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A Comparison of the Nutrient and Antinutrient Composition of Industrially Processed Zimbabwean *Jatropha curcas* and *Glycine max* Meals

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Abstract: In a study to compare the nutritive and Anti-nutritional Factors (ANFs) composition of industrially processed shelled *Jatropha curcas* (Physic nut) kernels and soyabean (*Glycine max*) seed; samples of industrially processed Soyabean Meal (SBM) generated from the traditional industrial hexane extraction method were used. Samples of *J. curcas* Meal (JCM) were derived from double solvent extraction of shelled *J. curcas* kernels in a hexane-ethanol extraction system followed wet extrusion (126°C, 2 atmospheres, 10 min contact time) and then re-extraction with hexane. The re-extracted JCM was then heated with pressurized steam at 121°C for 30 min before dried samples were used in the laboratory analyses. Significant differences ($p<0.05$) in both the nutrient and ANFs existed between the seed meals. The JCM had a significantly higher ($p<0.05$) Crude Protein (CP) with 577.00 g kg^{-1} DM versus 470.80 g kg^{-1} DM in SBM. Similarly JCM had a higher ($p<0.05$) ash, calcium and phosphorus content with 119.7, 12.4 and 22.26 g kg^{-1} DM, respectively versus the 73.8, 3.43 and 7.31 g kg^{-1} DM, respectively in SBM. The SBM and JCM registered statistically similar levels of Acid Detergent Fibre (ADF); however JCM had a significantly higher ($p<0.05$) Neutral Detergent Fibre content (NDF) at 177.30 g kg^{-1} DM with the SBM having 125.60 g kg^{-1} DM Neutral Detergent Fibre. The JCM had a residual Phorbol Esters (PEs) concentration of 0.8 mg g^{-1} that was equivalent to a decrease of 87.69% from the 6.5 mg g^{-1} PEs content in raw shelled *Jatropha curcas* kernels. The SBM registered 19.40 TUI mg^{-1} as trypsin inhibitor activity while the JCM did not show any such activity. Both meals did not cause agglutination and haemolysis of erythrocytes indicating that lectins and saponins were completely inactivated during the industrial processing of each meal.

Key words: Plant protein sources, *Jatropha curcas*, *Glycine max*, anti-nutritional factors

INTRODUCTION

In Zimbabwe the major protein source in pig and poultry rations is soyabean (*Glycine max*) in the form of hexane extracted Soyabean Meal (SBM). Soyabean production in the country is chiefly the preserve of a few commercial farmers who are endowed with irrigation facilities that ensure successful soyabean production inspite of the high incidences of mid-season dry spells that characterize Zimbabwe. Feed costs in pig and poultry production account for 60 to 80% of the production costs, with the protein component taking up 65% of the feed costs[1]. This scenario means the little tonnage produced translates into very costly pig and poultry feeds since there is no complement and or competitor to soyabean that can be produced by more farmers at a cheaper cost. As such the country is deprived of enough animal protein intake that could be realized from the high off-takes that are characteristic of pig and poultry production. Small stock (pigs and poultry) is regarded as the female gender's animals; thus a shortage of cheaper feeds deprives the much-needed empowerment of the female gender of the country.

The Physic nut, *Jatropha curcas*, (*Euphorbiaceae* family) was introduced in the Eastern parts of Zimbabwe from Mozambique in pre-colonial Zimbabwe[2]. Gaydou *et al.*[3] and Heller [4] reported that the Physic nut grows well in marginal areas. The chemical composition of *J. curcas* kernels varies with source, but intervarietal differences are known to be insignificant[5]. The proximate

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composition (nutrient) of *J. curcas* seed meal (JCM) is reported to be comparable to SBM. *Jatropha curcas* meal with 1-2% residual oil has a Crude Protein (CP) content of up to 640 g kg^{-1} (of which 95% is true protein) compared to 470 g kg^{-1} CP in SBM[5,6]. However, like soyabean and other legumes that have a high nutritional value, *J. curcas* cakes and or meals also contain Antinutritional Factors (ANFs) in the form of lectins, saponins, phytates and protease inhibitors[6-8]. In addition to the ANFs stated above, *J. curcas* cakes and or meals have been found to contain toxic thermo-stable but lipo-soluble Phorbol Esters (PEs). Phorbol esters that are reported to auto-oxidize under normal storage conditions, seem to be stabilized by some compounds in the *Jatropha* oil. Phorbol esters are reported to mimic Diacylglycerate (DAG), a key substrate in cell growth and development, thus they stimulate Protein Kinase C (PKC), whose natural activator is DAG. Since PKC is involved in signal transduction and developmental processes of cells and tissues, the toxicity and cocarcinogenic effects of PEs is due to their stimulation of PKC. Phorbol esters have to be removed if the oil and seed cake and or meal are to be used for human and animal consumption, respectively.

