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Anti-Nutritional Metabolites and Effect of Treated *Jatropha curcas* Kernel Meal on Rumen Fermentation *in vitro*

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Abstract: *Jatropha curcas* Linn. seed upon oil extraction resulted in a protein enriched byproduct called kernel meal. The presence of anti-nutritional metabolites in high levels renders the meal unsuitable as an animal feed. This research was carried out to determine the effects of physical and chemical treatments on the level of anti-nutritional metabolites present in the kernel meal. The effects of treated kernel meal on rumen microbial fermentation were evaluated *in vitro*. Proximate analysis of kernel meal obtained from *J. curcas* grown in Malaysia showed 61.8% crude protein, 9.7% NDF and 4.8% ADF. The anti-nutritional metabolites analyses showed high levels of total phenolic (3.9 mg g^{-1} DM), total saponin (1.9 g 100 g^{-1} DM), phytic acid (9.1%), trypsin inhibitors (34.2 mg g^{-1} DM), lectin activity (102.7 mg mL^{-1}) and phorbolesters (3.0 mg g^{-1} DM). Combination of hydrothermal treatment, alkali and oxidizing agents alleviated the levels of anti-nutritional metabolites. Phenolic compounds, saponin and phorbolesters levels declined significantly ($p<0.05$) while the level of phytic acid did not decrease. Trypsin inhibitors and lectin activity were fully inactivated. The level of phorbolesters decreased by 76.7% in treatment with heat, 3% (w/w) NaOH and 10% (v/w) NaOCl. *In vitro* fermentation by rumen microbes showed significant ($p<0.05$) decrease in fermentation parameters when chemically treated kernel meal was used as substrates while physically treated kernel meal did not affect the fermentation parameters significantly.

Key words: *Jatropha curcas* Linn., lectin, phorbolesters, saponin, trypsin inhibitor, rumen fermentation *in vitro*

INTRODUCTION

Jatropha curcas Linn. plant belongs to the Euphorbiaceae family. It is a multipurpose small tree or shrub with various economic importance due to its several industrial and medicinal applications (Kulkarni *et al*., 2005). This plant has drawn considerable interest due to its seed's oil becoming a potential source of biodiesel. *Jatropha curcas* plantation in a large scale has been established in a few countries including Malaysia to overcome the shortage of fossil fuel.

In line with the biodiesel production, a byproduct called kernel meal which is high in protein is produced. Kernel meal has the potential as animal protein supplement (Makkar *et al*., 1998a). However, the presence of high levels of anti-nutritional factors (ANFs) in some genotypes renders the meal unsuitable as an animal feed. The major ANFs in *J. curcas* kernel meal according to Rakshit *et al.* (2008) are lectin and phorbolesters. Lectins are heat labile and easily inactivated but attempts to remove phorbolesters completely were unsuccessful.

Since, currently there are no available agronomically improved varieties with low levels of ANFs, efforts to remove them in the kernel meal should be considered. Therefore, this research was carried out to determine the effects of physical and chemical treatments on levels of anti-nutritional metabolites present in the kernel meal. Consequently, the effects of treated kernel meal on rumen microbial fermentation were evaluated *in vitro*.

MATERIALS AND METHODS

Seed collection and preparation of kernel meal: Mature seeds of *J. curcas* Linn. obtained from the Malaysian Agriculture Research and Development Institute

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(MARDI) were air dried and stored in a plastic container at 4°C. The kernels were ground by using a mechanical grinder followed by oil extraction with Soxhlet apparatus using petroleum ether (boiling point 40-60°C) for 16 h (AOAC, 1990). Defatted kernel (kernel meal) was air dried at room temperature and kept in screw cap bottle at 4°C.

Chemical analyses: The dry matter (DM) content of samples was determined by oven drying to a constant weight at 105°C. Chemical analyses were conducted according to AOAC (1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by Van Soest *et al.* (1991).

Trypsin inhibitor activity: Trypsin inhibitor activity was determined according to Smith *et al.* (1980). The enzyme was added last as suggested by Liu and Markakis (1989) and the results were expressed as milligrams of pure trypsin inhibited g DM^{-1}.

Lectin (phytohemagglutinin activity): The meal was extracted according to Aregheore *et al.* (1998) and lectin was determined by using hemagglutination assay as described by Makkar *et al.* (1999). Lectin was reported as reciprocal of the minimum amount of the sample in mg mL^{-1} of the assay which produced agglutination.

