JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Evaluation of Detoxification Methods on Toxic and Antinutritional Composition and Nutritional Quality of Proteins in *Jatropha curcas* Meal

Jianhui Xiao,[†] Hui Zhang,[*,†] Liya Niu,[‡] Xingguo Wang,[†] and Xia Lu[†]

[†]State Key Laboratory of Food Science and Technology and School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, People's Republic of China

[‡]School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, People's Republic of China

ABSTRACT: The *Jatropha curcas* meal was detoxified by different methods, and the effect of detoxification was evaluated in this study. The method that hydrolysis of enzymes (cellulase plus pectinase) followed by washing with ethanol (65%) had a significant ($p < 0.05$) effect on the toxin, antinutritional components, and nutritional quality of proteins. After this treatment, the phorbolesters (PEs) were decreased by 100%. The antinutritional components (phytates, tannins, saponins, protease inhibitor, and lectin activities) were decreased to tolerable levels, which were lower than those in soybean meal. The crude protein in detoxified meal was 74.68%, and the total content of amino acids was 66.87 g/100 g of dry matter. The *in vitro* protein digestibility (IVPD) increased from 82.14 to 92.37%. The pepsin-insoluble nitrogen was only 4.57% of the total nitrogen, and about 90% of the protein was true protein. The protein-digestibility-corrected amino acid score (PDCAAS) of the meal was 0.75. The results showed that this treatment was a promising way to detoxify *J. curcas* meal, and the nutritional quality of detoxified meal can be simultaneously enriched and improved.

KEYWORDS: *Jatropha curcas*, detoxification, phorbolesters, antinutritional components

INTRODUCTION

In recent years, biodiesel has been receiving considerable attention as a renewable source of energy. Biodiesel can be produced by transesterification of plant oils or animal fats. One of the non-edible feedstocks that has received great attention as a source of renewable energy is *Jatropha curcas*. *J. curcas* seeds contain 40−55% oil, which can be easily converted into biodiesel that meets American and European standards.[1,2]

After extraction of oil, the *J. curcas* meal is rich in protein between 50 and 64%. Except for lysine, all other essential amino acids in the meal have been reported to be higher concentrations than those of the Food and Agriculture Organization (FAO) reference pattern suggested for preschool children.[3] However, the *J. curcas* meal was found to be toxic to mice,[4] rats, calves, sheep, goats,[5,6] humans,[7] and chickens,[8] which greatly restrict its use. Some antinutritional components, such as saponin, phytate, trypsin inhibitor, glucosinolates, amylase inhibitors, flavonoids, vitexine, isovitexine,[9] and cyanogenic glucosides, as well as toxic/irritant compounds, such as curcin, β-D-glycosides of sitosterol, and 12-deoxy-16-hydroxy phorbol,[10−14] were reported in *J. curcas* meal. Apart from these, phorbolesters (PEs) present at high levels in the meal had been identified as the main toxic agent responsible for toxicity. If the toxin can be removed, *J. curcas* meal could be used as a protein source for livestock feeds.

Various methods, water leaching, autoclaving, and acid and alkali treatments, were adopted to detoxify *J. curcas* meal, and the detoxified meal was used to feed animals. However, a significant reduction in the feed intake, growth, and nutrient balance was found in the animals, and reduced protein digestibility in growing goat kids was observed.[15,16] The purpose of this study was to investigate various methods, especially enzyme treatment, to obtain a method that cannot only effectively remove or degrade the toxin and antinutritional components but also improve the nutritional quality of the detoxified meal.

MATERIALS AND METHODS

Materials. *J. curcas* seeds were procured from Sichuan province, China. Phorbol-12-myristate-13-acetate, standard phytic acid, diosgenin, and tannic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Cellulase and pectinase (the enzyme activities of the cellulase and pectinase were 126 filter paper units (FPUs) and 1.8 units/mg, respectively) were obtained from Novozymes A/S (Wuxi, China). All other chemical solvents used were of analytical grade.

