

Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats

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ABSTRACT

Seeds from a toxic variety of *Jatropha curcas* (Capo Verde, Nicaragua) were processed, defatted and ground to obtain the meal. The meal was subjected to heat and 14 different chemical treatments to detoxify the meal of lectin and phorbolsters. Heat treatment inactivated lectin, but not phorbolster. One of the treatments reduced phorbolsters to a tolerable level of 0.09 mg/g. The treated meals with other ingredients were used in diets to assess acceptance and nutritive value of detoxified *Jatropha curcas* meal in two experiments. Experiments 1 and 2 had twelve (12) male weanling rats each, Sprague Dawley strain, 28-30 days old, pre-experimental average body weights of 8379±7.2 and 84.6±6.4 g, respectively. They were divided into three groups according to body weight and fed casein diet (control) and two diets in which *Jatropha curcas* was the protein source. In experiment 1, the rats accepted diet 1, but did not fully accept diet 2. Food intake, growth rate, protein efficiency ratio (PER) and food transformation index (TI) were significantly better ($P<0.05$) in diet 1 than in the casein and diet 2. In experiment 2, casein diet was better ($P<0.05$) in food intake, growth rate, PER and TI than diets 1 and 2. Food intake with *Jatropha* meal was significantly reduced and the rats had drastic body weight loss ($P<0.05$) and this might be due to the presence of phorbolsters in the diets. Generally, the presence of phorbolsters in food has significant effect on its acceptance. *Jatropha* meal obtained from treatment 3 had a crude protein (CP) content of 68%, far higher than the CP content of most oilseed meals (soyabean). This treatment seems a better method of detoxifying *Jatropha curcas* meal for livestock but in economic terms it is expensive to produce a meal from it.

Keywords: *Jatropha curcas* meal, phorbolster, lectin, food intake, growth rate, PER, TI, rat.

1 INTRODUCTION

Jatropha curcas belongs to the Euphorbiaceae family. It is a multipurpose tree (has medicinal properties and also as an oilseed) of significant economic importance. The plant is widely distributed in the wild or semi-cultivated in Central and South America, Africa, India and South East Asia (Cano-Asseleih, 1986; Cano-Asseleih *et al.* 1989). The seed weighs about 0.75 g and the kernel contained protein and lipid contents of 27 – 32% and 58 – 60% respectively (Aderibigbe *et al.* 1997). In addition to being a source of oil, *Jatropha* also provides a meal that serves as a highly nutritious and economic protein supplement in animal feed, if the toxins are removed (Becker & Makkar, 1998). *Jatropha curcas* meal (fully defatted) has a protein content of between 53 – 58% CP.

Studies with animals (Adam, 1974; Ahmed & Adam, 1979a, b; El Balwi *et al.* 1995; Liberalino *et al.* 1988) have shown that the seeds are toxic. The seeds of *Jatropha curcas* are known to be toxic to mice (Adam, 1974) and rats (Stirpe *et al.* 1976). Liberalino *et al.* (1988) found a high degree of toxicity in the raw, cooked or roasted seeds of *Jatropha curcas*.

They found that all rats fed on diets containing different nut fractions died; with raw nuts death occurred within 23 days, with raw or cooked nut oil, within 68 days; and with roasted nuts, within 14 days. Ahmed & Adam (1979a) fed *Jatropha curcas* to six calves by at doses of 2.5, 1 and 0.25 g/kg once, and to two other calves

at 0.025 g/kg up to 14 days. The onset and manifestations of toxicity in the six calves was rapid and death occurred within 19 hours of administration. The two calves that received daily the lowest dose of *Jatropha curcas* showed signs of poisoning and died within 10-14 days. The clinical signs of diarrhoea, dyspnoea, dehydration and loss of condition were well correlated with the pathological findings. There was an increase in aspartate aminotransferase, ammonia and potassium, and decrease in total protein and serum of *Jatropha* poisoned calves.