Phytic acid: Samples were extracted with 3.5% (w/v) HCl and the extract was further purified by AG1-X8 chloride anion exchange column. Colorimetric determination of phytic acid was carried out at 500 nm by using Wade reagent (Vaintraub and Lapteva, 1988).

Total saponins: Total saponin was determined according to Makkar *et al.* (1999). The absorbance was read at 544 nm and results expressed as diosgenin equivalent g DM^{-1}.

Phorbolesters: Phorbolesters were determined according to Makkar *et al.* (2007). The sample was extracted with methanol and an aliquot was loaded on a High-Performance Liquid Chromatograph (HPLC) Agilent-1200 series instrument equipped with a UV-Vis photodiode array (DAD) detector and analytical column Agilent Reverse-phase, C18 LiChrospher 100, 250×4 mm I.D and 5 μm pore size. Solvents used were deionized water and acetonitrile. The absorbance was read at 280 nm and peaks were observed at 24.4, 25.5, 26.5 and 26.9 min. The results were expressed as equivalent to phorbol-12-myristate 13-acetate as the standard which was detected at 29.8 min.

Total phenolics: Total phenolics was determined colorimetrically according to Makkar (2003) and the results were expressed as tannic acid equivalents g DM^{-1}.

Sample treatments: Hydrothermal, alkali and oxidizing agents were applied to remove the ANFs. About 100 g of kernel meal were weighed in a glass beaker and moisture content of the sample was adjusted to 66% (Makkar *et al.*, 1998b) with either distill water or solution containing alkali (NaOH, $Ca(OH)_2$, KOH) or oxidizing agent (NaOCl) to give a final concentration of 3% (w/w) or 10% (v/w), respectively. Then samples were autoclaved for 30 min at 121°C, 15 psi (hydrothermal treatment). Samples were washed with distill water (1:10) and freeze dried. Physical and chemical treatments are shown in Table 1.

***In vitro* fermentation:** Two rumen fistulated mature cows were maintained on mixed forage and concentrate of 60:40 ratio fed twice daily. Rumen liquor was collected before the morning feed from both fistulated cows and strained through four layers of muslin gauze into a pre-warmed bottle at 39°C. Treated and untreated (control) *J. curcas* kernel meal were milled to pass through 1 mm sieve and used as substrates.

About 200 mg of substrate were weighed into 100 mL calibrated glass syringes. The incubation medium was prepared as described by Menke and Steingass (1988) and 30 mL was dispensed anaerobically into each syringe. Syringes were incubated at 39°C for 24 h. *In vitro* gas production (GP) was measured in triplicate at 2, 4, 8, 12 and 24 h. In each incubation run, three blanks were included to correct the GP values for gas released from endogenous substrates and three standard hays to check the quality of the inoculum. Apparent dry matter (ADM) and organic matter (OM) degradability of substrates were determined. Metabolizable energy (ME) was estimated

Table 1: *Jatropha curcas* Linn. kernel meal treatments

Treatments	[1]Chemical	[2]Hydrothermal
a	-	-
b	-	+
c	NaOH	+
d	$Ca(OH)_2$	+
e	KOH	+
f	NAOH+NaOCl	+
g	$CaOH_2$+NaOCl	+
h	KOH+NaOCl	+
i	NaOCl	+

- Not applied; +Applied; [1]Alkali at 3.0% (w/w) and oxidizing agent at 10% (v/w); [2]Autoclaved at 121°C for 30 min at 15 psi; a = kernel meal, b = hydrothermal treatment (HT); c = HT+3.0% (w/w) NaOH; d = HT+3.0% (w/w) KOH; e = HT+3.0% (w/w) $Ca(OH)_2$, f = HT+3.0% (w/w) NaOH+10.0% (v/w) NaOCl, g = HT+3.0% (w/w) KOH+10.0% (v/w) NaOCl, h = HT+3.0% (w/w) $Ca(OH)_2$+10.0% (v/w) NaOCl, I = HT+ 10.0% (v/w) NaOCl

according to Menke and Steingass (1988). The volatile fatty acids (VFAs) which include acetic, isobutyric, butyric, propionic, valeric, isovaleric and caproic acid were determined by Gas Chromatograph (Agilent 6890 A) which was equipped with a capillary column packed with 10% (w/v) PEG 600 on Shimalate TPA 60/80. pH of fermenting fluid for each syringe was determined immediately after 24 h incubation.

Phenol hypochlorite color reaction method described by Chaney and Marbach (1962) was used to determine the ammonia nitrogen. Total gas values were corrected based on blank gas volume which contained only rumen fluid. Cumulative gas production data were fitted to the model by Orskov and McDonald (1979) using NEWAY Excel version 5.0 package (Chen, 1997).