The aim of the study was to determine and compare the nutrient and anti-nutrient composition of industrially processed Zimbabwean *J. curcas* provenance seed meal and the traditionally solvent extracted SBM.

MATERIALS AND METHODS

Sourcing of materials: A sample of commercially (hexane extracted) produced SBM used in the experiment was obtained from Olivine Industries Limited Zimbabwe. *J. curcas* seed was procured from Agri-Seed Services Zimbabwe.

Industrial processing of *Jatropha curcas* seed: Initially all the *J. curcas* seed was shelled using a motorised sheller. The resultant kernels were processed at Pymarc Pvt. Ltd and Speciality Animal Feed Company, both of Zimbabwe. The shelled kernels were then minced in preparation for the solvent extraction. Minced kernels were soaked in 95% hexane for 8 h followed by 3 cycles of extraction at 30°C, each of 45 min duration. The hexane-extracted kernels were then shade-dried to a constant air-dry weight over two days under a mean ambient temperature of 22°C. The dried kernels were then extracted with 95% ethanol at 35°C for 45 min. The ethanol extraction step was aimed at removing most of the lipo-soluble PEs as they have been observed to be highly soluble in ethanol[5]. The ethanol-extracted meal (still in the extraction pots) was heated with pressurised steam at 90°C for 30 min to distil off and recover the ethanol after which it was sun-dried for three days. The sun-dried *J. curcas* meal was wet extruded (20% moisture, 126°C, 2 atmospheres with a contact time of 10 min). The extrusion helped break fat globules enclosed in the cell walls, thus exposed residual oil to solvents. The extruded meal was re-extracted with hexane, again at 30°C. The re-extracted meal, while still in the extraction pots, was heated with steam at 121°C for 30 min. primarily to inactivate lectins and trypsin inhibitors[5] and secondarily to distil off and recover the hexane. The resultant *J. curcas* Meal (JCM) after air-drying in the sun was then used to source samples used in the nutrient and anti-nutrient determinations.

Chemical analyses of the seed meals

Proximate analysis: SBM and JCM samples milled through a 1 mm screen were used in the determination of Dry Matter (DM), Crude Protein (CP), Ether Extract (EE) and ash content using the Official Methods of Analysis of the Analytical Chemists[9]. Calcium and phosphorus content of the seed meals was determined by wet chemistry as stipulated by the AOAC[9]. The fibre fraction, that is, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) was determined as described by Van Soest *et al.*[10]. The gross energy values of the meals were estimated using a CP 400 adiabatic bomb calorimeter. Each analysis was done in decaplicate.

Determination of anti-nutritional factors

Total phenolics determination: Two hundred milligram samples milled through a 1 mm screen of SBM and JCM were weighed into test tubes. Each sample was extracted using 8 mL of 7:3 mixture of acetone: water v/v for 20 min, repeated three times in a sonicator. The supernatant was filtered through glass-cintered crucibles after each extraction. On the third extraction the entire contents of the tube were emptied into the crucible. After filtering into graduated flasks, the volume of the extracts was made up to 30 mL by adding aqueous acetone. The extract was then used to determine total Phenolics via precipitation by trivalent ytterbium as described by Reed *et al.*[11].

Insoluble proanthocyanidins (unextractable condensed tannins) determination: A sample of 10 mg of the dry residue from the extraction above was weighed into 25 mL test tubes. A 5 mL aliquot of the butanol-HCL reagent (butanol-HCL 95: 5, v/v) was added to each test tube and boiled at 98-100°C for 1 h. After allowing test tubes to cool to room temperature, absorbance was read a 550 nm.

Lectin activity assay: Lectin activity was determined using the haemagglutination test as described by Gordon

and Marquardt[12]. Modifications to the procedure included utilization of rabbit, sheep and pig erythrocytes in place of bovine erythrocytes. Both undigested and protease (Bromelain) digested erythrocytes were used.

Trypsin inhibitor activity determination: Trypsin Inhibitor Activity (TIA) of the meal was determined as outline by Gaborit *et al.*[13].

Phorbol esters estimation: Samples of SBM and JCM had their PEs estimation done as described by Aderibigbe *et al.*[14]. The determination was done in the Proyecto Biomasa Laboratory of Professor Nikolas Foidl in Austria.

Experimental design and data analysis: A complete randomized design with ten replicates per variable analyzed was used. Data analysis for the proximate composition parameters as well as for the Total Phenolics (TPs) and Insoluble Proanthocyanidins (IPA) was subjected to analysis of variance using Genstat Release, Version 7.1[15]. Mean separation was done using the Least Significance Procedure.