In another experiment, Ahmed & Adam (1979b) studied the sequential development of the clinical signs and lesions in the organs of desert sheep and Anglo-Nubian goats dosed with *Jatropha curcas* seeds at 0.05, 0.5 and 1 g/kg/day. Diarrhoea, reduced water consumption, dehydration, sunken eyes, inappetence and loss in condition were the important signs of poisoning in the sheep and goats.

El-Badwi *et al.* (1995) reported high mortality and severe pathological changes in Brown Hixec chicks fed diet containing 0.5 % *Jatropha curcas* seed. The above observations have therefore restricted its use as a food or feed source. The toxic or irritant compounds isolated from *Jatropha* seeds include curcin, a lectin (Siegel 1893; Felke, 1913; Stirpe *et al.* 1976), flavonoids, vitexine and isovitexine (Mourque *et al.* 1961) and 12-deoxy-16-hydroxyphorbol (Adolf *et al.* 1984). Lectin was thought responsible for the toxicity of *Jatropha* (Cano-Asseleih,

1986; Cano-Asseleih *et al.* 1989), however, recent reports have shown that lectin is not the major toxic principle in *Jatropha curcas* meal (Aderibigbe *et al.* 1997; Aregheore *et al.* 1998). The meal of *Jatropha curcas* has a high activity of trypsin inhibitor and lectin but these can be reduced by heat treatment (Aderibigbe *et al.* 1997).

The high concentration of phorbol esters present in *Jatropha* seed has been identified as the main toxic agent responsible for *Jatropha curcas* toxicity (Makkar & Becker, 1997a, b). It is not possible to destroy phorbol esters by heat treatment because they are heat stable and can withstand roasting temperature as high as 160⁰ C for 30 min. however, it is possible to reduce its concentration in the meal by chemical treatments (Makkar & Becker, 1997a, b).

This research therefore intends to investigate (1) the effect of heat and different chemical treatments employed in the detoxification of a meal of a toxic variety (Cape Verde, Nicaragua) of *Jatropha curcas*; and (2) carry out nutritional evaluation of the detoxified meals using rats. The parameters measured were growth rate, food intake, protein efficiency ratio (PER), and food transformation index (TI).

2 MATERIALS AND METHODS

2.1 PROCESSING OF THE TOXIC SEEDS FOR MEAL

The seeds used were from Nicaragua (a toxic variety, Capo Verde). The seeds were dehulled, ground and completely defatted in a Soxhelt apparatus using petroleum ether (b. p. 40 – 60⁰ C). The defatted and ground kernel is referred to as the meal.

After taking the defatted meal out of the soxhelt apparatus it was evenly spread on a tray lined with aluminum foil. The tray was kept over night in a fume cupboard to get rid of any remaining petroleum ether and for the defatted meal to get dry.

2.2 DETOXIFICATION PROCEDURES

(i) *Treatment with Methanol*

Approximately 300 g of the defatted meal was weighed into a 1000 ml capacity beaker and 600 ml of distilled water was added to bring the moisture level to 66%.

The mixture was made into a paste using a glass-stirring rod. This was covered with aluminum foil and placed in an autoclave at 121⁰C for 30 min.

The autoclaved sample was removed and allowed to cool at room temperature. The sample was later placed in a freezer and lyophilized the following day. After lyophilization it was ground with a simple laboratory mill to produce the meal.

(ii) *Treatment with Sodium hydroxide + sodium hypochlorite*

300 g of the defatted meal was weighed into a 1000 ml capacity beaker and mixed with 4 % sodium hydroxide (NaOH) to form a paste. This was followed by the intermittent addition of 30 ml of sodium hypochlorite (NaOCl) to the paste in a fume cupboard using a variable microliter pipette (Research Typ 3110, Labortechnik Griesinger). In between the addition of NaOCl, the paste

was thoroughly mixed to bring the moisture content to approximately 66%. Consequently the beaker was covered with aluminum foil, placed inside autoclavable disposable bags and autoclaved at 121⁰ C for 30 min.