Statistical analyses: Data were analysed using the General Linear Models (GLM) procedure of SAS (2003) in a Completely Randomized Design (CRD) and the means were compared with Duncan's multiple range test.

RESULTS

Chemical constituents: Chemical analyses of local *J. curcas* kernel and kernel meal are shown in Table 2. The DM content of kernel and kernel meal were similar. Fat content of kernel was 55.7%. Crude protein in kernel and kernel meal were 29.5 and 61.8%, respectively. Oil extraction decreased the OM but increased the ash, NDF and ADF.

Chemical constituents of treated samples: Table 3 shows the chemical constituents of samples after various treatments. Chemical treatments decreased the OM while increased the crude protein, ash, NDF, ADF and DM lost significantly ($p<0.05$). Gross energy was not affected by treatments.

Anti-nutritional metabolite contents: Anti-nutritional metabolite contents of untreated and treated samples are shown in Table 4. The highest level of anti-nutritional metabolites were in untreated kernel meal and the values decreased significantly ($p<0.05$) upon various treatments. The total phenolic and saponin contents decreased up to 66.6 and 63.1% in treatments g and h, respectively. Phytic acid level in kernel meal was 9.1% and heat treatment decreased the total phytic acid concentration by 14.3%. However, chemical treatments increased phytic acid concentration significantly ($p<0.05$).

Trypsin inhibitor and lectin activity were fully inactivated in all treated samples. Phorbolesters level in kernel meal was 3.0 mg g^{-1} DM and the amount was significantly ($p<0.05$) reduced by treatments. The lowest phorbolesters content was observed in treatment f which showed 76.7% loss when compared to the control.

***In vitro* rumen fermentation:** Fermentation parameters are shown in Table 5. Apparent DM and OM degradability in kernel meal were 67.3 and 59.3%, respectively. Hydrothermal treatment did not affect the DM and OM degradability. However, combination of hydrothermal treatment with alkali and oxidizing agents significantly ($p<0.05$) reduced the DM and OM degradability.

Metabolizable energy (ME) in kernel meal and hydrothermal treatment were similar but other treatments decreased the ME values significantly ($p<0.05$). All fermentation parameters which consisted of $IVGP_{24}$, NH_3-N, VFA varied among treatments ($p<0.05$) except pH which did not show any significant difference.

As shown in Table 5, the $IVGP_{24}$ was suppressed significantly ($p<0.05$) as a result of hydrothermal and chemicals treatments. The highest $IVGP_{24}$ was observed in kernel meal with a value of 33.0 mL and the lowest

Table 2: Chemical analysis of *J. curcas* (on DM basis)

Item	Kernel	Kernel meal
DM (%)	94.2±0.5	94.2±0.5
OM (%)	96.0±0.0	91.6±0.0
Ash (%)	3.9±0.0	8.3±0.0
CP (%)	29.5±0.3	61.8±0.1
Fat (%)	55.7±0.4	ND
NDF (%)	4.3±0.5	9.7±0.5
ADF (%)	2.9±0.1	4.8±0.2
GE (MJ kg^{-1})	29.1±0.2	17.7±0.1

ND: Not Determined

Table 3: Effect of physical and chemical treatments on chmical constituents of samples

Sample	DM lost (%)	Organic matter (%)	Ash (%)	Crude protein (%)	NDF (%)	ADF (%)	Gross energy (MJ kg^{-1})
a	ND	90.30^{a}	9.60^{d}	61.8^{e}	9.70^{e}	4.80^{b}	17.70
b	4.60^{d}	90.00^{a}	9.90^{d}	62.5^{e}	10.40^{e}	5.30^{ab}	17.70
c	25.80^{a}	89.00^{b}	10.90^{c}	67.1^{c}	20.00^{a}	6.00^{a}	17.30
d	24.00^{b}	87.80^{c}	12.10^{b}	70.8^{b}	18.70^{b}	6.50^{a}	17.00
e	19.50^{c}	85.40^{d}	14.50^{a}	64.6^{d}	17.80^{b}	6.20^{a}	17.10
f	26.80^{a}	87.60^{c}	12.00^{b}	67.7^{c}	20.90^{a}	6.60^{a}	17.60
g	25.10^{ab}	87.90^{c}	12.00^{b}	71.8^{b}	19.10^{a}	6.30^{a}	17.60
h	20.60^{c}	86.80^{c}	13.10^{b}	67.8^{c}	18.20^{b}	6.40^{a}	17.30
i	20.80^{c}	90.10^{a}	9.80^{d}	75.1^{a}	18.10^{cd}	6.40^{a}	17.50
SEM	0.48	0.28	0.28	0.31	0.37	0.34	0.24