Detoxification Method. *J. curcas* seeds were deshelled and ground. The ground kernel was defatted using a screw extruding−expanding pretreatment technique at 90 °C, followed by petroleum ether extraction [boiling point (bp) = 40−60 °C]. The defatted ground kernel of *J. curcas* meal was served as the control. The meal was treated by the following methods: (i) The sample was not treated further as a control. (ii) The sample was only treated with enzymes (cellulase plus pectinase) for 1 h. The procedure was carried out under this condition: cellulase (5 mg/g) and pectinase (10 mg/g) at 50 °C and pH 4.5−5.0 for 1 h. Hydrolysis was stopped by heated at 105 °C for 15 min. Hydrolysates was clarified by centrifugation (4500*g* for 15 min), and then the residue was freeze-dried and designated as a. (iii) After treated by step ii, the sample was washed using 60% methanol (5:1, v/v) and 65% ethanol (5:1, v/v), respectively. The procedure was at 50 °C with

Received: January 9, 2011
Accepted: March 7, 2011
Revised: February 28, 2011
Published: March 16, 2011

ACS Publications © 2011 American Chemical Society

constant stirring for 1 h. Then, the solvent was removed by centrifugation (4500*g* for 15 min) and recovered. The residue was freeze-dried and designated as b and c, respectively. (iv) The sample was only washed by 90% methanol (10:1, v/v) and 90% ethanol (10:1, v/v), respectively. The procedure was at 50 °C with constant stirring for 2 h. Then, the solvent was removed by centrifugation (4500*g* for 15 min) and recovered. The residue was freeze-dried and designated as d and e, respectively.

Determination of Proximate Composition. Moisture content, crude protein (CP), lipid, neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash content were determined in accordance with the standard methods of the Association of Official Analytical Chemists (AOAC).[17] Gross energy (GE) was estimated by an adiabatic bomb calorimeter (IKA C7000).

Determination of Toxin and Antinutritional Components. *Determination of PEs by High-Performance Liquid Chromatography (HPLC).* The sample (2 g) was weighed and subsequently extracted with methanol as described by Makkar and Aderibigbe.[18] The PE content was determined by HPLC (Agilent Technologies, Santa Clara, CA). The four PE peaks appearing between 26 and 31 min were identified at 280 nm. The results were expressed as equivalent to a standard (phorbol-12-myristate-13-acetate), which appeared between 34 and 36 min.

Determination of Phytohemagglutinating Activity. The lectin was conducted by a hemagglutination assay in round-bottomed wells of microtiter plates using 1% (v/v) trypsinized rabbit blood erythrocyte suspension in phosphate buffered saline (PBS) (10 mM, pH 7.0).[19] One hemagglutinating unit (HU) was defined as the least amount of material per milliliter in the last dilution giving positive agglutination.

Determination of the Trypsin Inhibitor Activity (TIA) and Phytic Acid and Saponin Contents. TIA was determined essentially according to Liu et al.[20] Phytic acid was determined by a colorimetric procedure described by Vaintraub et al.[21] Total saponin was determined using a spectrophotometric method described by Hiai et al.[22]

Determination of Total Phenolics. Total phenolics were extracted and determined by spectrophotometric methods described by Makkar.[23] Total phenolics were quantified by the Folin−Ciocalteu reagent, and results were expressed as tannic acid equivalents.

