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EVALUATION OF THE NUTRITIONAL QUALITY OF NONTOXIC KERNEL FLOUR FROM *JATROPHA CURCAS* L. IN RATS

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ABSTRACT

The origin of *Jatropha curcas* L. is in Central America, probably Mexico, although it is also distributed in South America, Africa and Asia. In Mexico, it grows as nontoxic and toxic *J. curcas* genotypes. In this work, the protein quality including protein efficiency ratio (PER), net protein ratio (NPR) and true digestibility (TD) of nontoxic genotype defatted flour was assessed using Wistar rats. The probed diets contained flour (3), flour-lysine, 1% (4), flour-phytase, 500 FTU (5) and two control diets: nitrogen-free (1) and casein (2). The rats were fed for 28 days. The PER (1.37, 1.77 and 1.61) and NPR (1.80, 2.29 and 2.12) obtained values for diets (3.4 and 5) were lower than those obtained for casein (2.07 and 2.46), respectively. No statistical differences were found in TD.

PRACTICAL APPLICATIONS

Jatropha curcas defatted flour may be used in the food industry for the development of diets for human and animal consumption. Besides achieving fortification of foods that are made from wheat, which has a low protein content, with the addition of *Jatropha* meal, these foods will improve the protein quality of many food products.

INTRODUCTION

In Mexico, *Jatropha curcas* L. grows wild in tropical and semi-tropical climates, at elevations of 0–1,650 m.a.s.l., in infertile and sandy soils. On spreading, the seeds germinate within 3–5 days. Interestingly, only Mexico has reported a nontoxic *J. curcas* genotype. The seeds of this nontoxic genotype are traditionally used in Veracruz, Puebla and Hidalgo States of Mexico to prepare a variety of traditional dishes (Makkar *et al*. 1997; Martínez-Herrera *et al*. 2006).

It is noteworthy that seeds of toxic and nontoxic genotypes cannot be differentiated morphologically so that special care must be exercised to differentiate these two types of seeds. The toxic seeds contain compounds known as phorbol esters that are highly toxic and when they are consumed can cause dizziness, vomiting, severe diarrhea and can even lead to death. Only through gas chromatography methods has it been possible to detect and quantify the phorbol esters (Goel *et al*. 2007). The seeds of *J. curcas* from Mexico have high protein (25–30%) and oil (55–62%) content, depending on the agro-climatic characteristics of the region. The oil seed can be converted into biodiesel. After oil extraction from dehulled kernels, the remaining meal contains 60% protein, and these proteins have a good amino acid balance. When it is compared with the Food and Agriculture Organization Reference Protein pattern for a growing child, the sulfur amino acid content is higher and lysine is the only limiting amino acid. The *in vitro* protein digestibility is above 80% (Martínez-Herrera *et al*. 2006). The seed contains some anti-nutritional compounds such as trypsin inhibitors, lectins and phytates. Trypsin inhibitors and lectins are inactivated by heat treatments, while phytate is heat stable (Makkar *et al*. 1997; Martínez-Herrera *et al*. 2006). A wealth of evidence exists showing the beneficial effect of several seeds: soybean

(Carroll and Kurowska 1995), sesame (*Sesamum indicum*) (Rajamohan and Kurup 1997), buck wheat (*Fagopyrum esculentum*) (Tomotake *et al*. 2001), lupine (*Lupinus albus* L.) (Sirtori *et al*. 2004), quinoa (*Chenopodium quinoa* Wild L.) (Takao *et al*. 2005) and cowpea (Frota *et al*. 2008). Both of them have been reported to exhibit cholesterol-lowering effects. A number of different mechanisms may explain the favorable effects of these seeds on cholesterolemia. A reduction in the intestinal absorption of cholesterol and/or bile acids, an increase in plasma cholesterol clearance through enhanced hepatic low-density lipoprotein (LDL) receptor activity and changes in the hepatic biotransformation of cholesterol can all be implicated. The meal kernel of *J. curcas* has been tested as feed ingredients for fish (*Nile tilapia*) after moist heat treatment (Makkar and Becker 1999; Martinez Herrera *et al*. 2007), but studies have not been conducted using rats to evaluate the protein quality and cholesterol-lowering effects of kernel meal obtained after removing the oil. The objective of this study was to evaluate the protein quality of nontoxic *J. curcas* flour and its cholesterol content in rats.

