Fermentability and Digestibility of Ration Containing Crude Curcin Extract of *Jatropha curcas* L. Seed Meal

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Abstract

Jatropha (J.) curcas L. seed meal (JCSM) is a byproduct of J. curcas L. oil extraction. This JCSM contains high protein concentration (37.56% DM) and ether extract (35.02% DM) which makes a potential source of animal feed. However, JCSM utilization can be limited by the presence of antinutrients such as curcin or lectin, and phorbolester. These antinutrients, especially the curcin, can be digested differently by microbes from the rumen fluid of cattle and buffalo. Therefore, an experiment was conducted to study fermentability and digestibility (in vitro) of ration that contain crude curcin extracted from Jatropha curcas L. seed meal by microbes from the rumen fluid of cattle and buffalo. Factorial randomised block designs were used in fermentability (4x2x2), and digestibility (4x2) studies; three replications were used in both experiments. The treatments were levels of crude curcin extracted from JCSM (0, 1, 2 and 3% v/w) that were added into rations as factor A, microbes from the rumen fluid of cattle and buffalo as factor B, and incubation times (0 and 3 h) as factor C in fermentability study; factors A and B were also applied as treatments in digestibility experiment. Variables measured were ammonia and total VFA concentrations, total bacterial and protozoal populations, and dry matter (DM) and organic matter (OM) digestibilities. Data were analysed using analysis of variance, and differences in treatment means were examined with contrast or polynomial orthogonal. The results showed that effects of addition levels of crude curcin extracts into rations were not significant on all variables measured, except the total protozoal population ($P<0.01$). There were differences between the rumen fluid of cattle and those from the rumen fluid of buffalo on total bacterial population, DM and OM digestibilities ($P<0.01$). Ammonia and total VFA, and total bacterial population were increased at 3h incubation time ($P<0.01$). It is concluded that addition of crude curcin extracts from JCSM up to 3% (v/w) did not produced negative effects on ration fermentability and digestibility, except for protozoal population. The greater numbers of bacteria in the rumen fluid of buffalo than those in the rumen fluid of cattle cause higher DM and OM digestibilities.

Keywords: curcin, digestibility, fermentability, Jatropha curcas L. seed meal

Introduction

Jatropha (*J*.) *curcas* L. seed meal (JCSM) is a byproduct of *J. curcas* L. oil extraction and has potential source as animal feed due to its production potential and nutrient contents as protein and energy sources. In Indonesia, the production was about 0.4 ton/ton dry seed with 200 - 300 l oil production (Brodjonegoro *et al*., 2005). JCSM without husk contained 86.26% dry matter (DM), 37.56% crude protein (CP), 35.02% ether extract (EE), 7.23% crude fibre (CF), 12.47% nitrogen free extract (NFE),7.71% ash, 16.30% NDF, 15.86% ADF, 4.51% lignin and 0.01% silica (DM basis). Nut husk inclusion in oil extraction increased DM and fibre fractions, but reduced CP and EE contents; the husk contained high fibre fractions (Tjakradidjaja *et al*., 2007). The fibrous fractions and presence of curcin and phorbolester as the main antinutrients (Martinez-Herrera *et al*., 2006; Makkar *et al*., 1998) limited its use, but antinutrient effects varied among JC provenances, location, *etc* (Makkar and Becker, 1997; Makkar *et al*., 1997).

JCSM is, then, more suitable for ruminants than for monogastric. The presence of rumen microbes, especially the bacteria, has enable the ruminants to degrade CF or antinutrients developed by adaptation to feeds that were consumed; however, these varied among rumen microbes and ruminants (McDonald *et al*., 2002). Tolerance of rumen microbes from goat, sheep, cattle and buffalo to JCSM antinutrients had been studied (Tjakradidjaja *et al*., 2008; Tjakradidjaja *et al*., 2010). Goat rumen bacteria were more able to degrade JCSM and tolerate its antinutrients within 0-12 h fermentation with proteolytic bacteria are important in degrading antinutrients having protein structure. These led to extract curcin from JCSM and study its effects on ration fermentability and digestibility. Its addition up to 3% (v/w) did not affect fermentability, rumen fluid of goat had greater total bacterial population with no change in protozoal population at 3 h incubation, but lower DM and OM digestibility compared to sheep (Tjakradidjaja *et al*., 2011). The effects can be different using rumen fluids of large ruminant. Therefore, an experiment was conducted to study fermentability and digestibility (*in vitro*) of ration containing JCSM crude curcin extract by microbes from cattle and buffalo rumen fluids.

Materials and Methods

Materials were JCSM with husk, crude curcin extract, cattle rumen fluids obtained from slaughter house in Bogor and the buffalo from fistulated animal in BATAN, and ration. The ration was elephant grass (*Pennisetum purpureum*), ground corn and concentrate= 50 : 25 : 25% w/w). Crude curcin was extracted with Stirpe *et al*. (1976) method modified due to limitations in availability of experimental apparatus. Fermentability study was done following the first stage of Tilley and Terry method (1963) modified by Sutardi (1979); the two stage method was used

in digestibility study. Ammonia and total VFA concentration was determined, respectively, by micro diffusion Conway and steam distillation method (General laboratory procedure, Department of Dairy Science, 1966). Ogimoto and Imai (1981) method was used to count total bacterial (serially dillution method) and protozoal populations.

