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Degradability of dry matter and crude protein and rumen fermentation characteristics of *Jatropha curcas* kernel meal with different detoxification treatments

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ABSTRACT

The present study aimed to explore the degradability of dry matter (DM) and crude protein (CP) of *Jatropha curcas* kernel meal with different detoxification treatments in the rumen. Moreover, the impacts of the *Jatropha curcas* kernel meal on rumen fermentation characteristics were also discussed. *Jatropha curcas* kernel meal adopted in current work was treated as: *J. curcas* kernel meal no treatment (JNT); *J. curcas* kernel meal extraction treatment (JET); *J. curcas* kernel meal heat treatment (JHT), and *J. curcas* kernel meal high pressure treatment (JPT). The *in situ* degradability of DM and CP of JHT was compared with those of soybean meal and rapeseed meal for cattle in Experiment I. The *in vitro* degradability and rumen fermentation characteristics of JNT, JET, JHT and JPT for wethers were investigated in Experiment II. Collectively, after treatments of JHT, JET and JPT, the phorbol ester (PE) content was decreased. In addition, the CP content was higher, of which the degradability was more than 90% after 72 h of *in situ* incubation. The *Jatropha curcas* kernel meal with different treatments had no negative effects on the rumen fermentation characteristics after *in vitro* incubation. It was concluded that *Jatropha curcas* kernel meal with detoxification treatments could be used as a potential protein feed.

Keywords: Detoxification treatment, *In situ*, *In vitro*, Jatropha, Rumen fermentation

The seed kernel of Jatropha (*Jatropha curcas* L.) contains about 63% oil (Akbar *et al.* 2009). Therefore, Jatropha has been widely planted as raw material for bio-oil industry (Openshaw 2000). With the increase of Jatropha production, its by-products (oil residue and so on) have also increased. The whole Jatropha contains anti-nutritional factors due to the presence of inner phorbol ester (PE) (Goel *et al.* 2007). There is also a great deal of PE in *Jatropha curcas* kernel meal, which cannot be completely degraded (Makkar *et al*. 1998). Therefore, more studies should be carried out on how to degrade PE. Many reports have shown that PE can be effectively degraded by alkalization treatment (Rakshit *et al.* 2008), high pressure treatment, extraction treatment and heat treatment (Makkar *et al.* 1998). In our previous experiment, after the sheep are fed with 5% of heat-treated *Jatropha curcas* kernel meal, the palatability of sheep becomes poor, while no negative effects are found on their bodies (Lv *et al.* 2020). This finding has proved that *J. curcas* kernel meal has the potential to be used as protein feed for ruminants. However, the degradability of the *J. curcas* kernel meal after various treatments and its effects on rumen fermentation characteristics remain largely unexplored.

Many uncertainties still exist in the utilization of *Jatropha curcas* kernel meal as a protein feed. In addition, some reports have shown that the PE contents of *J. curcas* kernel meal from different varieties and areas are different (Makkar *et al.* 1997). It is possible to use *Jatropha curcas* kernel meal with a lower PE content as a safe protein feed through further detoxification treatments. Soybean meal and rapeseed meal (belonging to oil residue) are also common raw materials of protein feed. Comparisons of the feed characteristics and degradability among soybean meal, rapeseed meal and *J. curcas* kernel meal may contribute to the development of better application methods of *J. curcas* kernel meal.

Although *Jatropha curcas* kernel meal contains higher protein contents, the degradability of dry matter (DM) and crude protein (CP) in rumen remains largely unclear. The impacts of *J. curcas* kernel meal with different detoxification treatments on the degradability, fermentation characteristics and methane production in rumen need to be further studied. Therefore, we compared the degradability of DM and CP in *Jatropha curcas* kernel meal,

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soybean meal and rapeseed meal in rumen by *in situ* incubation (Experiment I). Moreover, we also investigated the degradability of *Jatropha curcas* kernel meal with different treatments by *in vitro* incubation and its effects on rumen fermentation characteristics (Experiment II).

MATERIALS AND METHODS

This experiment was approved by the Committee on Animal Experimentation (License No. 24121) and carried out under the institutional guidelines for animal experiments of Yamagata University.