Nutritional Quality Evaluation. *Determination of Amino Acid.* The amino acid composition was determined using an amino acid analyzer (Agilent Technologies, Santa Clara, CA).[23]

Determination of the in Vitro Protein Digestibility (IVPD). The IVPD was measured using a multi-enzyme technique and calculated using the following regression equation: $y = 234.84 - 22.56x$, where y was IVPD (%) and x was pH of the protein suspension after 20 min of digestion with the enzyme solution.[24]

Determination of Buffer-Soluble Nitrogen, Non-protein Nitrogen, and Pepsin-Insoluble Nitrogen. The sample (5 g) was homogenized in 100 mL of phosphate buffer (0.05 M, pH 7.0) using an ultraturrax at 10000*g* for 20 min and then filtered. A total of 10 mL of the filtrate was mixed with 10 mL of 20% trichloroacetic acid (TCA), refrigerated overnight, and centrifuged (3000*g* for 10 min) to collect the supernatant. An aliquot (10 mL) of the supernatant was analyzed for non-protein nitrogen, and total soluble nitrogen was analyzed using the Kjeldahl analysis. Pepsin-insoluble nitrogen was determined as described by Makkar et al.[23] Results were expressed as grams of CP per 100 g of dry matter (DM).

Determination of the Chemical Score. The chemical score was calculated using the following formula:[25]

$$\frac{\text{grams of essential amino acid in test protein}}{\text{grams of total essential amino acid in test protein}} \times \frac{\text{grams of total essential amino acid in egg protein}}{\text{grams of essential amino acid in egg protein}}$$

Determination of the Essential Amino Acid Index (EAAI) and Biological Value (BV). EAAI was calculated according to the method by Oser,[26] and BV was calculated using the formula by Oser.[27]

$$\text{BV} = 1.09(\text{EAAI}) - 11.7$$

Determination of the Nutritional Index (NI). NI was calculated using the formula of Crisan and Sands.[28]

$$\text{NI} = \frac{\text{EAAI} \times \text{percent protein}}{100}$$

Determination of the Protein-Digestibility-Corrected Amino Acid Score (PDCAAS). PDCAAS was calculated using the following formula:[29]

$$\text{PDCAAS} = \frac{\text{amount of amino acid in test protein}}{\text{amount of amino acid in reference protein}} \times \text{digestibility of test protein}$$

Statistical Analysis. All of the values, except the content of amino acid, were expressed as the mean of triplicate determinations (standard deviation) and subjected to one-way analysis of variance using SPSS (version 13.0) software. Means were separated by Fisher's protected least significant difference ($p < 0.05$).

RESULTS AND DISCUSSION

Proximate Composition. Results for the proximate compositions of all of the treated samples were given in Table 1. The DM varied from 93.61 to 97.85%. The moisture was very low (<6%), which could prevent deterioration of meal, thus improving the shelf life of this byproduct. The fiber (<10% NDF and <8% ADF) in the current study was lower than that in soybean meal (17.2% NDF and 12.2% ADF).[30] Similar to previous publication results, the GE was similar to that in the soybean meal.[31] Thus, the treated meal has a better nutrient profile than the control.

Toxin and Antinutritional Components. Results for the toxin and antinutritional component levels of all of the treated samples were given in Table 2. All of the PEs were removed ($p < 0.05$) from the defated meal after treatment with 60 or 65% ethanol or methanol. In general, PEs are known to activate protein kinase C, which in turn activates a cascade of signal transduction reactions causing tumor promotion. Abud-Aguye et al.[32] had reported that feeding mice with PEs as low as 1 mg/kg of body weight caused death.

The residue of TIA in the control sample was 3.15 mg/g. TIA is generally considered to be a toxic factor in *J. curcas* seeds. TIA interferes with the normal functioning of pancreatic proteolytic enzymes in non-ruminants, leading to severe growth depression.[33] Data indicated that different treatments had a significant ($p < 0.05$) effect on TIA. The residues of TIA in all samples in the present study were lower than that reported by Aderibigbe et al. (5 mg/g).[31]

Lectin is also considered to be another toxic factor in *J. curcas* seeds.[34] The residue of lectin activity in the control sample was very close to that reported by Makkar, Aderibigbe, and Becker.[18] In comparison to the control sample, the values of lectin activity in samples b (1.49 mg/mL) and c (1.35 mg/mL) were decreased significantly ($p < 0.05$). The lectin activity can be easily inactivated when the temperature was high.[31]