a = kernel meal, b = hydrothermal treatment (HT), c = HT+3.0% (w/w) NaOH, d = HT+3.0% (w/w) KOH, e = HT+3.0% (w/w) $Ca(OH)_2$, f = HT+3.0%(w/w) NaOH+10.0% (v/w) NaOCl, g = HT+3.0% (w/w) KOH +10.0% (v/w) NaOCl, h = HT+3.0% (w/w) $Ca(OH)_2$+10.0% (v/w) NaOCl, i = HT+10.0% (v/w) NaOCl; Means with different superscripts within column are significantly different ($p<0.05$); ND: Not Determined; All the data are based on DM

Table 4: Effect of various treatments on ANFs content in *J. curcas* kernel meal

Treatments*	Total phenolics[a] (mg g^{-1})	Total saponin[b] (g/100g)	Phytic acid[c] (%)	Trypsin inhibitor[d] (mg g^{-1})	Lectin activity[e] (mg mL^{-1})	Phorbolestersf (mg g^{-1})
a	3.90^{a}	1.90^{a}	9.10^{b}	34.20^{a}	102.70^{a}	3.00^{a}
b	3.50^{b}	1.70^{b}	7.80^{c}	0.30^{b}	1.20^{b}	1.30^{d}
c	2.80^{c}	1.50^{c}	11.20^{a}	0.30^{b}	1.20^{b}	1.30^{d}
d	1.80^{e}	0.90^{d}	11.00^{a}	0.20^{b}	1.20^{b}	1.50^{cde}
e	1.40^{ef}	0.90^{d}	11.00^{a}	0.10^{b}	1.20^{b}	1.70^{bc}
f	2.40^{d}	1.40^{c}	11.30^{a}	0.30^{b}	1.20^{b}	0.70^{f}
g	1.30^{f}	0.80^{d}	10.80^{a}	0.30^{b}	1.20^{b}	1.30^{d}
h	1.50^{ef}	0.70^{e}	10.80^{a}	0.20^{b}	1.20^{b}	1.40^{de}
i	1.80^{e}	1.50^{c}	8.80^{b}	0.20^{b}	1.20^{b}	1.60^{cd}
SEM	0.11	0.01	0.14	0.60	0.12	0.07

[a]Tannic acid equivalents; [b]Diosgenin equivalents; [c]Phytic acid g/100 g DM; [d]mg of pure trypsin inhibited/g sample; [e]Reciprocal of minimum amount of the sample required to show the agglutination after two fold dilution in 1 mL of final assay medium; [f]Equivalent to phorbol 12-myristate,13 acetate a = kernel meal, b = hydrothermal treatment (HT), c = HT+3.0% (w/w) NaOH, d = HT+3.0% (w/w) KOH, e = HT+3.0% (w/w) $Ca(OH)_2$, f = HT+3.0% (w/w) NaOH +10.0% (v/w) NaOCl, g = HT+3.0% (w/w) KOH+10.0% (v/w) NaOCl, h = HT+3.0% (w/w) $Ca(OH)_2$+10.0% (v/w) NaOCl, I = HT+10.0% (v/w) NaOCl; Means with different superscript within column are significantly different ($p<0.05$)