Jatropha curcas kernel meal treatment and sample preparation: The *Jatropha curcas* kernel meal no treatment (JNT) from Indian was supplied as experimental materials. Before *in vitro* incubation, the ground samples were detoxified by the following three different treatments: 1) *Jatropha curcas* kernel meal extraction treatment (JET), the sample was extracted with diethyl ether and recycled after 16 h; 2) *Jatropha curcas* kernel meal heat treatment (JHT), the sample was heated at 180°C for 30 min; 3) *Jatropha curcas* kernel meal high pressure treatment (JPT), the sample was incubated at 0.235 MP for 1 h in a high-pressure sterilizer (MLS-3780F, Tech Jam Corporation, Osaka, Japan).

*Experiment I (*in situ *incubation):* Two female Japanese black cattle (with inserted rumen fistula, average weight 563±15 kg) were used in the experiment, and they were fed with 80% of reed canary grass (*Phalaris arundinacea* L.) hay and 20% of commercial concentrate at maintenance energy level (2.0% DM of their body weight). The animals were given free access to clean drinking water, and mine salt was supplied at 8:00 AM and 16:00 PM every day, respectively. In order to keep the rumen environment stable, the *in situ* incubation was carried out after 2 weeks of feeding according to above-mentioned feeding conditions. Briefly, a nylon bag (BG1020, size: 10 cm × 20 cm, Sanshin, Yokohama Japan) containing 5 g sample was put into the rumen through a rumen fistula, followed by incubation for 3, 6, 12, 24, 48 and 72 h. After the *in situ* incubation, the bag was washed with running water and put into a drying oven at 60°C for 3 days, and then the CP and degradability of samples were analyzed.

*Experiment II (*in vitro *incubation):* The treated samples of *Jatropha curcas* kernel meal were ground into 0.5 mm powder and chosen as experimental materials. Two adult wethers (average initial body weight, 72.5 kg) with rumen cannula were adopted as ruminal fluid donors. These animals were fed once with following basal diets: 50% reed canary grass (*Phalaris arundinacea* L.) hay and 50% commercial concentrate at maintenance energy level (2.0% DM of their body weight), and free access to clean drinking water was given at 9:00 AM every day. Ruminal fluid was harvested through the rumen cannula after 2 h of feeding and placed into plastic bottles. These fluids were filtered through four layers of cheese cloth and equally pooled. The combined filtrate was mixed with CO_2-bubbled McDougal's artificial saliva (McDougal 1948) at a ratio of 1:4 (v/v). Subsequently, 50 mL buffered ruminal fluid was transferred to a 128 mL serum bottle containing 0.5 g sample, and flushed with O_2-free CO_2. The tubes were capped with a butyl rubber stopper and sealed with an aluminum cap. The *in vitro* incubation was carried out in triplicate at 39°C for 3, 6 and 12 h in a water bath with a reciprocal shaker (100 strokes/min).

Chemical analysis: Samples of *Jatropha curcas* kernel meal were ground using a mill with a 0.5 mm screen. The moisture, ash, CP, ether extract (EE), and crude fibre levels were measured using conventional methods (AOAC 1999). The levels of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the method described by Van Soest *et al.* (1991).

Briefly, 25 µL of formaldehyde solution (35%) was injected into serum bottles to stop fermentation at the end of the *in vitro* incubation, and then the bottles were immediately sealed and cooled at room temperature. An air syringe was used to collect the gas samples in serum bottles, and the collected samples were injected into a gas chromatograph (GC323; GL Sciences, Tokyo, Japan) consisting of a thermal conductivity detector and a stainless steel column (WG-100 SUS, 1.8 m × 6.35 mm outer diameter) under the following conditions: column oven temperature, 50°C; injector temperature, 50°C; and detector temperature, 50°C. The methane content of each serum bottle was calculated. A glass electrode *p*H meter (D-21; Horiba, Kyoto, Japan) was employed to determine the *p*H of the fluid. Ammonia-nitrogen (NH3-N) content was measured using a previously established method (Conway 1962). To determine the total volatile fatty acid (VFA), ruminal fluid was steam-distilled and titrated using sodium hydroxide (Hamada 1971). Dried VFA salt was separated, and gas chromatography (G-5000A; Hitachi, Tokyo, Japan) consisting of a thermal conductivity detector and a stainless-steel column (Unisole F-200, 3.2 mm × 2.1 m, Hitachi, Tokyo, Japan) was adopted to determine the content of VFA under the following conditions: column oven temperature, 140°C; injector temperature, 210°C; and detector temperature, 250°C.