The beaker content was allowed to cool, placed in a freezer and lyophilized. The dry paste was removed from the beaker and ground using a simple laboratory mill to give the meal.

(iii) *Sodium hydroxide treatment followed by washing with distilled water*

300 g of the defatted meal was weighed into a 1000 ml capacity beaker, followed by the addition of approximately 4% NaOH solution to form a paste. The paste was heat treated, lyophilized and milled as described in treatment 2. Consequently, the lyophilized meal was washed with distilled water three times, prior to milling.

(iv) *Sodium hydroxide treatment followed by washing with water*

The NaOH treated meal was similar to that mentioned above in treatment iii. The NaOH treated meal was washed four times with water instead of 92% methanol that was used as in treatment iii.

In all excluding the methanol treatment, there were thirteen meals that were chemically treated followed by heating at 121⁰ C for 30 min. (see table 1 for the various treatments).

2.3 PHORBOLESTERS AND LECTIN ASSAYS

To determine the concentration of phorbol esters and lectin meals of *jatropha* were subjected to different chemical treatments. The quantification of phorbol esters was carried out by the procedures described by Makkar *et al.* (1997), while lectin was carried out by hemagglutination assay (Aregheore *et al.* 1998).

2.4 EXPERIMENT 1

2.4.1 Diets, feeding and management

From the treated meals (Table 1), treatments 3 and 7 were selected for testing in experiment 1 because they contained the lowest concentrations of phorbol esters. The meals were compounded with other ingredients to formulate the diets that are shown in Table 2. The source of protein in the diets were casein (control); 92% methanol treated *Jatropha* meal (diet 1), and 4.0% NaOH (w/w) + 10 % NaOCl (v/w) treated *Jatropha* meal (diet 2). Other ingredients used in the diets were DL – methionine, (only for the casein diet), soya oil, cellulose, sucrose, rice starch, vitamin and mineral mixtures. All the diets were formulated to contain 10% CP. The ingredients were mixed with a food processor, pelleted and later dried in a forced air oven at 50⁰ C for 3 days.

2.4.2 Animals, experimental design and housing

Twelve (12) male weanling rats (28 – 30 days of age) of the Sprague Dawley strain were purchased from an animal farm (Charles River Deutschland GmbH, Sandhofer, Sulzfeld) and used in the growth study. The rats with the initial average body weight of 83.7 ± 7.2 g were fed a laboratory standard diet (Altromin Standard diets, Germany) for 4 days, to get acclimatized to the new

environment. After which they were weighed and divided into three equal treatment groups of 2 rats per group and 4 per treatment in organic glass cages. The housing provided had the following conditions, controlled lighting of 12:12 h of light:dark, temperature from 22 – 23⁰ C and relative humidity of approximately 51%.

The rats were given 4 days to adjust to the experimental diets before the 7-day growth trial. The three diets were weighed out daily and offered *ad libitum* at 9:00 h to the rats, and water was given free choice.

The food that was not consumed within 24 h was weighed and discarded, prior to determining daily food intake. Data for initial and final body weights were used to calculate weight gain/loss of the rats.

During the experimental period (7 days) daily faecal production were collected, weighed and kept in 50 x 27 mm, 20 ml glass bottles in deep freezer for future analysis. On day 7 (the last day of the experiment), food was withdrawn 6 h from the rats before they were weighed and sacrificed.

2.5 EXPERIMENT 2

2.5.1 Diets, feeding and management

In experiment 2, two diets including a casein diet (control) were used. The meals were obtained from (i) 4.0% NaOH treatment followed by 2 x washing with 92% methanol (diet 1); and diet 2 (4.0% NaOH treatment followed by washing 4 x with distilled water).

The assumptions in the use of these meals were that with 4.0% NaOH treatment and washing as in treatment 3 (Table 1), the concentration of phorbol-ester would be reduced and rats may accept the diets prepared from the

meals. The meals with other ingredients were used for the diets. The diets were processed as described in experiment 1. Table 3 presents particulars of the diets.