Table 5: *In vitro* ruminal fermentation parameters of samples

	Treatments									
Items	a	b	c	d	e	f	g	h	i	SEM
DM degradability (%)	67.30^{a}	68.90^{a}	59.30^{b}	61.40^{b}	56.30^{c}	60.20^{b}	60.50^{b}	56.80^{c}	61.00^{b}	0.79
OM degradability (%)	59.30^{a}	61.40^{a}	54.50^{b}	54.10^{b}	51.60^{c}	55.50^{b}	54.80^{b}	51.70^{c}	55.80^{b}	0.74
Total gas (mL/24 h)	33.10^{a}	32.30^{a}	24.50^{b}	24.70^{b}	22.90^{c}	24.30^{b}	24.10^{b}	22.70^{c}	23.70^{bc}	0.39
pH	6.60	6.60	6.70	6.60	6.60	6.60	6.60	6.60	6.60	0.02
Ammonia N (mg/100 mL)	15.20^{ab}	16.30^{a}	15.50^{ab}	15.80^{ab}	15.10^{b}	15.30^{ab}	15.90^{ab}	15.10^{b}	15.90^{ab}	0.31
Total VFA (mM)	57.00^{a}	56.90^{a}	45.20^{b}	46.40^{b}	45.50^{b}	45.20^{b}	45.30^{b}	44.10^{b}	45.40^{b}	1.57
ME (MJ kg^{-1} DM)	10.60^{a}	10.50^{a}	9.70^{b}	9.80^{b}	9.10^{d}	9.50^{bcd}	9.60^{bc}	9.10^{cd}	9.80^{b}	0.14
Gas production parameters*										
a (mL)	0.70^{c}	1.40^{a}	1.20^{ab}	1.30^{a}	1.40^{a}	1.10^{ab}	1.20^{ab}	0.90^{bc}	1.10^{ab}	0.10
b (mL)	40.40^{a}	41.70^{a}	36.80^{b}	38.30^{b}	38.10^{b}	37.70^{b}	36.60^{b}	37.10^{b}	37.90^{b}	0.61
c (% h^{-1})	0.19^{a}	0.10^{b}	0.06^{c}	0.06^{c}	0.04^{c}	0.04^{c}	0.05^{c}	0.05^{c}	0.05^{c}	0.01
a+b (mL)	41.60^{b}	43.60^{a}	38.00^{c}	39.00^{c}	39.10^{c}	38.90^{c}	37.80^{c}	37.90^{c}	39.10^{c}	0.60

a = kernel meal, b = hydrothermal treatment (HT), c = HT+3.0% (w/w) NaOH, d = HT+3.0% (w/w) KOH, e = HT+3.0% (w/w) $Ca(OH)_2$, f = HT+3.0% (w/w) NaOH+10.0% (v/w) NaOCl, g = HT+3.0% (w/w) KOH +10.0% (v/w) NaOCl, h = HT+3.0% (w/w) $Ca(OH)_2$+10.0% (v/w) NaOCl, i = HT+10.0% (v/w) NaOCl; Means in each row with different superscripts are significantly different ($p<0.05$); *a, b, c and a+b were calculated from exponential equation $p = a+b\ (1-e^{-ct})$; a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction (b), (a+b) = potential extent of gas production

was in treatment h with a value of 22.7 mL. The ammonia nitrogen values were not significantly different among treatments. Total VFAs was not affected by hydrothermal treatment but when combined with chemical treatments the amounts were significantly ($p<0.05$) reduced. Chemical treatments significantly affected ($p<0.05$) gp parameters (Table 5). Table 6 shows the proportion of VFAs after 24 h of fermentation. Significant differences were not observed in all fatty acids except for butyric acid which showed a significant decrease ($p<0.05$) when compared to the control.

DISCUSSION

Chemical constituents: Chemical constituents of kernel meal obtained from local *J. curcas* seed showed protein and fiber content with values suitable as animal feed. Upon treatments, DM and OM decreased with the concomitant increase in crude protein, ash, NDF and ADF as shown in Table 3. Removal of soluble fraction during washing stage resulted in the increase in percentage values. These findings were in agreement with the results reported by Martinez-Herrera *et al.* (2006) who showed that physical and chemical treatments increased the crude protein, ash, crude fiber and NDF content of *J. curcas* kernel meal.

Anti-nutritional metabolite contents of treated samples: Reduction of phenolic compounds would be an advantage since, they are known for their interaction with proteins and subsequent effect upon feed intake, DM and protein degradability in ruminant.

In the present study, total phenolic compounds were reduced significantly ($p<0.05$) in all treatments with treatment e, g and h showing the lowest content of phenolic compounds. The mechanism of reduction in total phenolic compounds was not clear, however it could be attributed to the washing, transformation, decomposition of phenolics and formation of phenolic-protein complex under thermal, chemical and pressure conditions. Saponins are steroid or triterpene glycoside compounds. Previous researches reported the beneficial effects of saponin such as defaunation of the rumen and

Table 6: Molar proportion of volatile fatty acids after 24 h of fermentation with various substrates