Briefly, 1 g of the ground samples were mixed with 10 mL alcohol (Kanto Chemical Co. Inc. Tokyo, Japan) and allowed to stand for 12 h. After it was vibrated with ultrasonic waves for 30 min, the mixture was subjected to centrifugation. Supernatant was filtered through a 0.45 nm membrane prior to further analyses. LC-10A system API-4000 (Shimadzu Corporation, Kyoto, Japan) was used in analysis meter. Column was Intertsil ODS-3 (GL Sciences Inc. Tokyo, Japan), the column temperature was 40°C, velocity was set at 0.2 mL/min, and dose was 5 µL. The accuracy was determined to be 10 ppm under LC/MS (Wilhelm and Martin 2000, Hipal Gaudani *et al*. 2009).

Statistical analysis: The general linear model procedure (SAS Institute, Cary, NC, USA) (1995) was adopted for the statistical analyses. Data of fermentation characteristics, *in vitro* DM degradability, CP degradability, methane and VFA production of *Jatropha curcas* kernel meal with

Table 1. Chemical composition of the rapeseed meal and treated *Jatropha curcas* kernel meal

	JNT	JET	JHT	JPT
Crude protein (DM %)	26.7	26.6	25.9	26.3
Ether extract (DM %)	6.2	0	5.4	5.6
Neutral detergent fibre (DM %)	44.7	56.6	50.1	53.5
Phorbol esters (ug/g)	30.7	<1	<1	<1

JNT, *Jatropha curcas* kernel meal no treatment; JET, *Jatropha curcas* kernel meal extraction treatment; JHT, *Jatropha curcas* kernel meal heat treatment; JPT, *Jatropha curcas* kernel meal high pressure treatment; DM, Dry matter.

different treatments were analyzed by one-way analysis of variance (ANOVA). Differences (P<0.05) between means were identified by Tukey's test.

RESULTS AND DISCUSSION

Following the treatments of JHT, JPT and JET, the PE content of *Jatropha curcas* kernel meal was reduced. The CP level of *Jatropha curcas* kernel meal was about 26%, which was not changed after different treatments (Table 1).

*Experiment I (*in situ *incubation)*: Experiment I revealed that the DM degradability of *Jatropha curcas* kernel meal was significantly lower compared with soybean meal and rapeseed meal, and about 60% of DM was degraded after 72 h of incubation (Fig. 1). Fig. 2 shows the CP changes of the soybean meal, rapeseed meal and *Jatropha curcas* kernel meal during *in situ* incubation. In the initial stage of *in situ* incubation (0–12 h), the CP degradability of *Jatropha curcas* kernel meal was low. However, the degradation of CP in rumen was increased after 12 h of *in situ* incubation, which was similar to that of soybean meal and rapeseed meal, about 80% of CP was decomposed after 48 h of *in situ* incubation, and more than 90% of CP was degraded after 72 h of *in situ* incubation.

*Experiment II (*in vitro *incubation):* Experiment II showed that after 3 or 12 h of *in vitro* incubation, the degradability of DM of JHT was lower compared with the

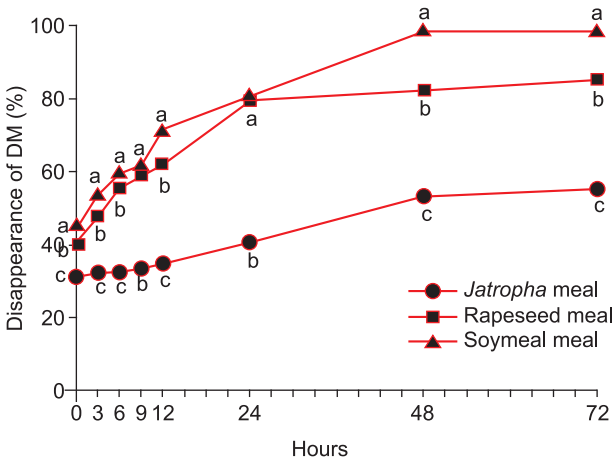

Fig. 1. Changes in disappearance of DM in the soybean meal, rapeseed meal and *Jatropha* meal. DM, dry matter; Different letters in same time denote significant differences (P< 0.05).