2.5.2 Animals, experimental design and housing

Twelve male rats with an average pre-experimental body weight of 84.6 ± 6.4 g, 28 days old were used in a trial that lasted 7 days. Rats were divided into three groups of four and subjected to the same management procedures as in experiment 1. Initial body weights of the rats were recorded at the beginning and end of the experiment and were used to compute weight gain/loss. In both experiments 1 and 2, food intake was regarded as the total amount consumed daily by each rat, and this was determined by weighing the amounts of diet given, refused and spilled. The Thomas-Mitchell (1924) biological Techniques were used to calculate protein efficiency ratio (PER) and food transformation index (TI).

2.6 Analytical procedures

The crude protein (CP) of the untreated, defatted and chemically treated *Jatropha* meals was determined in accordance to AOAC (1980) procedure. The following parameters were determined for each experiment – food intake (expressed as dry matter), body weight gain/loss, protein efficiency ratio (PER), and food transformation index (TI).

Data on food intake, growth rate, PER and TI were analyzed by standard analysis of variance, (Steel & Torrie, 1980) and where significant difference occurred, Bonferroni *t*-statistics were used for comparison among means (Gill, 1978).

Table 1 Concentrations of phorbol-ester and lectin in heat and chemically treated meals of a toxic variety of *Jatropha curcas*

Treatments	Phorbol-ester	Lectin
1) Untreated	1.78	102 ^a
2) Control (defatted, heated)	1.78	1.17 ^b
3) Control (treated followed by 4 times washing with 92% methanol)	0.09	nil
Chemical treatments followed by heating at 121⁰ C for 30 min		
4) 2.5% NaOH (w/w) + 10% NaOCl* (v/w)	0.22	nil
5) 3.0% NaOH (w/w) + 10% NaOCl* (v/w)	0.22	nil
6) 3.5% NaOH (w/w) + 10% NaOCl* (v/w)	0.14	nil
7) 4.0% NaOH (w/w) + 10% NaOCl (v/w)	0.13	nil
8) 2.0% NaOH (w/w)	0.89	nil
9) 2.5% NaOH (w/w)	0.34	nil
10) 3.0% NaOH (w/w)	0.29	nil
11) 3.5% NaOH (w/w)	0.18	nil
12) 2.0% NaOH (w/w) + 15% NaOCl* (v/w)	0.46	nil
13) 2.0% NaOH (w/w) + 20% NaOCl* (v/w)	0.47	nil
14) 2.0% NaOH (w/w) + 25% NaOCl* (v/w)	0.24	nil
15) 4.0% NaOH followed by 2 x washing with 92% methanol	not determined	nil
16) 4.0% NaOH followed by 4 x washing with dist. H ₂ O	not determined	nil

Phorbol-esters (phorbol-12-myristate 13-acetate equivalent). Lectin (inverse of minimum amount of the sample in mg/ml of the assay which produced agglutination).^{a,b} values within anti-nutritive factor (lectin) for each variable of different superscript differ (P<0.05); * % active Cl 6-13 (Riedel-de Haen AG, Seelze, Germany)

3 RESULTS

3.1 CONCENTRATION OF ANTI-NUTRITIONAL FACTORS (ANFS)

Table 1 presents the final concentration of phorbolsters and lectin obtained for each treatment - heat-treated meals followed by different chemical treatments. The concentrations of phorbolsters (phorbol-12-myristate 13-acetate equivalent) in both untreated and heat treated meals at 121⁰ C, 30 min, 66% moisture was 1.78 mg/g. The concentrations of lectin, (inverse of minimum amount of the sample in mg/ml of the assay which produced agglutination) for both meals were 102 mg/ml and 1.17 mg/ml, respectively (Table 1).