MATERIALS AND METHODS

Materials

Nontoxic *J. curcas* seeds were collected from Pueblillo, Veracruz, Mexico in August 2006. Seeds were manually dehulled to obtain the kernels. The kernels were ground into a flour mill (Cyclotec™, Foss, Eden Prairie, MN). The *Jatropha* meal was defatted with petroleum ether (60–65C) for 12 h. After that, *Jatropha* meal was autoclaved at 121C for 20 min at 88% moisture and then lyophilized (Lyovac GT2, Finn-Aqua Santasio-Sohlberg GmbH, Hurth, Germany).

Methods

Proximate Composition. The following Association of Official Analytical Chemist methods (A.O.A.C. 1995) were used to determine proximate composition of defatted and autoclaved meal: protein (NX6.25, method 955.04), lipids (method 920.39), neutral detergent fiber (method 985.29) and ash (method 923.03), and carbohydrate content was estimated by difference.

Determination of Antinutritional Compounds of *J. curcas* Flour. Trypsin inhibitor activity was determined according to method (Smith *et al*. 1980) and the enzyme was added at last (Liu and Markakis 1989). The phytic acid content was determined by a colorimetric procedure (Vaintraub and Lapteva 1988). Suitable aliquots were diluted with distilled water to make 3 mL and then used for the assay. Results are expressed as g/100 g phytic acid, using phytic acid (sodium salt, Sigma, St. Louis, MO) as a standard. Total saponin content was determined using a spectrophotometric method (Hiai *et al*. 1976). The concentration of saponins was read using a standard curve of different concentrations of diosgenin in 80% aqueous methanol and expressed as g/100 g diosgenin equivalents. Analysis of the lectin content was conducted by hemagglutination assay in round-bottomed wells of microtiter plates using 1% (v/v) trypsinized cattle blood erythrocyte suspension in saline phosphate buffer, pH 7.0 (Makkar *et al*. 1997). The hemagglutination activity was defined as the minimum amount of the *Jatropha* flour (in milligram per milliliter of the assay medium), which produced agglutination. The minimum amount was the material per milliliter of the assay medium in the highest dilution that was positive for agglutination. One hemagglutinating unit was defined as the least amount of material per milliliter in the last dilution giving positive agglutination (Grant *et al*. 1983). Extraction and estimation of phorbol esters were determined by high performance liquid chromatography (Makkar *et al*. 1998). The analytical column was a reverse phase C18 LiChrospher 100, 5 mm (250 × 4 mm internal diameter, Merck KGaA, Darmstadt, Germany), column protected with a guard column containing the same material as the main column according to the procedure outlined (Makkar *et al*. 1998). The results are expressed as equivalent to a standard phorbol-12-myristate 13-acetate, which appeared between 34 and 36 min.

Biological Evaluation. Protein quality evaluation was assessed using a set of diets prepared with thermal treatment of *J. curcas* defatted flour (autoclave 121C, 15 min, 66% moisture) (diet 3); diet 3 added 1% lysine (diet 4) and diet 3 added 500 FTU phytase (diet 5). Diets 1 and 2 were protein-free diet and casein-containing diets, respectively. Each protein diet was tested using growing Wistar rats (10 per diet), weighing 45 ± 5 g at the beginning of the study and randomly located in individual cages. The cages were housed in a room at 20 ± 1C and 55% relative humidity, under 12-h light/12-h dark cycles. Diets had the following composition: 10 g of protein, 9 g of fat, 2 g of vitamin mix, 5 g of mineral mix, 5 g of cellulose and 69 g of corn starch to complete 100 g. Corn oil was used as the fat source. The vitamin and mineral mixes were AIN-93-VX and AIN-936-MX and were obtained from Harland Tekland Laboratory Animal Diets (Madison, WI). Food and water were given *ad libitum*. Feed intake was recorded everyday. Weight gain was recorded weekly; protein efficiency ratio (PER) was determined for a period of 28 days. Net protein ratio (NPR) was measured for an 8-day period during 18–26 test days. Feed and fecal nitrogen contents were analyzed by micro-Kjeldahl method. Apparent digestibility (%) was