RESULTS AND DISCUSSION

Significant differences ($p<0.05$) were observed between the proximate composition of SBM and JCM. The JCM had significantly higher ($p<0.05$) CP, ash, calcium and phosphorus content (Table 1). The JCM had a significantly higher ($p<0.05$) content of TPs at 36.70 g kg^{-1} DM with SBM having 11.20 g kg^{-1} DM. Soyabean meal had a significantly higher ($p<0.05$) relative IPA content with 1.13 AU g^{-1} DM while JCM registered an absorbance of 0.81 AU g^{-1} DM (Table 2).

None of the meals caused agglutination of either protease digested or undigested erythrocytes from sheep, pig and rabbit. Furthermore JCM did not exhibit any Trypsin Inhibitor Activity (TIA). However SBM registered some TIA with a mean 19.40 TUI mg^{-1}. The JCM had a mean 0.80 mg g^{-1} DM PEs concentration while SBM had none.

The proximate composition of JCM is in agreement with the findings of Makkar *et al.*[6] that reported that extracted *J. curcas* meals tend to have higher CP and mineral levels compared to SBM. The CP content in JCM of 577 g kg^{-1} DM is in consistent with the ranges of 564 to 638 g kg^{-1} DM reported by Makkar and Becker [5]. Chivandi *et al.*[16] indicated that JCM derived from unshelled *J. curcas* kernels had a CP value of 284.7 g kg^{-1} DM; much lower than the mean of 470 g kg^{-1} DM reported in SBM. The CP values in *J. curcas* only surpass those of SBM provided there has been shelling and oil extraction from the *J. curcas* kernels. The ash, calcium and phosphorus content of SBM were significantly lower ($p<0.05$), with 73.8, 3.43 and 7.31 g kg^{-1} DM, respectively, compared to 119.7, 12.4 and 22.26 g kg^{-1} DM, respectively in JCM. Makkar and Becker[5] reported an ash content of between 96 and 104 g kg^{-1} DM in JCM, thus making the 119.7 g kg^{-1} DM in this experiment a reasonable figure. The slightly higher ash content in JCM found in this study could have emanated from differences in the soil nutrient status in which the *J. curcas* grew and or from the varietal differences within the *J. curcas* plants. The small amounts of shell fragments that fell into the shelled seed could also have contributed to the slight increase in the ash content of JCM since shell material is known to contain complexes of calcium and other minerals. The 73.8, 3.43 and 7.31 g kg^{-1} DM ash, calcium and phosphorus, respectively reported in SBM in this study is on average in agreement with mean values in SBM of 62.0, 3.67 and 6.8 g kg^{-1} DM, respectively as reported McDonald *et al.*[17].

McDonald *et al.*[17] reported an NDF and ADF content in SBM of 125 and 91 g kg^{-1} DM, respectively. These values are closely related to the 125.6 and 94.5 g kg^{-1} DM reported in this study. Interesting to note is that the ADF for SBM and JCM of 94.5 and 101.9 g kg^{-1} DM, respectively is statistically not different. The similarity offers opportunity to make comparison in monogastric animals feeding trials more valid as one replaces SBM with JCM of similar ADF content. The 177.3 g kg^{-1} DM neutral detergent fibre in JCM that is significantly higher ($p<0.05$) than that in SBM (at 125.0 g kg^{-1} DM) (Table 1) could have been due to the more fibrous shell flakes that fell with the *Jatropha* kernels during the shelling process. The relative TP content of the meals was higher ($p<0.05$) in JCM with 36.7 g kg^{-1} DM compared with 11.2 g kg^{-1} DM in SBM (Table 2). The converse was true for IPA with SBM showing a higher absorbance at 1.13 AU g^{-1} DM versus 0.807 AU g^{-1} DM shown with JCM. The ytterbium precipitation method used in the determination of TP is not specific since it is known to measure some moieties other than total Phenolics [11]. It becomes difficult to allude that the TP values reflect entirely TP. However, Makkar and Becker[5] reported negligible amounts of TP in JCM using the Foila-Ciocalteu reagent. The relative IPA for both SBM and JCM if worked backwards (raw absorbance values) will show there are no IPA in SBM and JCM. This observation is in agreement with[5] who reported absence of IPA in JCM.