The concentration of lectin in defatted and heat-treated meal was significantly lower (P<0.05) than in the untreated meal. Chemical treatments consequently, removed all traces of lectin in the various meals. However, the concentration of phorbolster did not change even when the meals were subjected to heat treatment at 121⁰ C for 30 min. Treatments 3 and 7 reduced the concentrations of phorbolsters to levels of 0.09 and 0.13 mg/g (as phorbol-12-myristate 13-acetate equivalent),

respectively. However, other chemical treatments did not reduce the concentration of phorbolsters.

3.2 FOOD INTAKE, GROWTH RATE, PER AND TI OF RATS

Table 4 presents data on food intake, growth rate, PER and TI of rats fed the different diets in experiment 1. All the parameters investigated were affected by the kind of chemical treatments applied to the meal used. For example rats on diet 2 had very low food intake, growth rate, PER and TI (P<0.05) compared to the rats fed diet 1. Consequently rats on diet 2 were very weak, emaciated and sometimes strike against the walls of the cages and were sacrificed before the termination of the experiment.

However, the rats fed diet 1 had the same level of food intake as the rats on the casein diet (control diet) and they exhibited no symptoms of ill health. Body weight gain, food intake, PER and TI were best in the rats in diet 1 above the casein fed rats. The rats in the casein diet had diarrhoea (production of sausage like pellet faeces sometimes covered with mucus), after the third day. Besides, those rats in casein diet, the rats in diet 3 had low live weight gain. It was however, observed that the poor performance was due to the fact that the casein used as control had expired, and this might have been responsible

Table 2 Percentage composition of diets used in experiment 1

Ingredients (%)	Diets		
	Casein	1	2
Casein	9.83	-	-
DL-Methionine	0.52	-	-
<i>Jatropha</i> (1) +	-	16.0	-
<i>Jatropha</i> (2) ++	-	-	16.0
Soya oil	5.0	5.0	5.0
Cellulose	4.0	4.0	4.0
Sucrose	10.0	10.0	10.0
Rice starch	62.22	56.57	56.57
Vitamin mix *	2.0	2.0	2.0
Mineral mix *	6.43	6.43	6.43
Total	100.00	100.00	100.00

+ Heat treated and 4 x washing of treated meal (autoclaved at 121⁰ C for 35 min) with 92% Methanol

++ 4.0% NaOH (w/w) + 10% NaOCl (v/w) treated meal

*Vitamin and mineral mix, products of Alma Futter, Allgäu, Germany.

Table 3 Percentage composition of diets used in experiment 2

Ingredients (%)	Diets+		
	Casein	1	2
Casein	9.83	-	-
DL-Methionine	0.52	-	-
<i>Jatropha</i> (1) +	-	16.0	-
<i>Jatropha</i> (2) ++	-	-	16.0
Soya oil	5.0	5.0	5.0
Cellulose	4.0	4.0	4.0
Sucrose	10.0	10.0	10.0
Rice starch	62.22	56.57	56.57
Vitamin mix *	2.0	2.0	2.0
Mineral mix *	6.43	6.43	6.43
Total	100.00	100.00	100.00

+ 4.0% NaOH (w/w) and 2 x washing with 92% Methanol

++ 4.0% NaOH (w/w) and 4 x washing with distilled water; *Vitamin and mineral mix, products of Alma Futter, Allgäu, Germany

Table 4 Food intake, growth rate, PER and TI of rats fed diets containing treated meals of a toxic variety of *Jatropha curcas* (experiment 1)

Parameters	Diets+		
	Casein	1	2
Initial av. wt. (g)	86.9 ± 7.2	85.8 ± 12.5	75.5 ± 10.1
Final av. wt. (g)	88.7 ± 5.3	102.5 ± 15.3	56.8 ± 10.2
Body wt gain/loss (g)	1.8 ± 4.7 ^b	16.7 ± 3.6 ^a	-18.7 ± 0.9 ^c
Daily wt gain (g)	0.26 ± 0.7 ^b	2.4 ± 0.5 ^a	-2.7 ± 0.2 ^c
Daily food intake (g)	14.1 ± 1.8 ^a	13.6 ± 0.8 ^a	2.9 ± 0.5 ^b
Protein efficiency ratio (PER)	1.8 ^a	1.7 ^a	-0.9 ^b
Food transformation index (TI)	54.2 ^a	1.5 ^c	10.2 ^b