	Treatments									
VFA concentration (Molar %)	a	b	c	d	e	f	g	h	i	SEM
Acetic acid	55.6	56.1	56.2	55.8	56.0	56.1	56.2	56.2	55.9	0.19
Propionic acid	20.7	21.4	21.8	21.7	21.2	21.6	21.3	21.5	21.6	0.37
Isobutiryc acid	2.5	2.3	2.7	2.3	2.3	2.7	2.5	2.5	2.1	0.15
Butyric acid	13.4^{a}	11.1^{b}	10.0^{c}	9.7^{c}	9.5^{c}	10.1^{c}	9.8^{c}	9.4^{c}	9.3^{c}	0.25
Isovaleric acid	6.8	6.9	6.9	7.2	6.9	7.1	7.2	7.1	7.2	0.15
Valeric acid	2.2	2.2	2.3	2.4	2.3	2.5	2.5	2.4	2.5	0.06
Caproic acid	0.9	0.6	0.7	0.7	0.6	0.6	0.6	0.7	0.9	0.12
Acetic/propionic	2.6	2.6	2.5	2.5	2.6	2.6	2.6	2.6	2.5	0.04

a = kernel meal, b = hydrothermal treatment (HT), c = HT+3.0% (w/w) NaOH, d = HT+3.0% (w/w) KOH, e = HT+3.0% (w/w) $Ca(OH)_2$, f = HT+3.0% (w/w) NaOH+10.0% (v/w) NaOCl, g = HT+3.0% (w/w) KOH +10.0% (v/w) NaOCl, h = HT+3.0% (w/w) $Ca(OH)_2$+10.0% (v/w) NaOCl, i = HT+10.0% (v/w) NaOCl; Means in each row with different superscript are significantly different ($p<0.05$)

manipulation of the end products of fermentation but harmful effects were also observed at higher concentrations (Wina *et al*., 2005). Total saponin in kernel meal of local *J. curcas* seed was lower (1.9 g/100 g) compared to kernel meal of toxic and non toxic varieties with the values of 2.6 and 3.4 g/100 g, respectively (Makkar and Becker, 2009). Total saponin as shown in Table 4 in kernel meal decreased significantly ($p<0.05$) as a result of treatments and washing. Since, the total saponin content of local *J. curcas* kernel meal was lower than that of the soybean meal (4.7 g/100 g, Makkar *et al*., 1998a) therefore, it might not cause any harmful affect on animal performance.

Phytic acid has anti-nutritional properties due to its ability to chelate minerals resulting in mineral deficiency which may cause various diseases. The phytic acid in mongastric nutrition is more important than polygasteric since polygastric animal benefits from microbial phytase which allows digestion of phytate (Liao *et al*., 2005). Phytic acid level in kernel meal was 9.1% which could be considered safe as the non-toxic varieties contain about 8.9% phytic acid (Makkar and Becker, 2009). As shown in Table 4, chemical treatments did not reduce the phytic acid content and similar effect was observed by Martinez-Herrera *et al*. (2006). Probably chemical treatments reduced the solubility of phytic acid and the increase in the percentage values of phytic acid were due to the loss in DM of treated samples.

Trypsin inhibitors inhibit pancreatic proteases resulting in impaired protein digestion, hypertrophy and hyperplasia of the pancreas followed by depression in growth rate (Makkar and Becker, 2009). However, they contribute towards seeds resistance to insect and pathogenic microorganisms (Valueva and Mosolov, 2004). Trypsin inhibitors in kernel meal was 34.2 mg g^{-1} DM (Table 4) higher that those toxic varieties with the value of 21.31 mg g^{-1} DM reported by Makkar and Becker (2009). High content of trypsin inhibitor in *J. curcas* kernel meal might be attributed to the genotype. Hydrothermal treatment of 121°C for 30 min at 15 psi fully inactivated the trypsin inhibitors. Similar observation was made by Martinez-Herrera *et al*. (2006). The inactivation of trypsin inhibitor could be attributed to denaturation of their structural proteins and loss of water solubility due to heat treatment.

Lectins agglutinate red blood cells, impair lipid, carbohydrate and protein metabolism, hypertrophy or atrophy of organs and tissues (Vasconcelos and Oliveira, 2004). As shown in Table 4, lectin content of kernel meal in the present study was comparable to other varieties including toxic and non-toxic genotypes containing 102 mg mL^{-1} according to Makkar and Becker (2009). Since, lectins are protein. therefore, heat treatment inactivated them by denaturing their structures.

Phorbolesters are tetracyclic diterpenoids known for their tumor promoting activity through mimicking the action of diacyl glycerol (activator of protein kinase C) leading to over expression of protein kinase C and tumor generation (Goel *et al*., 2007). Phorbolesters content of *J. curcas* kernel meal was 3.0 mg g^{-1} DM (Table 4), considered harmful to animals since the toxic varieties contain 2.7 mg g^{-1} DM according to the category described by Makkar and Becker (2009). All treated samples showed significant ($p<0.05$) reduction of phorbolesters to <2.7 mg g^{-1} DM.