TABLE 1. CHEMICAL COMPOSITION OF WHOLE FLOUR, DEFATTED FLOUR AND DEFATTED FLOUR AFTER HEAT TREATMENT (g/100 g DRY MATTER BASIS FOR ALL EXCEPT GROSS ENERGY)

Sample Pueblillo	Gross energy (MJ/kg)	Moisture (%)	Crude protein (%)	Oil (%)	Ash (%)	Fiber (%)	NDF (%)
Whole flour	31.1 ± 0.2	4.66 ± 0.3	31.1 ± 0.2	57.4 ± 0.1	4.7 ± 0.2	3.2 ± 0.1	3.7 ± 0.2
Defatted flour	19.0 ± 0.1	9.70 ± 0.3	63.7 ± 0.0	0.5 ± 0.1	9.8 ± 0.2	4.9 ± 0.1	9.7 ± 0.3
Defatted flour after heat treatment	19.1 ± 0.2	5.7 ± 0.2	65.7 ± 0.1	0.7 ± 0.1	9.7 ± 0.3	5.2 ± 0.2	13.1 ± 0.3

The results represent the average of independent ± SD.
NDF, neutral detergent fiber; SD, standard deviation.

determined according to Eggum (1976). True digestibility (TD) was corrected for endogenous excretion of nitrogen.

The trial was performed for 28 days using Wistar rats (National School of Biological Sciences – National Polytechnic Institute): 50% females and 50% males. The rats were kept in cages (one per rat) and were located randomly to six diets with 10 replicates for each dietary treatment. Weekly, the variation in weight of each rat and the food eaten, food not consumed and wasted were recorded. Feces were stored and the nitrogen content was determined by the Kjeldahl method. After 28 days of the experiment, animals were sacrificed and blood samples were obtained. After the blood sampling, the rats were sacrificed. Using dissection equipment, the liver was extracted, washed with distilled water and weighed on a watch glass. The weight of livers from all animals was recorded and averaged for each group. Liver weight as a percentage of final body weight of the rodent was determined (Arjmandi *et al*. 1992).

Total Cholesterol, Total Triglycerides and High-Density Lipoproteins. Total cholesterol, total triglycerides and high-density lipoproteins (HDLs) were determined according to Biocromatic ABBOTT-VP (Abbott VP analyzer, Abbott Diagnostics, Dallas, TX) methodology (Anon 1979). The VP analyzer is a microcomputerized instrument for biological fluids. The blood in microfuge vials was centrifuged at 10,000 g for 15 min in a Beckman Microfuge TM II. Serum was separated from the pellet with the help of a Pasteur pipette and deposited in a vial.

Statistical Analysis

The obtained data were analyzed statistically with analysis of variance and Student's *t*-test in order to find significant differences between groups at the level of 0.05% using the statistical package SigmaStat 2.03 (Systat, Richmond, CA).

RESULTS AND DISCUSSION

Chemical Composition

The oil content of *J. curcas* kernels was 57.4 g/100 g and the protein content was 31.1 g/100 g (Table 1). Makkar *et al*. (1997) reported values of 19–29 and 43.9–59.0 g/100 g for protein and oil contents, respectively, in *J. curcas* kernel from different countries. The oil content of *J. curcas* seeds is comparable with the contents obtained for some conventional oilseeds: safflower (29.3%), sunflower (19.5%) and peanut (30.3%) (Gama *et al*. 2010; Alencar 2011; Ullah and Bano 2011). This unconventional seed may be an alternative to obtain oil and protein for human and animal consumption. The proportions of ash and fiber in kernel were 4.7 and 3.2 g/100 g, respectively, which are similar to other reports (Makkar *et al*. 1998; Makkar and Becker 1999).

The proportions of ash and fiber were 4.7 and 3.2%, respectively, which are similar to those previously reported (Makkar *et al*. 1998); however, in some parts of Nigeria, the proportion of fiber has been reported in higher concentration (Akintayo 2004).