Table 1. Effect of Detoxification Treatments on Proximate Compositions (%) and GE (MJ/kg)[a]

	treatments					
	control	a	b	c	d	e
DM	93.61 a (2.76)	95.34 a (2.51)	97.85 a (3.63)	96.82 a (1.31)	97.71 a (1.22)	96.47 a (3.06)
lipid	1.43 b (0.41)	1.52 ab (0.09)	1.73 ab (0.28)	1.87 a (0.18)	1.55 ab (0.11)	1.62 ab (0.11)
crude ash	9.91 a (0.31)	6.72 b (0.02)	6.73 b (0.03)	6.79 b (0.80)	7.07 b (0.36)	6.74 b (0.04)
crude fiber	5.09 a (0.52)	4.57 ab (0.61)	4.19 b (0.12)	4.22 b (0.29)	5.15 a (0.07)	5.07 a (0.09)
NDF	9.01 a (0.74)	7.95 b (0.68)	6.91 c (0.42)	6.09 c (0.91)	8.48 ab (0.04)	8.26 ab (0.01)
ADF	7.71 a (0.11)	6.65 b (0.04)	4.57 c (0.67)	4.69 c (0.15)	6.78 b (0.09)	6.49 b (0.26)
GE	18.12 a (0.41)	19.23 a (1.04)	19.01 a (1.21)	18.73 a (1.06)	18.45 a (1.07)	18.56 a (1.74)

[a] All values are the mean of triplicate determinations (standard deviation). Values followed by different letters are significantly different ($p < 0.05$). a, enzymatic treatment; b and c, enzymatic treatment + 60% methanol and 65% ethanol treatments, respectively; and d and e, 90% methanol and 90% ethanol treatments, respectively.

Table 2. Effect of Detoxification Treatments on Toxic and Antinutritional Components[a]

	treatments					
	control	a	b	c	d	e
PEs[b]	2.88 a (0.11)	2.47 b (0.24)	0.00 d (0.00)	0.00 d (0.00)	0.98 c (0.04)	1.16 c (0.08)
TIA (mg/g)	3.15 a (0.13)	2.93 a (0.01)	2.55 b (0.09)	2.47 b (0.06)	3.09 a (0.12)	3.12 a (0.24)
lectin activity (mg/mL)[c]	3.43 a (0.01)	3.41 a (0.17)	1.49 bc (0.00)	1.35 c (0.04)	1.46 bc (0.12)	1.62 b (0.10)
saponins (g/100 g)[d]	2.67 a (0.13)	2.62 a (0.09)	1.14 c (0.05)	0.93 d (0.09)	1.58 b (0.11)	1.46 b (0.06)
total phenolics (g/100 g)[e]	0.32 a (0.21)	0.28 ab (0.03)	0.12 bc (0.11)	0.09 c (0.02)	0.18 abc (0.06)	0.14 abc (0.07)
phytic acid (%)	10.04 a (0.39)	9.11 b (0.26)	7.01 c (0.31)	6.85 c (0.41)	7.28 c (0.23)	8.83 b (0.12)

[a] All values are the mean of triplicate determinations (standard deviation). Values followed by different letters are significantly different ($p < 0.05$). a, enzymatic treatment; b and c, enzymatic treatment + 60% methanol and 65% ethanol treatments, respectively; d and e, 90% methanol and 90% ethanol treatments, respectively. [b] Equivalent to phorbol-12-myristate 13-acetate. [c] Minimum amount of the sample required to show agglutination in 1 mL of final assay medium. [d] Diosgenin equivalents. [e] Tannic acid equivalents.