In this study, sodium hydroxide was found to be the best alkali to remove the phorbolesters. The efficiency of phorbolesters removal depends on the extent of saponification in residual oil followed by washing process. Accordingly, lower level of phorbolesters was observed in treatment c compared to d and e. Sodium hypochlorite was used as an oxidizing agent and combined with hydrothermal and alkali treatments could remove phorbolesters by 76.7% in treatment f.

Various removal efficiency based on combination of heat and chemical treatments were reported e.g., 75% total phorbolesters by Martinez-Herrera *et al*. (2006), 87% by Aregheore *et al*. (2003) and 94% by Rakshit *et al*. (2008) where the initial phorbolesters amount in those studies were 3.85, 1.78 and 1.35 mg g^{-1} DM, respectively.

***In vitro* rumen fermentation:** Although, hydrothermal treatment could reduce the ANFs but it did not improve the apparent DM, OM degradability estimated ME and $IVGP_{24}$ significantly (Table 5). These results were in agreement with Aderibigbe *et al.* (1997) who observed that hydrothermal treatment (80% moisture at 160°C for 60 min) on *J. curcas* kernel meal did not affect ruminal *in vitro* apparent OM degradability and estimated ME. On the other hand, combination of hydrothermal and chemical treatments suppressed the apparent DM, OM degradability estimated ME and $IVGP_{24}$ which might be due to loss of soluble fraction, protein denaturation or alteration of chemical constituents (Table 3) of the kernel meal upon processing.

Similarly De Oliveira *et al.* (2010) showed that alkali treatment denaturated the soluble proteins of castor seed meal which possibly reduced protein solubility and rate of ruminal degradation. As shown in Table 5, pH was not affected significantly by treatments. Ammonia nitrogen among treatments were similar except for treatments e and h ($p<0.05$). Combination of alkali and oxidizing treatments in e and h treatments markedly decreased the apparent dry and organic matter degradability ($p<0.05$) which resulted in low levels of ammonia nitrogen in treatments e and h.

This result was supported by Waltz and Loerch (1986) who observed low level of rumen ammonia nitrogen when soybean meal was treated with 5 M sodium hydroxide. Hydrothermal treatment did not affect the total VFAs significantly and similar observation was also reported for heat treated cotton seed by Pires *et al.* (1997). Getachew *et al.* (2004) illustrated the positive correlation between total gas and VFAs production. Lower gp was observed in hydrothermal alkali and oxidizing treatments and accordingly lower total VFAs lead to lower degradation rate of samples. Table 5 shows that hydrothermal alkali and oxidizing treatments increased the gp from fraction (a) of kernel meal significantly ($p<0.05$).

It was possibly due to the increase in soluble fraction of substrates at initial stage of fermentation. Gas production from fraction (b) of kernel meal (Table 5) was not affected by hydrothermal treatment, however alkali and oxidizing agent treatments reduced the gp from this insoluble fraction which indicated a reduction in degradation. Low rate of fermentation of the insoluble fraction of treated kernel meal might be beneficial for synchronization of nitrogen release with available fermentable metabolizable energy to achieve high yield of rumen microbial biomass. The treatments did not influence each VFA concentration significantly except for butyric acid which was significantly ($p<0.05$) higher in kernel meal and hydrothermal treatment as compared to others.

CONCLUSION

Chemical analyses revealed the similarity of local *J. curcas* Linn. seed to other genotypes from different regions. Anti-nutritional metabolite analyses indicated that the *J. curcas* seed used in the present study could be classified under the toxic variety. Combination of hydrothermal, alkali and oxidizing treatments alleviated the anti-nutritional metabolites in the kernel meal to the safe levels except for the phorbolesters which was only reduced to 0.7 mg g^{-1} DM. *In vitro* rumen fermentation showed that chemical processing resulted in low degradability of kernel meal and *in vitro* gp study did not indicate any positive effects of reduction of anti-nutritional metabolites on rumen microbial fermentation.

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REFERENCES

AOAC, 1990. Official Methods of Analysis of the AOAC. 15th Edn., Association of Official Analytical Chemists, Arlington VA.

Aderibigbe, A.O., H.P.S. Makkar, K. Becker and N.N. Foidl, 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.

Aregheore, E.M., K. Becker and H.P.S. Makkar, 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.

Aregheore, E.M., K. Becker and H.P.S. Makkar, 2003. Detoxification of a toxic variety of Jatropha curcas using heat and chemical treatments, and preliminary nutritional evaluation with rats. South Pacific. J. Nat. Sci., 21: 50-56.