Determination of Antinutritional Compounds of *J. curcas*

The trypsin inhibitor activity decreased by 98% after thermal treatment (Table 2). These digestive enzyme inhibitors adversely affect the nutritional quality of contained seed proteins. To improve the nutritional quality, inhibitors are usually inactivated by thermal treatment during food processing, which modifies inhibitor conformational structure (Khokhar and Chauhan 1986). The residual activity in the thermally treated flour represents about 2% found in the unthermal *J. curcas* flour; however, most meals keep 5–20% of original activity of trypsin and chymotrypsin inhibitor (Friedman and Brandon 2001). When thermal treatments are prolonged to destroy 100% of the protease inhibitor activity, damage of the protein quality may occur (Friedman and Brandon 2001).

Phytic acid is considered as an antinutrient mainly due to its ability to bind essential dietary minerals as well as proteins and starch and consequently decrease their bioavailability in humans (Phillippy *et al*. 2002). With respect to this compound, it appears that thermal treatment decreased slightly (6%) the phytic acid in *J. curcas* flour. Ibrahim *et al*. (2002) reported that thermal treatment decreased this compound in cowpea seeds by 6–11%.

TABLE 2. ANTINUTRITIONAL FACTORS OF *J. curcas* FLOUR DEFATTED AND WITH HEAT TREATMENT

Sample	TI (mg/g simple)*	Phytic acid (%)	Saponins (g/100 g)†	Lectins (mg/mL)‡	PE (mg/g)§
Defatted flour	36.4 ± 0.49[a]	9.1 ± 0.06[a]	2.77 ± 0.06[a]	5.89[a]	ND
Defatted flour heat treatment	0.53 ± 0.01[b]	8.5 ± 0.31[b]	2.41 ± 0.06[a]	0.35[b]	ND

Different letters in columns represent significant difference ($P < 0.05$).

* TI, miligram of pure trypsin inhibited per gram of sample.

† Diosgenin equivalents.

‡ Minimum amount of the sample required to show the agglutination after twofold dilution in 1 mL of final assay medium.

§ Equivalent to phorbol 12-myristate, 13-acetate.

ND, not detected; PE, phorbol esters; TI, trypsin inhibited.

The content of saponins found in *J. curcas* was 2.77 g/100 g, which is reduced by 13% by thermal treatment. Cooking and processing can have a significant effect on the levels of saponins in legumes. Interestingly, the results are not necessarily the same for all legumes. Soaking and cooking studies on chickpeas and lentils suggest that 2–5% of saponin content can be lost from chickpeas during cooking; however, a much larger, 6–14%, saponin can be lost from lentils (Ruiz *et al*. 1996). The method of cooking has a significant effect on saponin loss, with autoclaving having a large effect (Jood *et al*. 1986). Some saponins are thermolabile and may interconvert or degrade (e.g., soyasaponin VI forms soy saponin I with increased cooking time) (Price *et al*. 1986; Shi *et al*. 2004). In terms of human health, the biological significance of such interconversions is unclear. Phorbol esters were not detected in the samples; this seeds are consumed by humans and used as chicken feed.

Heat treatment improves the quality of the protein of *J. curcas*, as in many seeds, increasing the digestibility and absorption of them in addition to completely inactivating trypsin inhibitors and lectins that may have adverse health effects. So if you intend to use flour for food, *J. curcas* heat treatment and the addition of lysine and phytase are essential to have a better quality product.

Biological Evaluation

The PER (Table 3) value in the diets was statistically lower ($P < 0.05$) than that obtained for casein (2.07 ± 0.3), but it was within the levels reported for cereals and legumes (0.9–2.1). The PER value obtained for diet 4, which was supplemented with lysine, was 1.77 ± 0.20, followed by diet 5 of 1.61 ± 0.32 (added phytase), and finally the diet 3 (1.37 ± 0.31). These values were higher than reported for maize (1.2) and soybeans (1.4) (Friedman and Brandon 2001); treatments such as cooking, soaking, germination and fermentation are known to eliminate several antinutritional factors and to improve legume nutritional value, transforming it into a protein-rich food (Rozan *et al*. 1997). In this case, it is worth mentioning that cooking improves the PER value, the addition of limiting amino acid (Martínez-Herrera *et al*. 2006) can enhance their value (29%), while adding phytase can decrease the concentration of phytic acid, a compound that is found in significant quantities in the seeds of *J. curcas* (Martínez-Herrera *et al*. 2006). The high level of phytate present in *Jatropha* meals might decrease the bioavailability of minerals (especially Ca^{2+} and Fe^{2+}). Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interacting with enzymes such as