+ Heat treated meal (autoclaved at 121⁰ C for 35 min) and 4 x washing with 92% methanol

+ 4.0 % NaOH (w/w) + 10% NaOCl (v/w) treated meal

$$\text{PER} = \frac{\text{weight gained (g/rat/day)}}{\text{protein intake (g/rat/day)}}$$

$$\text{TI} = \frac{\text{food intake (g/rat/day)}}{\text{Weight gain (g/rat/day)}}$$

^{a, b, c:} values in the same row with different superscript differ significantly (P<0.05).

Table 5 Food intake, growth rate, PER and TI of rats fed diets containing treated meals of a toxic variety of *Jatropha curcas* (experiment 2)

Parameters	Diets+		
	Casein	1	2
Initial av. wt. (g)	84.6 ± 2.2	84.9 ± 2.0	84.4 ± 2.1
Final av. wt. (g)	129 ± 5.3	86.6 ± 2.0	79.8 ± 1.9
Body wt gain/loss (g)	44.4 ± 3.1 ^a	1.7 ± 0.0 ^b	-4.6 ± 0.2 ^c
Daily wt gain (g)	6.3 ± 0.4 ^a	0.2 ± 0.0 ^b	-6.6 ± 0.3 ^c
Daily feed intake (g)	14.1 ± 1.8 ^a	2.1 ± 0.2 ^b	-2.2 ± 0.3 ^c
Protein efficiency ratio (PER)	4.4 ± 0.2 ^a	0.9 ± 0.0 ^b	-0.3 ± 0.1 ^c
Food transformation index (TI)	2.3 ^b	10.5 ^a	-4.4 ^c

+ 4.0% NaOH (w/w) and 2 x washing with 92% Methanol

+ 4.0% NaOH (w/w) and 4 x washing with distilled water

$$\text{PER} = \frac{\text{weight gained (g/rat/day)}}{\text{protein intake (g/rat/day)}}$$

$$\text{TI} = \frac{\text{food intake (g/rat/day)}}{\text{Weight gain (g/rat/day)}}$$

^{a, b, c:} values in the same row with different superscript differ significantly (P<0.05).

for the low live-weight gain and the sausage like pellet faeces voided by the rats offered it.

In experiment 2, casein from a different source (Sigma-Aldrich Chemie, Steinheim, Germany) was used as the control diet. Comparatively, rats in the casein diet had higher food intake, growth rate, PER and TI than the rats in diets 1 and 2 (table 5). The meals used for diets 1 and 2 were treated with 4% NaOH (w/w) followed by washing 2 x with 92% methanol or 4 x washing with distilled water, respectively.

These rats had poor food intake, body weight gain, PER and TI (P<0.05) compared to the rats in the casein diet. The poor performance of the rats in diets 1 and 2 was comparable to the results obtained for diet 2 rats in experiment 1.

4 DISCUSSION

The untreated meal and the fully defatted meal had CP values of 25.6% and 52.8%, respectively. The methanol treated meal had 68.0% CP, while the NaOH + NaOCl treated meal had 55% CP.

The increase in CP in methanol treated meal could be due to proportionate higher loss of non-protein components. The heat treatment did not decrease the concentration of phorbolsters and this confirmed the earlier reports of Makkar & Becker (1997a, b), that heat alone cannot inactivate it. However, with the additional chemical treatments, the concentration of phorbolsters was reduced and the most effective among all the treatments carried out were treatments 3 and 7 (Table 1).

The results suggested that the concentration of 0.13 mg/g phorbol esters present in the *Jatropha curcas* meal that made up 16% of diet 3 has a significant adverse effect on food intake and growth rate of rats. The reduced food intake, loss in body weight, low PER and TI may have resulted from higher concentrations of phorbol esters in the test diets compared to the control.