Makkar and Becker[5], Nell *et al.*[7] reported presence of phyto-haemaglutinins in raw *J. curcas* and soyabean

Table 1: Mean values for Dry Matter (DM; g kg^{-1}), Crude Protein (CP; g kg^{-1}), Ether Extract (EE; g kg^{-1}), Ash (g kg^{-1}), Calcium (Ca^{2+}; g kg^{-1}), Phosphorus (PO_4^{3-}; g kg^{-1}), Neutral Detergent Fibre (NDF, g kg^{-1}), Acid Detergent Fibre (ADF; g kg^{-1}) and Gross Energy (GE; MJ kg^{-1})

	Parameter								
	DM	CP	EE	Ash	Ca^{2+}	PO_4^{3-}	NDF	ADF	GE
SBM	922.1^a	470.8^a	19.3^a	73.8^a	3.43^a	7.31^a	125.6^a	95.4^a	19.3^a
JCM	883.4^b	577.0^b	30.2^b	119.7^b	12.4^b	22.26^b	177.3^b	101.9^a	16.0^a
G/Mean	902.7	523.9	24.7	96.8	7.9	14.8	151.5	98.6	17.6
Sig level	**	**	**	**	**	**	**	ns	ns
S.E.D	1.897	4.72	3.86	2.06	0.225	0.1606	3.86	6.87	1.335
C.V.(%)	0.3	1.1	19.1	2.6	3.5	1.3	3.1	8.5	9.3
LSD	5.27	13.11	10.72	5.71	0.63	0.45	10.71	19.08	3.71

^{a,b} Within column means with different superscripts are different at $p<0.05$; **Significant at $p<0.05$; ns Non-significant

Table 2: Mean Total Phenolics (TP; g kg^{-1}) and Insoluble Proanthocyanidins (IPA; AU/g DM)

	Parameter	
	TP	IPA
SBM	11.2^a	1.13^a
JCM	36.7^b	0.807^b
Grand Mean	23.9	0.968
Significance Level	**	**
S.E.D	4.41	0.0328
C.V. (%)	22.6	4.2
LSD	12.25	0.0911

^{a,} Within column means with different superscripts are different at $p<0.05$
**Significant at $p<0.05$

seed. In this experiment the meals' failure to cause agglutination of either the protease digested or undigested erythrocytes from sheep, pig and rabbit blood points to complete inactivation of lectins. Makkar *et al.*[6] and Nell *et al.*[7] also indicated presence of saponins in raw *J. curcas* and soyabean seeds. Saponins are known to cause haemolysis of erythrocytes[18]. In this study during the lectin assay no haemolysis was observed suggesting that the processing managed to inactivate or completely remove saponins from both meals. Absence of trypsin inhibitor activity in JCM is a pointer to the efficacy of the moist-heat-treatment (used in the processing of *J. curcas* kernels) in inactivating the trypsin inhibitors that are known to be thermo-labile[7]. The 19.40 TUI mg^{-1} registered by SBM indicates that not all the trypsin inhibitor activity in SBM was destroyed during processing. However 19.40 TUI mg^{-1} in SBM is by far lower than the 51.60 TUI mg^{-1} reported by Gaborit *et al.*[13] in raw soyabean seed. The reduced activity in the processed SBM indicates that although most of the trypsin inhibitors were inactivated the processing methodology used in generating SBM was not efficient in as far as inactivating trypsin inhibitors. The residual 0.8 mg g^{-1} DM PEs in the JCM is far lower than the 6.5 mg g^{-1} DM PEs concentration reported by Chivandi *et al.* [16] in raw *J. curcas* kernels. The 87.69% reduction in PEs concentration in JCM following the industrial processing leaves a window of opportunity to fine tune the processing procedure and bring the concentration of toxic PEs in the meal to 0.11 mg g^{-1} DM reported by Makkar and Becker[5] in the Mexican non-toxic varieties. If complete detoxification were achieved, Zimbabwe would exploit the high CP potential of *J. curcas* to boost pig and poultry production.

The cost of processing (detoxifying) *J. curcas* kernels seems astronomical considering the cost of chemicals (solvents) and machinery required. However the many by-products, for example, oil (37% of kernel weight) that can be used as bio-diesel, pesticides and molluscides that can be precipitated from the oil or extracted from other parts of the plants and biogas (from fermentation of *J. curcas* fruit shells) can off-set the high processing cost. This gives the plant tremendous potential to uplift the livelihoods of rural communities since it grows well in marginal areas where still most of the communal farmers of Zimbabwe reside. The study revealed that processing of both JCM and SBM managed to completely inactivate lectins in the seed meals. However JCM was not completely detoxified since it still contained 0.8 mg g^{-1} PEs, a concentration higher than 0.11 mg g^{-1} DM PEs concentration in non-toxic Mexican varieties. This therefore calls for more research into the detoxification of *J. curcas* kernels so that the resultant meal can be used in livestock production.

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