The phytic acid content was significantly ($p < 0.05$) affected by the treatments b−e but only slightly affected by the other treatments (Table 2). The high level of phytic acid present in *J. curcas* meal might decrease the bioavailability of minerals (especially Ca^{2+} and Fe^{2+}). Phytic acid have also been implicated in decreasing protein digestibility by forming complexes and also interacting with enzymes, such as trypsin and pepsin.[35] Phytic acid in all processed samples was very close to those reported by Aderibigbe et al.[31]

Saponin in sample c was 0.93 g/100 g, which was lower than that reported in soybean meal (4.7 g/100 g).[9] The treatments b, d, and e can reduce the total saponins by about 50% (Table 2). However, treatment c significantly ($p < 0.05$) decreased the total saponins by about 70%. Vasconcelos et al.[30] had reported that saponins were not destroyed by cooking. The reduction of saponins, resulting from treatment c, was probably due to them being extracted along with ethanol and the cell wall being destructed by enzymes (cellulase plus pectinase). Treatment c was the most effective way to remove the saponins compared to the other treatments.

It is well-known that the enzymes (cellulase plus pectinase) can destroy the plant cell wall, which is helpful to release intracellular components. Therefore, the method of hydrolysis of enzymes (cellulase plus pectinase) followed by solvent washing can be used to detoxify the PEs and antinutritional factors in *J. curcas* meal. The data in the present study validate the effect of enzymes. Treatment c will be a promising way to detoxify *J. curcas* meal.

Nutritional Quality Evaluation. Results for the levels of CP, buffer-soluble nitrogen, non-protein nitrogen, pepsin-insoluble nitrogen, and IVPDs of all of the treated samples were given in Table 3. The CP increased from 59.64 to 74.86%. The CP in sample c was the highest at 74.86%, as compared to soybean meal (45.7%). The buffer-soluble nitrogen and non-protein nitrogen were 7.32−8.42 and 4.48−5.61 g of CP/100 g of dry matter, respectively. Only 4.34−9.0% (as a percentage of total CP) of the total nitrogen of all samples was non-protein nitrogen, which, in jojoba, soybean, sunflower, and rapeseed meals, were 21−30, 2.9−7.8, 5.0, and 6.9%, respectively,[36] suggesting the presence of a high level (90%) of true protein in the present study. The pepsin-digestible nitrogen of sample c was very high (about 95%) (Table 3). The value of IVPDs in the control sample was 82.14%. This low digestibility may be possibly due to the high content of TIA presented in the *J. curcas* meal and the denatured protein. Treatment c improved protein digestibility by 10% (92.37%) better than treatments a, b, d, and e. It may even be possibly due to the lower levels of tannins, which inhibit the trypsin digestion in sample c, and the cell wall being destroyed by cellulase and pectinase.

Results for the levels of amino acid composition of all of the treated samples and soybean meal were given in Table 4. In comparison to soybean meal, the data revealed an almost similar pattern for all essential amino acids, except lysine and sulfur-containing amino acids. The PDCAAS (Table 5) value of detoxified meal obtained from treatment c was 0.75, which was calculated from the amino acid composition analyzed (except the sulfur-containing amino acids) and the reported value of

Table 3. Effect of Detoxification Treatments on Buffer-Soluble Nitrogen, Non-protein Nitrogen, Pepsin-Insoluble Nitrogen, and IVPDs (g of CP/100 g of DM)[a]

	treatments					
	control	a	b	c	d	e
CP	59.64 e (0.25)	63.43 d (0.61)	68.52 b (0.22)	74.86 a (0.91)	62.82 d (0.06)	64.74 c (0.52)
IVPD	82.14 d (0.76)	85.21 c (0.4)	88.61 b (0.8)	92.37 a (0.82)	82.13 d (0.48)	84.38 cd (0.19)
pepsin-insoluble nitrogen	4.03 c (0.18)	4.31 ab (0.04)	4.35 ab (0.13)	4.57 a (0.19)	4.21 bc (0.04)	4.26 bc (0.24)
buffer-soluble nitrogen	7.69 bc (0.1)	7.32 c (0.1)	7.79 b (0.03)	8.42 a (0.06)	7.56 bc (0.5)	7.64 bc (0.08)
non-protein nitrogen	4.48 d (0.06)	4.53 d (0.09)	4.82 c (0.01)	5.61 a (0.02)	4.94 b (0.09)	4.80 c (0.02)