It has been reported that in rat food intake is influenced by a variety of factors, such as the (i) amino acid pattern of the dietary protein, (ii) taste, (iii) smell and (iv) texture of the diet (Temler et al 1983). The low food intake of rats in diet 2, (Experiment 1) and in diets 1 and 2 (experiment 2) could be attributed to probably such factors as taste, smell and texture, but not to the amino acids pattern of the *Jatropha curcas* meal. Except for low lysine and the sulphur-amino acids, *Jatropha curcas* meal has a good balance of amino acids (Becker, 1996; Makkar & Becker 1997a). The poor performance observed in the rats therefore could not be attributed to the quality of protein in the detoxified meals used in the different diets, but due to the above-mentioned factors.

The low performance observed in rats fed the *Jatropha curcas* meals suggest that rats cannot tolerate phorbol esters concentration of 0.13 mg/g present in the *Jatropha curcas* meal used in experiment 2 that was not well accepted by the rats (experiment 1).

The rats in this diet had poor utilisation of ingested food, emaciated body conformation and loss in body weight. The performance data of the rats confirmed the reports of Makkar & Becker (1997a, b) that the presence of phorbol esters in food has significant effect on its' acceptance.

The *Jatropha curcas* meals obtained from treatments 15 and 16, were chemically treated with 4.0% NaOH followed either by washing two times with 92% methanol or four times with distilled water, respectively (Experiment 2). These meals had a strong NaOH odour and this affected food acceptance, intake and finally food utilisation for growth.

Body weight gain is relative to diet intake therefore it could be assumed that the weight gain obtained in the rats fed diet 1 (experiment 1) was due to better utilisation of ingested food. Also the low food intake and loss in body weight observed in rats in diet 2 (experiment 1) and those in diets 1 and 2 (experiment 2) seems to indicate that the treatments used for these meals were not effective in eliminating the adverse effects of phorbol esters. With a high phorbol esters concentration, the acceptance of *Jatropha curcas* meal was always low (Makkar & Becker 1997a).

5 CONCLUSION

Phorbol esters was reduced to a tolerable level of 0.09 mg/g when *Jatropha curcas* meal was heat-treated and washed 4 x with 92% methanol (treatment 3, table 1). The meal derived from it had a CP content 68%, which was far higher than the crude protein content of most oilseed meals e.g soyabean (45.7% CP).

Heat treatment at 121⁰ C, 30 min (with 66% moisture) followed by 4 times washing with 92% methanol seems a better means of detoxifying *Jatropha curcas* meal and this confirm the earlier report of Makkar & Becker (1997a).

Jatropha curcas meal as plant protein source can substitute for conventional meals such as the expensive soyabean meal.

This treatment is promising, but in economic terms it is expensive to produce *Jatropha curcas* meal from it. However, it can be exploited by small-scale industry. The price can be reduced if the methanol is recovered.