TABLE 3. NUTRITIONAL EVALUATION OF NONTOXIC *J. curcas* FLOUR

	Casein	Diet 3	Diet 4	Diet 5
PER*	2.07 ± 0.31[a]	1.37 ± 0.19[b]	1.77 ± 0.20[c]	1.61 ± 0.32[d]
NPR* (second week)	2.66 ± 0.59[a]	1.89 ± 0.32[b]	2.61 ± 0.40[a]	2.31 ± 0.39[d]
NPR* (fourth week)	2.46 ± 0.36[a]	1.80 ± 0.14[b]	2.29 ± 0.20[c]	2.12 ± 0.26[d]
TD*	89.41 ± 4.73[a]	96.71 ± 2.5[b]	94.98 ± 5.46[c]	95.43 ± 3.83[c]

* Average of 10 repetitions ± standard error.

Different letters in columns represent significant difference ($P < 0.05$).

Diet 2, casein (control); diet 3, flour *J. curcas* (JCH) with treatment (autoclave 121C/15 min, 88% humidity); diet 4, JCM-aqueous heat treated more lysine (1%); and diet 5, JCM with more phytase treatment (0.1%) (500 FTU).

JCH, Jatropha curcas with heat treatment without lysine and phytase; JCM, Jatropha curcas meal with heat treatment more lysine and diet 5, JCM Jatropha curcas meal with heat treatment more phytase; PER, protein efficiency ratio; NPR, net protein ratio; TD, true digestibility.

TABLE 4. LIPID PROFILE OF WISTAR RATS FED DIFFERENT DIETS

Diet	Cholesterol* (mmol/L)	HDL cholesterol* (mmol/L)	LDL cholesterol* (mmol/L)	Triglycerides* (nmol/L)
1	22.07 ± 1.58[a]	19.92 ± 0.85[a]	3.20 ± 0.43[a]	0.71 ± 0.24[a]
2	19.20 ± 1.52[a]	19.54 ± 1.02[a]	1.80 ± 0.41[a]	0.433 ± 0.07[b]
3	20.51 ± 0.74[a]	19.12 ± 0.79[a]	2.19 ± 0.48[a]	0.463 ± 0.07[a]
4	20.67 ± 1.26[a]	20.47 ± 1.55[a]	0.23 ± 0.07[b]	0.43 ± 0.09[b]
5	23.02 ± 1.35[a]	19.29 ± 1.61[a]	3.56 ± 0.55[a]	0.39 ± 0.07[b]

* Determinations represent the average of 10 independent determinations ± SD.

Different letters in columns represent significant difference ($P < 0.05$).

Diet 1, free of nitrogen; diet 2, casein (control); diet 3, flour *J. curcas* (JCH) with treatment (autoclave 121C/15 min, 88% humidity); diet 4, JCM-aqueous heat treated more lysine (1%); and diet 5, JCM with more phytase treatment (0.1%) (500 FTU).

JCH, Jatropha curcas with heat treatment without lysine and phytase; JCM, Jatropha curcas meal with heat treatment more lysine and diet 5, JCM Jatropha curcas meal with heat treatment more phytase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

trypsin and pepsin (Reddy and Pierson 1994); however, even if the PER value obtained was higher than in the original sample, that obtained for the sample with limiting amino acid was less, so it is likely that the addition of both would improve considerably the value of *Jatropha* protein.