[a] All values are the mean of triplicate determinations (standard deviation). Values followed by different letters are significantly different ($p < 0.05$) a, enzymatic treatment; b and c, enzymatic treatment + 60% methanol and 65% ethanol treatments, respectively; d and e, 90% methanol and 90% ethanol treatments, respectively.

Table 4. Effect of Detoxification Treatments on Amino Acid Compositions (g/100 g of DM)[a]

	treatments					
amino acid	control	a	b	c	d	e
essential						
cystine	0.47	0.52	0.49	0.66	0.34	0.38
methionine	0.64	0.71	0.69	0.96	0.49	0.58
valine	2.83	3.13	4.62	5.69	4.41	4.22
isoleucine	2.52	2.27	4.05	4.37	3.73	3.69
leucine	3.91	4.32	4.54	5.39	4.11	4.06
arginine	5.82	6.14	7.58	8.66	6.81	6.83
phenylalanine	2.51	2.76	3.57	3.97	3.32	3.26
histidine	1.30	1.43	1.67	2.28	1.88	1.51
lysine	1.38	1.48	2.61	3.41	2.42	2.37
threonine	1.96	2.16	2.83	3.28	2.58	2.47
non-essential						
aspartic acid	4.86	5.36	5.82	6.41	5.11	5.31
proline	2.89	3.09	1.93	2.13	1.69	1.73
serine	2.48	2.68	3.08	3.35	2.72	2.81
glutamic acid	8.98	9.88	6.99	7.67	4.86	6.38
glycine	2.43	2.37	3.04	3.67	2.84	2.77
alanine	2.65	2.92	3.34	3.93	2.41	3.05
tyrosine	1.44	1.58	1.01	1.04	0.88	0.92
totals	49.07	52.8	57.89	66.87	50.6	52.45

[a] a, enzymatic treatment; b and c, enzymatic treatment + 60% methanol and 65% ethanol treatments, respectively; d and e, 90% methanol and 90% ethanol treatments, respectively.

Elvin-Lewis.[36] The seed protein was rich in glutamic and aspartic acids and similar to conventional oilseed proteins. Results for nutritional indices of all of the treated samples were given in Table 5. Data showed that treatment c can increase the nutritional quality of protein. The detoxified meal obtained by treatment c has a relatively high content of protein (74.86%) and essential amino acids, good digestibility (92.37%), high value of PDCAAS (0.75), and no antinutrients and toxic factors. Therefore, it could be inferred that the detoxified meal has a high availability of protein to animals.

CONCLUSIONS

Detoxification methods for *J. curcas* meal are very important because of the high content of toxin, antinutritional components, and the difficulty in their digestion. The effective use of protein in *J. curcas* meal as a source of food/feed, especially in a developing country, is also important. Therefore, a simple and inexpensive detoxification technique that changes the composition of *J. curcas* meal and improves its acceptability is necessary.

On the basis of the results of this study, it can be concluded that no single method can remove or eliminate all of the antinutrients and toxic factors. A combination of hydrolysis of enzymes (cellulase plus pectinase) and washing with ethanol (65%) brought about all of the desirable changes in *J. curcas* meal. This treatment significantly reduced PEs and antinutritional components, such as phytates, tannins, the flatulence-causing factors, saponins, protease inhibitor, and lectin activities. Among all of the varieties studied, the detoxified meal by this treatment had better nutritional indices (protein quality) than the other treatments. In addition, the 65% ethanol treatment is more economic and toxicologically feasible than the methanol treatment. It can be concluded that this treatment was a promising way to detoxify *J. curcas* meal.