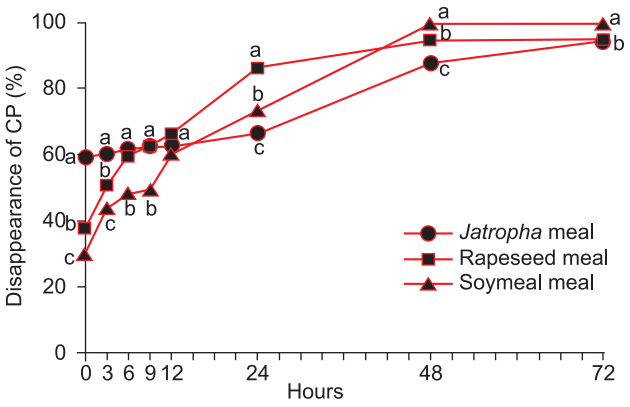

Fig. 2. Changes in disappearance of CP in the soybean meal, rapeseed meal and *Jatropha* meal. CP, crude protein; Different letters in same time denote significant differences (P < 0.05).

other treatments. The CP degradability was increased with the increase of *in vitro* incubation time. After 12 h, the degradability of JNT, JET, JHT and JPT was 41.5%, 42.6%, 26.7% and 29.5%, respectively. The degradability of CP of JET was the highest. After 3, 6 and 12 h of *in vitro* incubation, gas was not produced. However, the methane production showed the lowest value with JNT, while the highest value was found with JET. There was no significant difference in *p*H after four treatments. After 3 or 6 h of incubation, the total VFA production was the highest in JET, while its lowest value was found in JHT. After 12 h of *in vitro* incubation, there was no difference in total VFA production among the four treatments. After 3 or 6 h of *in vitro* incubation, the total VFA production of JPT exhibited a descending tendency. Propionic acid production showed a lower ratio with JHT (Table 2).

Jatropha curcas kernel meal has the potential to be used as a protein feed, and many studies have been focussed on the application test of animal feeding (Liberalino *et al.* 1988, Chivandi *et al.* 2000, Gadir *et al.* 2003). In the experiments, the PE content of *Jatropha curcas* kernel meal was significantly decreased after treatments of JET, JHT and JPT, which was in agreement with the investigations of Makkar *et al*. (1998) and Devappa *et al.* (2010). In addition, the PE level of untreated *Jatropha curcas* kernel meal was lower compared with the other related reports (Goel *et al*. 2007, Rakshit *et al*. 2008). Some reports have shown that the PE level of *Jatropha curcas* kernel meal is affected by species, growth conditions and oil process (Makkar *et al*. 1997). In our experiments, the CP level of *Jatropha curcas* kernel meal was decreased after JHT or JPT treatment, which could be attributed to the loss of water-soluble protein under the condition of high-pressure steam or high temperature. The degradability of DM and CP was regarded as the basic standard to assess the animal diets.

In our previous study, we have compared soybean meal, rapeseed meal and *Jatropha curcas* kernel meal in terms of the amino acid composition, and the impacts of short-term *in vitro* incubation on digestibility have also been discussed. Our results have shown that there is higher content of amino

Table 2. *In vitro* ruminal digestibility of DM and CP as well as gas, methane and VFA production of *Jatropha curcas* kernel meal treatment incubated for 3, 6 and 12 h, respectively