This same effect occurs in the values of NPR obtained, which were 2.29 ± 0.20, 2.12 ± 0.26 and 1.80 ± 0.14 for diets 4, 5 and 3, respectively, and for casein it was 2.46 ± 0.36. The NPR values were higher than PER values because NPR measures protein efficiency based on animal growth and maintenance needs, allowing evaluation of proteins that do not promote growth. The PER value, in contrast, considers only growth needs, meaning that even if a diet has a PER of zero, it can still meet maintenance needs. This value can therefore underestimate protein quality in many cases. Digestibility indicates the amount of protein nitrogen absorbed. In this case, the digestibility values (%) were the following: diet 3, 96.7 ± 2.5; diet 4, 94.9 ± 5.4; diet 5, 95.4 ± 3.8; and casein 89 ± 4.7, which were higher than values for maize (76%), amaranth (78%) and soybeans (78%) (Friedman and Brandon 2001). While not all protein is used, its digestibility is good, considering that the digestibility of plant protein is low (Table 4). Finally, in the livers of rats fed *J. curcas* containing diets, no abnormality was observed.

Although the etiology in the development of obesity, cardiovascular disease, diabetes and cancer is multifactorial, diet is now considered as a triggering factor of great importance, which is because the modification of diet can be a good strategy to reduce the prevalence of these health conditions (Liu *et al*. 2006).

Table 4 indicates the effect of the diets given to the different study groups, showing a significant decrease in LDL-cholesterol concentration of diet 4 group and a slight but definite increase in HDL-cholesterol concentration. This change could be considered beneficial in relation to its effect on cardiovascular disease (Blaha *et al*. 2008; Ranjan 2009).

Among factors that may explain the cholesterol-lowering effect are those observed in soybeans and other seeds, such as phytic acid, fiber, phytosterols, saponins, proteins, peptides and amino acid profiles (Frota *et al*. 2008). Proteins can directly alter the metabolism of cholesterol and also by conjugation with bile acids, causing an increase in fecal excretion (Choi *et al*. 2010). A casein – amino acid mixture produces a hypercholesterolemia similar to that of casein. This appears to be mainly due to lysine and methionine, although other essential amino acids probably contribute to the effect. Arginine appeared to counteract the hypercholesterolemic effects of other essential amino acids (Carroll and Kurowska 1995).

Furthermore, the saponins isolated from different plants such as soybeans, alfalfa and chickpea (Garjani *et al*. 2009) have been shown to have hypolipidemic effect, which is mainly by blocking the intestinal absorption of cholesterol and increasing its secretion (Garjani *et al*. 2009; Campos-Vega *et al*. 2010).

TABLE 5. RELATIONSHIP BETWEEN LIVER WEIGHT AND THE WEIGHT OF THE RAT

	Casein	Diet 3	Diet 4	Diet 5
Weight rat/liver	12.08 ± 2.80	10.04 ± 1.56	10.85 ± 1.50	11.04 ± 2.73

* Average of 10 repetitions ± standard error.

Diet 2, casein (control); diet 3, flour *J. curcas* (JCH) with treatment (autoclave 121C/15 min, 88% humidity); diet 4, JCM-aqueous heat treated more lysine (1%); and diet 5, JCM with more phytase treatment (0.1%) (500 FTU).

JCH, Jatropha curcas with heat treatment without lysine and phytase; JCM, Jatropha curcas meal with heat treatment more lysine and diet 5, JCM Jatropha curcas meal with heat treatment more phytase.

Finally, there was not significant change in the relative weights of livers dissected in this study (Table 5). This behavior could be assumed that the main effect of *J. curcas* flour would be more oriented to their action on cholesterol metabolism rather than its effect on bile secretion.

CONCLUSIONS

The *J. curcas* flour is a valuable protein-rich material, which may be considered for incorporation in food and feed, as their PER. NPR and TD values were higher than those of other seed meals. The feeding of diets containing *J. curcas* flour did not cause any adverse effects, suggesting it to be safe as a food or feed component. It may also be noted that *J. curcas* seeds from the nontoxic genotypes are being consumed by human for centuries in only some regions of Mexico. Heat treatment improves the quality of the protein of *J. curcas*, as in many seeds, increasing the digestibility and absorption of them in addition to completely inactivate trypsin inhibitors and lectins that may have adverse health effects. So if you intend to use flour for food, the *J. curcas* heat treatment and the addition of lysine and phytase are essential to have a better quality product. Meal supplemented with lysine had a higher value of PER (diet 4). It would be appropriate to conduct further studies on biological assessment of lysine and phytase supplemented *J. curcas* meal/flour, which is expected to increase the PER.

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