Table 5. Effect of Detoxification Treatments on Nutritional Indices[a]

	treatments					
	control	a	b	c	d	e
EAAI	65.78	65.99	76.47	82.28	74.57	72.92
BV	60	60.23	71.65	77.16	69.58	67.78
NI	32.27	41.86	52.4	61.59	46.84	47.2
PDCAAS	0.51	0.54	0.67	0.75	0.62	0.61

[a] a, enzymatic treatment; b and c, enzymatic treatment + 60% methanol and 65% ethanol treatments, respectively; d and e, 90% methanol and 90% ethanol treatments, respectively.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-139-211-77990. Fax: +86-510-853-29099. E-mail: zhanghui@jiangnan.edu.cn.

Funding Sources

The authors acknowledge the fund and technical services provided by the State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China.

REFERENCES

(1) Berchmans, H. J.; Hirata, S. Biodiesel production from crude *Jatropha curcas* L. seed oil with a high content of free fatty acids. *Bioresour. Technol.* **2008**, *99*, 1716–1721.

(2) Mohibbe Azam, M.; Waris, A.; Nahar, N. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. *Biomass Bioenergy* **2005**, *29* (4), 293–302.

(3) Liberalino, A.; Bambirra, E.; Moraes Santos, T.; Vieira, E. *Jatropha curcas* L. seeds: Chemical analysis and toxicity. *Arq. Biol. Tecnol.* **1988**, *31* (4), 539.

(4) Adam, S. Toxic effects of *Jatropha curcas* in mice. *Toxicology* **1974**, *2* (1), 67–76.

(5) Ahmed, O.; Adam, S. Effects of *Jatropha curcas* on calves. *Vet. Pathol.* **1979**, 16.

(6) Ahmed, O.; Adam, S. Toxicity of *Jatropha curcas* in sheep and goats. *Res. Vet. Sci.* **1979**, *27*, 89.

(7) Mampane, K.; Joubert, P.; Hay, I. *Jatropha curcas*: Use as a traditional Tswana medicine and its role as a cause of acute poisoning. *Phytother. Res.* **2006**, *1*, 50–51.

(8) El Badwi, S.; Mousa, H.; Adam, S.; Hapke, H. Response of brown hisex chicks to low levels of *Jatropha curcas*, *Ricinus communis* or their mixture. *Vet. Hum. Toxicol.* **1992**, *34*, 304.

(9) Makkar, H.; Becker, K.; Schmook, B. Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. *Plant Food Hum. Nutr.* **1998**, *52*, 31–36.

(10) Becker, K.; Makkar, H. Effects of phorbol esters in carp (*Cyprinus carpio* L.). *Vet. Hum. Toxicol.* **1998**, *40*, 82.

(11) Adolf, W.; Opferkuch, H.; Hecker, E. Irritant phorbol derivatives from four *Jatropha* species. *Phytochemistry* **1984**, *23*, 129–132.

(12) Ahmed, W.; Salimon, J. Phorbol ester as toxic constituents of tropical *Jatropha curcas* seed oil. *Eur. J. Sci. Res.* **2009**, *31*, 429–436.

(13) Mourgue, M.; Delphaut, J.; Baret, R.; Kassab, R. Study of the toxicity and localization of toxalbumin (curcin) in the seeds of *Jatropha curcas* Linn. *Bull. Soc. Chim. Biol.* **1961**, *43*, 517.

(14) Stirpe, F.; Pession-Brizzi, A.; Lorenzoni, E.; Strocchi, P.; Montanaro, L.; Sperti, S. Studies on the proteins from the seeds of *Croton tiglium* and of *Jatropha curcas*. Toxic properties and inhibition of protein synthesis in vitro. *Biochem. J.* **1976**, *156*, 1.

(15) Makkar, H.; Becker, K. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 773–787.