		Treatment				
	Time	JNT	JET	JHT	JPT	SEM
DM	3	23.80^{a}	23.70^{a}	18.50^{b}	23.60^{a}	0.82
digestibility (%)	6	26.10	26.70	22.40	26.90	1.02
	12	33.00^{b}	39.40^{a}	24.10^{c}	33.00^{b}	0.64
CP digestibility	3	28.10^{a}	29.10^{a}	19.10^{b}	17.90^{b}	0.92
(%)	6	29.40^{a}	32.70^{a}	27.00^{ab}	19.10^{b}	1.92
	12	41.50^{a}	42.60^{a}	26.70^{b}	29.50^{b}	1.25
Methane production	3	4.71^{b}	9.41^{a}	6.04^{b}	5.91^{b}	0.46
(L/kg DDM)	6	3.42^{c}	12.52^{a}	8.32^{b}	4.12^{c}	0.66
	12	2.08^{c}	13.81^{a}	6.93^{b}	3.44^{bc}	0.90
*p*H	3	6.81	6.81	6.82	6.83	0.01
	6	6.78	6.77	6.81	6.78	0.03
	12	6.74	6.61	6.69	6.69	0.04
Total VFA	3	3.12^{a}	3.13^{a}	2.82^{b}	3.11^{a}	0.02
(mmol/dl)	6	3.41^{b}	3.80^{a}	3.09^{c}	3.71^{a}	0.02
	12	4.22	4.51	4.21	3.89	0.21
Acetic acid	3	36.10^{a}	35.80^{a}	35.50^{a}	32.60^{b}	0.35
(A) (mol %)	6	32.60^{b}	35.70^{a}	33.40^{ab}	30.50^{b}	0.68
	12	27.60	29.90	29.40	29.70	0.64
Propionic acid	3	40.5^{ab}	39.40^{bc}	38.10^{c}	41.70^{a}	0.36
(P) (mol %)	6	40.00	36.70	38.10	41.10	2.12
	12	40.90^{a}	38.00^{ab}	36.30^{b}	40.10^{a}	0.80
Butyric acid	3	15.10^{b}	15.40^{b}	17.50^{ab}	20.50^{a}	0.78
(mol %)	6	18.50	18.90	19.80	20.30	2.23
	12	21.60^{b}	15.20^{c}	24.0^{a}	20.40^{b}	0.35
Isovaleric acid	3	3.71	4.10	4.41	5.31	0.62
(mol %)	6	5.82	5.72	7.29	7.22	1.15
	12	9.12	8.51	9.44	8.70	1.04
Acetic acid D	3	0.90^{a}	0.90^{a}	0.90^{a}	0.80^{b}	0.02
Propionic acid	6	0.80^{ab}	1.00^{a}	0.90^{ab}	0.70^{b}	0.04
	12	0.70^{b}	0.80^{a}	0.80^{a}	0.70^{ab}	0.02

^{a,b}Means with different letters in different treatment significantly differ (P<0.05). JNT, *J. curcas* kernel meal no treatment; JET, *J. curcas* kernel meal extraction treatment; JHT, *J. curcas* kernel meal heat treatment; JPT, *J. curcas* kernel meal high pressure treatment; SEM, standard error of the means; DM, dry matter; CP, crude protein; DDM, degradation dry matter; VFA, volatile fatty acid.

acid in *Jatropha curcas* kernel meal compared with soybean meal and rapeseed meal, and the DM degradability becomes lower after 6 h of *in vitro* incubation (Tagawa *et al*. 2013). Therefore, it is necessary to investigate the CP digestibility of *Jatropha curcas* kernel meal and its changes in rumen after long-term *in vitro* incubation for protein feed. Experiment I showed the changes of DM and CP in rumen during 72 h of *in situ* incubation. Especially, the higher CP degradability proved that the *Jatropha curcas* kernel meal might be adopted as a protein feed. We also observed that the degradation process of DM was slow, and its degradability was only 60%. Due to the effects of different heat treatments on the degradation of DM and CP in rumen, the nutrients in samples are changed (Mckinnon *et al*. 1991). The fast degradation parts of DM and CP could be converted into slow degradation parts or non-decomposition parts, which were caused by Maillard reaction. In addition, due to NDF binding to the true protein to generate neutral detergent insoluble crude protein (NDICP) during heat treatment, the effective degradable CP in the rumen would be decreased (Mckinnon *et al.* 1995). In the future study, we will investigate the changes of proteins with different characteristics in the rumen.

In the Experiment II, the degradability of DM and CP was increased with the increase of *in vitro* incubation time, and it was affected by different treatments. After JHT, the fibre degradation was affected by higher total acid detergent lignin (ADL) content in *Jatropha curcas* kernel meal, which contributed to lower degradability of DM and CP. In addition, the structures of proteins and amino acids can be changed, which can affect the rumen degradation (Liu *et al.* 2014). Some reports have shown that the diet treated by dry-heat treatment has a positive effect on ruminant absorbing protein, making trypsin inhibitor inactive and increasing the quantity of undegradable protein entering the small intestine (Demajanec *et al.* 1995). However, the excessively heated diets would show an opposite result (Makkar *et al*. 2008). Some reports have indicated that *Jatropha curcas* kernel meal treated by heat treatment is degraded about 23.3–43.9% after 24 h of *in vitro* incubation in rumen (Makkar *et al.* 1998). The data of the Experiment II revealed that *Jatropha curcas* kernel meal treated by heat treatment was degraded about 24.1% after 12 h of *in vitro* incubation, which was in agreement with the findings of Makkar *et al.* (2008). In a supplementary experiment, *in situ* incubation was used to detect the degradability of *Jatropha curcas* kernel meal. After 48 h of *in vitro* incubation, the DM degradability was about 43%, and the protein degradability was over 90%. Therefore, the CP of *Jatropha curcas* kernel meal had a good digestibility. In another feeding experiment, there were no differences in BUN in blood and VBN in rumen between the sheep fed with diets containing a little *Jatropha curcas* kernel meal and the sheep fed with the rapeseed meal, showing that the proteins of *Jatropha curcas* kernel meal digested in ruminant can be absorbed. The results showed that CP degradability of *Jatropha curcas* kernel meal was obviously lower after JPT treatment, which might be attributed to the loss of some water-soluble proteins during the high-pressure treatment, leading to the reduced total protein content of the raw material, while the high temperature destroyed the amino acid structure and degradability (González-Vega *et al.* 2011).