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REFERENCES

- Adam, S. E. I., 1974. Toxic effects of *Jatropha curcas* in mice. *2*, 67-76.
- Aderibigbe, A. O., Johnson, C. O. L. E., Makkar, H. P. S. and Becker, K., 1997. Chemical composition and effect of heat on organic matter and nitrogen degradability and some anti-nutritional components of *Jatropha* meal. *Anim. Feed Sci. Technol.* **67**, 223-243.
- Adolf, W., Opferkuch, H. J., Hecker, E., 1984. Irritant phorbol derivatives from four *Jatropha* species. *Phytochem.* **23** (1), 129-132.
- Ahmed, O. M. M. and Adam, S. E. I., 1979a. Effects of *Jatropha curcas* on calves. *Vet. Pathol.* **16**, 476-482.
- Ahmed, O. M. M., Adam, S. E. I., 1979b. Toxicity of *Jatropha curcas* in sheep and goats. *Research Vet. Sci.* **27**, 89-96.
- AOAC, 1980. *Official Methods of Analysis*. 13th Edition. Association of Official Agricultural Chemists, Washington, DC.
- Aregheore, E. M., Makkar, H. P. S. and Becker, K., 1998. Assessment of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. *J. Sci. Food Agric.* **77**, 349-352.
- Becker, K., 1996. Effect of different treatments on level of toxins in *Jatropha curcas* meal and effect of feeding on growth of fish (carp), rats and chickens. A report. *Institute for Animal Production in the Tropics and Subtropics* **480**, 27 pp. University of Hohenheim, Stuttgart, Germany.
- Becker, K., Makkar, H. P. S., 1998. Toxic effects of phorbol esters in carp (*Cyprinus carpio* L.) *Vet. Human Toxicol.* **40**, 82-86.
- Cano-Asseleih, L. M., 1986. Chemical investigation of *Jatropha curcas* L. seeds. *Ph.D. Thesis, University of London*, U. K. 290 pp.
- Cano-Asseleih, L. M., Plumbly, R. A. and Hylands, P. J. 1989. Purification and partial characterization of the hemagglutination from seeds of *Jatropha curcas*. *Jour. Food Biochem.* **13**, 1-20.
- El Badwi, S. M. A., Adam, S. E. I. and Hapke, H. J., 1995. Comparative toxicity of *Ricinus communis* and *Jatropha curcas* in Brown Hissex chicks. *Dtsch. Tierarztl. Wochenschr.* **102**(2), 75-77.
- Felke, J., 1913. Über die Giftstoffe der Samen von *Jatropha curcas*. *Landw. Vers. Stat.* **82**, 427-467.
- Gill, J. L., 1978. *Design and Analysis of experiments*. Vol. 1. Iowa State University Press, Ames, IO, USA.

- Liberalino, A. A. A., Bambirra, E. A., Moraes-Santos, T. and Viera, C. E., 1988. *Jatropha curcas* L. seeds. Chemical analysis and toxicity. *Arq. Biol. Technol.* **31**, 539-550.
- Makkar, H. P. S. and Becker, K., 1997a. *Jatropha curcas* toxicity: identification of toxic principle(s). In: *5th International symposium on poisonous plants*. May 19-23, 1997, San Angelo, Texas, USA.
- Makkar, H. P. S. and Becker, K., 1997b. Potential of *Jatropha* seed cake as protein supplement in livestock feed and constraints to its utilization. In: *Proceedings of Jatropha 97: International symposium on Biofuel and Industrial Products from Jatropha curcas and other Tropical Oil Seed Plants*. 23-27 Feb., 1997. Managua/Nicaragua, Mexico.
- Makkar, H. P. S., Becker, K., Sporer, F. and Wink, M., 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *Jour. Agric. Food Chem.* **45**, 3152-3157. 267-276.
- Mourque, M., Delphant Beret, R., Kassab, R., 1961. Etude de la toxicité et localisation de l'atoxalbumine (curcine) des graines de *Jatropha curcas* Linn., *Bull. Soc. Chim. Biol.* **41**, 517-531.
- Siegel, A., 1893. *Über die Giftstoffe zweier Euphorbiaceen*. Ph. D. Dissertation. Medicinal Faculty, Imperial Univ., Dorpat.
- Stirpe, F., Pession Brizzi, A., Lorenzoni, E., Strochi, P., Montanaro, L. and Sperti, S., 1976. Studies on the proteins from the seeds of *Croton tiglium* and *Jatropha curcas*. *Biochem. Jour.* **156**, 1-6.
- Steel, R. G. D. and Torrie, J. H., 1980. *Principles and Procedures of Statistics*. McGraw-Hill, N.Y.
- Temler, R. S., Ch. Dormond, A., Finot, P. A., 1983. Biological assessment of protein from different sources by protein efficiency ratio (PER) and by nitrogen retention. *Nutr. Report International* **28**,