be interesting to compare the lectin values of jatropha meal with the unheated soyabean meal. Amongst the jatropha meals, lectin activity was lowest in the meal of the non-toxic Mexico than in the other meals. The lectin assay used is based on haemagglutination of two-fold serially diluted extracts of the sample and sensitivity of this assay is ± 1 dilution. This implies that values of 51 and 102 by the haemagglutination method are separated by only one dilution and therefore are not much different from each other. Toxicity of *J. curcas* seeds is generally attributed to the presence of lectin in these seeds (Mourgue *et al.*, 1961; Stirpe *et al.*, 1976). Similar lectin values in the non-toxic Mexico and the toxic (Cape Verde and Nicaragua) varieties suggest that lectin is not the main toxic principle in jatropha seeds. Saponin content was higher for soyabean meal than for jatropha meals. Saponins from some plants produce adverse effects and from others beneficial effects (Price *et al.*, 1987; Liener, 1994). Saponins from soyabean are relatively innocuous (Liener, 1994). Phytate content of jatropha meals varied between 7.2% in Ife-Nigeria to 10.1% in Nicaragua variety. These values were extremely high especially when compared with 1.5% in soyabean meal. In addition, soyabean meal is considered to have high phytate content. These indicate that the consumption of jatropha meal can decrease the bioavailability of minerals, especially Ca and Zn. Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interacting with enzymes such as trypsin and pepsin (Reddy and Pierson, 1994).

Phorbol esters were present in high concentrations in the kernels of toxic seeds whereas a very low amount of phorbol esters was observed in those of the non-toxic variety (Table 10). Phorbol esters were also determined in three seed samples collected from three different trees (designated as NC 7 and NC 9 and NC 26) located in the backyards of farmers house. The NC 7 and NC 9 were from Quintana Roo and NC 26 was from Laguna Guerreo in Mexico. The seeds of both NC 7 and NC 9 are consumed by both humans and chicken but NC 9 is consumed very frequently and NC 7 less so. According to the farmer, consumption of seeds from NC 26 by humans causes diarrhea, giddiness and vomiting and therefore is not consumed. Phorbol esters in NC 7, NC 9 and NC 26 were 0.09, 0.03 and 2.49 mg g^{-1} kernel, respectively. These observations indicate that acceptance of jatropha seeds as food or feed is affected by the content of phorbol esters. The higher the phorbol esters,

the lower the acceptance of jatropha seeds. The immature seeds (greenish yellow stage) of NC 9 had a higher level of phorbol esters (0.15 mg g^{-1} kernel) suggesting higher toxicity of immature seeds as compared to mature seeds. The content of phorbol esters appears to vary from provenance to provenance, and, therefore, there is a need to promote the collection of non-toxic varieties of jatropha.

The phorbol esters content of oil from the Cape Verde toxic and the Mexican non-toxic variety was found to be 2.49 and 0.27 mg ml^{-1}, respectively. Therefore, long term toxicological studies by feeding diets containing oil from this non-toxic jatropha variety need to be conducted on rats or other laboratory animals before it can be recommended for human consumption. Phorbol esters were found to be responsible for purgative, skin-irritant effects and tumor promotion (Adolf *et al.*, 1984; Hirota *et al.*, 1988). Ingestion of plants in the families Euphorbiaceae and Thymelaeaceae that biosynthesize diterpene esters of the phorbol type cause severe symptoms of the toxicity in livestock (Kingsbury, 1964). The results of the present study suggest that toxicity of *J. curcas* seeds could be attributed to phorbol esters present in higher amounts in toxic varieties. The seeds of the non-toxic variety, after roasting, are consumed as peanuts by humans in Mexico without any apparent adverse effects, suggesting that the body's defense system can detoxify the low amounts of phorbol esters present in the non-toxic variety. However, a systematic epidemiological study needs to be conducted on the occurrence of cancer in the areas where these seeds are consumed, which might reveal the role, if any, of consumption of these low levels of phorbol esters in causing cancer.

CONCLUSION

J. curcas meals contain high true protein, high energy and low fiber. The estimated digestible organic matter and metabolizable energy in jatropha meals compare well with those in some conventional seed meals. These constituents in the meal from the non-toxic Mexico variety 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 seed/meal from the non-toxic Mexico variety is of as good a quality as those from the toxic varieties. The meals 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 phorbol esters in the seeds of non-toxic varieties from Mexico and about 20-times higher levels in toxic varieties, together with the fact that seeds of the non-toxic variety are consumed by humans in Mexico suggest that one of the toxic principles in seeds from toxic varieties is phorbol esters. Phytate constitutes a major single antinutritive component of jatropha meals which is not heat labile and can have adverse effects on 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 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 cake can be a good protein source for humans as well as livestock.

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