Although *Jatropha curcas* kernel meal was treated by different treatments, there was no gas production after the *in vitro* incubation. Gas production was caused by rumen fermentation and DM digestibility, which was attributed to the lower DM degradability and lower fermentation base NFC content. Methane production was affected by treatment method, and it was lower under the untreated condition. The methane level of untreated *Jatropha curcas* kernel meal was gradually decreased with the increase of *in vitro* incubation time. Therefore, PE might have an effect on

decreasing rumen microbial activity to inhibit the methane production. These results need to be verified in the future investigation. In addition, some reports have shown that tannin can inhibit the methane generation Puchala *et al.* (2005), and Makkar *et al.* (2007) have shown that *Jatropha curcas* kernel meal contains tannin. Therefore, the produced methane content can reflect tannin content of *in vitro* incubated samples. *Jatropha curcas* kernel meal treated by different treatments not only affected its residue toxicity, but also affected the contents of other materials.

Rumen fermentation can reflect the digestibility (Firkins *et al.* 1986), and it is indirectly related to base composition and treatments (Lei *et al*. 2018). In the Experiment II, the DM digestibility was different after different treatments. However, there was no significant difference in the *p*H in rumen at the same incubation time. The CP in base was easy to ferment, while the NDF was difficult to be used, and the difference of fiber content might affect the pH of rumen fluid (Nsahlai *et al*. 1994). Moreover, acetic acid and propionic acid showed significant differences, which might be caused by the phorbol and fiber contents of *Jatropha curcas* kernel meal with different treatments.

Collectively, after treatments of JHT, JET and JPT, the PE content was decreased. In addition, the CP content was higher, of which the degradability was more than 90% after 72 h of *in situ* incubation. The *Jatropha curcas* kernel meal with different treatments had no negative effect on rumen fermentation characteristics after *in vitro* incubation.

REFERENCES

Akbar E, Yaakob Z, Kamarudin S K, Ismail M and Salimon J. 2009. Characteristic and composition of *Jatropha curcas* oil seed from Malaysia and its potential as biodiesel feedstock. *European Journal of Scientific Research* **29**: 396–403.

AOAC. 1999. Association of Official Analytical Chemists. *Official Methods of Analysis of AOAC International*.16th edn. AOAC International, Gaithersburg, MD.

Chivandi E, Makuza S M, Erlanger K H, Mtimuni J P, Read J S and Tivapasi M. 2000. Effects of dietary *Jatropha curcas* on the haematology of weaned pigs. *Zimbabwe Veterinary Journal* **31**: 83–91.

Conway E J. 1962. *Microdiffusion Analysis and Volumetric Error*. 5th edn. Crosby Lockwood, London.

Demjanec B, Merchen N R and Cremin J D. 1995. Effect of roasting on site and extent of digestion of soybean meal by sheep: I. Digestion of nitrogen and amino acids. *Journal of Animal Science* **73**: 824–34.

Devappa R K, Makkar H P S and Becker K. 2010. Optimization of conditions for the extraction of phorbol esters from Jatropha oil. *Biomass Bioenergy* **34**: 1125–33.

Firkins J L, Berger L L, Merchen N R and Fahez G C Jr. 1986. Effects of forage particle size, level of feed intake and supplemental protein degradability on microbial protein synthesis and site of nutrient digestion in steers. *Journal of Animal Science* **62**: 1081–94.