Chaney, A.L. and E.P. Marbach, 1962. Modified reagents for determination of urea and ammonia. Clin. Chem., 8: 130-132.

Chen, X., 1997. Neway Excel: A Utility for Processing Data of Feed Degradability and *in vitro* Gas Production. XBC Laboratory, Rowett Research Institute, Aberdeen, UK.

De Oliveira, A.S., J.M.S. Campos, M.R.C. Oliveira, A.F. Brito and S.C.V. Filho *et al*., 2010. Nutrient digestibility, nitrogen metabolism and hepatic function of sheep fed diets containing solvent or expeller castorseed meal treated with calcium hydroxide. Anim. Feed Sci.Technol., 158: 15-28.

Getachew, G., P.H. Robinson, E.J. de Peters and S.J. Taylor, 2004. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. Anim. Feed Sci. Technol., 111: 57-71.

Goel, G., H.P.S. Makkar, G. Francis and K. Becker, 2007. Phorbol esters: Structure, biological activity and toxicity in animals. Int. J. Toxicol., 26: 279-288.

Kulkarni, M.L., H. Sreekar, K.S. Keshavamurthy and N. Shenoy, 2005. Jatropha curcas-poisoning. Ind. Crop Prod., 72: 75-76.

Liao, S.F., A.K. Kies, W.C. Sauer, Y.C. Zhang, M. Cervantes and J.M. He, 2005. Effect of phytase supplementation to a low-and a high-phytate diet for growing pigs on the digestibilities of crude protein, amino acids and energy. J. Anim. Sci., 83: 2130-2136.

Liu, K. and P. Markakis, 1989. Trypsin inhibitor assay as related to limited hydrolysis of inhibitors. Anal. Biochem., 178: 159-165.

Makkar, H.P.S. and K. Becker, 2009. Jatropha curcas, a promising crop for the generation of biodiesel and value-added coproducts. Eur. J. Lipid Sci. Technol., 111: 773-787.

Makkar, H.P.S., 2003. Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. Kluwer Academic Publisher, The Netherlands, pp: 102.

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

Makkar, H.P.S., K. Becker and B. Schmook, 1998b. Edible provenance of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. Plant Foods Hum. Nutr., 52: 31-36.

Makkar, H.P.S., K. Becker, H. Abel and E. Pawelzik, 1999. Nutrient contents, rumen protein degradability and antinutritional factors in some colour-and white-flowering cultivars of *Vicia faba* beans. J. Sci. Food Agric., 75: 511-520.

Makkar, H.P.S., P. Sidhuraju and K. Becker, 2007. Plant Secondary Metabolites (Methods in Molecular Biology). 1st Edn., Humana Press, New York, pp: 130.

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

Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim. Res. Dev., 28: 7-55.

Orskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., 92: 499-503.

Pires, A.V., M.L. Eastridge, J.L. Firkins and Y.C. Lin, 1997. Effects of heat treatment and physical processing of cottonseed on nutrient digestibility and production performance by lactating cows. J. Dairy Sci., 80: 1685-1694.

Rakshit, K.D., J. Darukeshwara, K.R. Raj, K. Narasimhamurthy, P. Saibaba and S. Bhagya, 2008. Toxicity studies of detoxified Jatropha meal (*Jatropha curcas*) in rats. Food Chem. Toxicol., 46: 3621-3625.

SAS, 2003. Statistical Analysis Institute (Version 9.1.3), SAS Institute Inc., Cary, NC, USA.

Smith, C., W. van Megan, L. Twaalfhoven and C. Hitchcock, 1980. The determination of trypsin inhibitor levels in foodstuffs. J. Sci. Food Agric., 31: 341-350.

Vaintraub, I.A. and N.A. Lapteva, 1988. Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. Anal. Biochem., 175: 227-230.

Valueva, T.A. and V.V. Mosolov, 2004. Role of inhibitors of proteolytic enzymes in plant defense against phytopathogenic microorganisms. Biochem. (Moscow), 69: 1305-1309.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Vasconcelos, I.M. and J.T. Oliveira, 2004. Antinutritional properties of plant lectins. Toxicon, 44: 385-403.

Waltz, D.M. and S.C. Loerch, 1986. Effect of acid and alkali treatment of soybean meal on nitrogen utilization by ruminants. J. Anim. Sci., 63: 879-887.

Wina, E., S. Muetzel and K. Becker, 2005. The impact of saponins or saponin-containing plant materials on ruminant production: A review. J. Agric. Food Chem., 53: 8093-8105.