(16) Martinez-Herrera, J.; Siddhuraju, P.; Francis, G.; Davila-Ortiz, G.; Becker, K. Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chem.* **2006**, *96*, 80–89.

(17) Association of Official Analytical Chemists (AOAC). In *AOAC Official Methods of Analysis*; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; pp 1048—1049.

(18) Makkar, H. P. S.; Aderibigbe, A. O.; Becker, K. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chem.* **1998**, *62*, 207–215.

(19) Gordon, J.; Marquardt, M. Factors affecting hemagglutination by concanavalin A and soybean agglutinin. *Biochim. Biophys. Acta, Biomembr.* **1974**, *332*, 136–144.

(20) Liu, K.; Markakis, P. Trypsin inhibition assay as related to limited hydrolysis of inhibitors. *Anal. Biochem.* **1989**, *178*, 159–165.

(21) Vaintraub, I.; Lapteva, N. Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Anal. Biochem.* **1988**, *175*, 227–230.

(22) Hiai, S.; Oura, H.; Nakajima, T. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Med.* **1976**, *29*, 116.

(23) Makkar, H.; Becker, K.; Sporer, F.; Wink, M. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J. Agric. Food Chem.* **1997**, *45*, 3152–3157.

(24) Satterlee, L.; Marshall, H.; Tennyson, J. Measuring protein quality. *J. Am. Oil Soc.* **1979**, *56*, 103–109.

(25) Food and Agriculture Organization (FAO). *Amino Acid Content of Foods and Biological Data on Proteins*; FAO: Rome, Italy, 1970.

(26) Oser, B. Method for integrating essential amino acid content in the nutritional evaluation of protein. *J. Am. Diet. Assoc.* **1951**, *27*, 396.

(27) Oser, B. An integrated essential amino acid index for predicting the biological value of proteins. *Protein Amino Acid Nutr.* **1959**, 281.

(28) Crisan, E.; Sands, A. The biology and cultivation of edible mushrooms. *Nutr. Value* **1978**, 137–168.

(29) Food and Agriculture Organization (FAO)/World Health Organization (WHO). *Protein Quality Evaluation: Report of Joint FAO/WHO Expert Consultation*; Bethesda, MD, Dec 4—8, 1989.

(30) Vasconcelos, I.; Siebra, E.; Maia, A.; Moreira, R.; Ferreira, N., A.; Campelo, G. J. A.; Oliveira, J. T. A. Composition, toxic and antinutritional factors of newly developed cultivars of brazilian soybean (*Glycine max*). *J. Sci. Food Agric.* **1997**, *75*, 419–426.

(31) Aderibigbe, A. O.; Johnson, C. O. L. E.; Makkar, H. P. S.; Becker, K.; Foidl, N. Chemical composition and effect of heat on organic matter- and nitrogen-degradability and some antinutritional components of *Jatropha* meal. *Anim. Feed Sci. Technol.* **1997**, *67*, 223–243.

(32) Abdu-Aguye, I.; Sannusi, A.; Alafiya-Tayo, R.; Bhusnurmath, S. Acute toxicity studies with *Jatropha curcas* L. *Hum. Exp. Toxicol.* **1986**, *5*, 269.

(33) Hajos, G.; Gelencser, E.; Pusztai, A.; Grant, G.; Sakhri, M.; Bardocz, S. Biological effects and survival of trypsin inhibitors and the agglutinin from soybean in the small intestine of the rat. *J. Agric. Food Chem.* **1995**, *43*, 165–170.

(34) Panigrahi, S.; Francis, B.; Cano, L.; Burbage, M. Toxicity of *Jatropha curcas* seeds from Mexico. *Nutr. Rep. Int.* **1984**, *29*, 1089–1100.

(35) Aregheore, E.; Makkar, H.; Becker, K. 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.* **1998**, *77*, 349–352.

(36) Elvin-Lewis, M. Non-traditional oilseeds and oils of India. *Econ. Bot.* **1988**, *42*, 540–540.