Gadir A, Onsa T O, Ali W E M, El Badwi S M A and Adam S E I. 2003. Comparative toxicity of *Croton macrostachys*, *Jatropha curcas*, *Piper abyssinica* seeds in Nubian goats. *Small Ruminant Research* **48**: 61–67.

Goel G, Makkar H P S, Francis G and Becker K. 2007. Phorbol esters: Structure, biological activity, and toxicity in animals. *International Journal of Toxicology* **26**: 279–88.

González-Vega J C, Kim B G, Htoo J K, Lemme A and Stein H H. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. *Journal of Animal Science* **89**: 3617–25.

Hamada T. 1971. Physicochemical analytical method for nutritional experiments, pp. 428–431. *Animal Nutrition Testing Method 1st edn*. (Ed) Morimoto H. Yokendo, Tokyo.

Hipal G, Mayank G, Nikhil G, Soham T, Prasad P, Girish G, Vamsi K K, Reddy M P, Sethiya B D and Rathod M R. 2009. Isolation and characterization of phorbol esters (toxin) from the *Jatropha curcas* L. *International Journal of Microbiology Research* **1**: 1–7.

Lei Y G, Li X Y, Wang Y Y, Li Z Z, Chen Y L and Yang Y X. 2018. Determination of ruminal dry matter and crude protein degradability and degradability kinetics of several concentrate feed ingredients in cashmere goat. *Journal of Applied Animal Research* **46**: 134–40.

Liberalino AAA, Bambirra E A, Moraes-Santos E A and Viera C E. 1988. *Jatropha curcas* L. seeds. Chemical analysis and toxicity. *Brazilian Archives of Biology and Technology* **31**: 539–50.

Liu Y, Song M, Maison T and Stein H H. 2014. Effects of protein concentration and heat treatment on concentration of digestible and metabolizable energy and on amino acid digestibility in four sources of canola meal fed to growing pigs. *Journal of Animal Science* **92**: 4466–77.

Lv R L, Horiguchi K, Tagawa S, Ichihara S and Yoshida N. 2020. Effect of inclusion heat treated Jatropha oil meal in wethers ration on feed intake, blood parameter and ruminal fermentation in short-term feeding. *Pakistan Journal of Zoology* **52**: 1647–55.

Makkar H P S, Becker K, Sporer F and Wink M. 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *Journal of Agriculture and Food Chemistry* **45**: 3152–57.

Makkar H P S, Becker K and Schmook B. 1998. Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. *Plant Foods for Human Nutrition* **52**: 31–36.

Makkar H P S, Francis G and Becker K.2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal* **1**: 1371–91.

Makkar H P S, Francis G and Becker K. 2008. Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. *Journal of the Science of Food and Agriculture* **89**: 1542–48.

McDougal E I. 1948. Studied on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochemical Journal* **43**: 99–109.

Mckinnon J J, Olubobokun J A, Christensen D A and Cohen R D H. 1991. The influence of heat and chemical treatment on ruminal disappearance of canola meal. *Canadian Journal of Animal Science* **71**: 773–80.

McKinnon J J, Olubobokun J A, Mustafa A, Cohen R D H and Christensen D A.1995. Influence of dry heat treatment of canola meal on site and extent of nutrient disappearance in ruminants. *Animal Feed Science and Technology* **56**: 243–52.

Nsahlai I V, Siaw D and Osuji P O. 1994. The relationships between gas production and chemical composition of 23 browses of the genus *Sesbania*. *Journal of the Science of Food*

and Agriculture **65**: 13–20.
Openshaw K. 2000. A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biomass Bioenergy* **19**: 1–15.
Puchala R, Min B R, Goetsch A L and Sahlu T. 2005. The effect of acondensed tannin-containing forage on methane emission by goats. *Journal of Animal Science* **83**: 182–86.
SAS. 1995. SAS/STAT User's Guide, Version 9.2 Edition. SAS Institute, Cary, NC, USA.
Tagawa S, Lv R, Horiguchi K, Yoshida N, Takahashi T, Ichihara S and Hishinuma S. 2013. Chemical composition, *in vitro* digestibility, gas production and methane production of Jatropha (*Jatropha curcas* L.) meal. *Japanese Journal of Grassland Science* **59**: 120–23. (Abstract).
Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.
Wilhelm H and Mittelbach M. 2000. Detoxification experiments with the seed oil from *Jatropha curcas* L. *Industrial Crops and Products* **12**: 111–18.