Three factors applied in fermentability study; factor A:JCSM crude curcin extract levels added into rations (0, 1, 2 and 3% v/w), factor B:rumen fluid sources (cattle and buffalo), and factor C:incubation time (0 and 3 h). Only factor A and B were treatments in digestibility study. Variables were concentrations of ammonia and volatile fatty acid (VFA), total bacterial and protozoal populations, and DM and organic matter (OM) digestibility coefficients. Experimental design and statistical analysis (analysis of variance, and orthogonal) were carried out based on Steel and Torrie (1993). Factorial randomised block design 4x2x2 and 4x2 were, respectively, used in fermentability and digestibility experiment with rumen fluids (three replications for each animals) were used as blocks.

Results and Discussion

Crude curcin extract and its effect on nutrient composition of rations

The extract in this experiment (370 ml/250 g dry weight) was smaller than that obtained by Tjakradidjaja *et al*. (2011) that was due to differences in JCSM sample and experimental condition during extraction. Addition levels at 1, 2 and 3% (v/w) were equal to addition of 0.67, 1.33 and 1.99% JCSM into treatment rations which were greater than that used by Ahmed and Adam (1979), 0.25% JCSM. Extract also contained saponin at 0.2% (Laboratory of Biofarmaka, IPB, 2008) that may also influence treatment effects. Extract addition slightly reduced DM (89.66 to 83.41-85.22%), NFE (55.13 to 51.65-52.10%) and TDN (68.71 to 66.75-67.77 %) contents of treatment rations, but increased CP (13.98 to 14.8 -15.56%) and CF (18.64 to 19.82-20.79%) contents; the effects did not follow linear patterns with extract levels. Increase in CP content was due to addition of protein structure of curcin, glycoprotein (Makkar and Becker, 2004; Juan *et al*., 2002; Aregheore *et al*., 2003).

Effects of treatments on fermentability, microbial population and digestibility

Table 1 showed that extract addition levels decreased significantly total protozoal population ($P<0.01$). Rumen fluid sources produced significant effects on microbial numbers ($P<0.01$). Incubation for 3 h increased ammonia ($P<0.01$) and total VFA ($P<0.05$) concentrations and total bacterial numbers ($P<0.01$) without changing protozoal numbers.

Results indicate that rumen bacteria were able to degrade protein including the extract, and ferment energy sources. The present ammonia concentration was

Table 1. Effects of treatments on all variables

Treatments	Variables					
	Ammonia (mM)	VFA (mM)	Bacterial population (x 10^8 colony forming unit/ml)	Protozoal population (x 10^5 cel/ml)	DM digestibility (%)	OM digestibility (%)
Curcin level addition[1,2]						
0% (v/w)	21.12±6.84	149.82± 12.95	0.98±0.77	0.78±0.05^{Aa}	41.62±5.43	39.97±6.50
1% (v/w)	21.26±4.92	155.77± 12.22	0.86±0.69	0.64±0.02^{Ab}	39.45±3.52	37.30±4.76
2% (v/w)	21.60±4.33	159.18± 12.26	0.50±0.11	0.57±0.02^{Bc}	36.33±5.55	34.24±7.32
3% (v/w)	22.19±3.26	164.85± 9.36	0.46±0.22	0.51±0.00^{Bc}	37.86±5.78	34.72±5.91
Rumen fluid sources[1,2]						
Cattle	21.63±0.83	153.19± 6.76	0.26±0.05^{A}	0.48±0.15^{A}	35.23± 2.56^{A}	32.23± 2.92^{A}
Buffalo	21.46±0.19	161.62± 5.87	1.14±0.47^{B}	0.77±0.08^{B}	42.40± 2.19^{B}	40.89± 2.56^{B}
Incubation time[1,2]						
0 h	18.12± 1.50^{A}	149.13± 7.31^{a}	0.38± 0.06^{A}	0.61±0.10	-	-
3 h	24.96± 0.67^{B}	165.68± 0.67^{b}	1.02±0.49^{B}	0.64±0.13	-	-

[1]Means with different superscript in capital letter within column differ significantly at (P<0.05)
[2]Means with different superscript in capital letter within column differ significantly at (P<0.01).

greater than that obtained from JCSM fermented by cattle and buffalo rumen fluids (Tjakradidjaja *et al*., 2008). Ammonia concentration had also been used to show ability of rumen microbes to degrade ricin from castor seed meal (*Ricinus communis* L.) at levels 0.42-1.68 mg/ml within 3-12 h incubation (de Oliviera *et al*., 2010), but rumen microbes could not degrade phorbolester (Makkar and Becker, 2010). Ability of rumen microbes degrading antinutrient with protein structure still depended on its concentration. Protozoal number reduction also occurred in other study (Tjakradidjaja *et al*., 2011). Protozoal cell was damaged and lysed by curcin and saponin (Fardiaz, 1992; Juan *et al*., 2002) due to greater sensitivity of protozoal cell, eukaryotes, to curcin and saponin than bacterial cell, prokaryotes (Makkar *et al*., 1998; de Oliviera *et al*., 2010). Sample used caused differences in microbial numbers between the ruminants. Less microbial population in cattle rumen fluid

because cattle was not fed properly before being slaughtered; the greater microbial numbers in buffalo was due to regular feeding. Other factors were differences in growth rates, enzyme activities (Bathia *et al*., 1980; Pradhan, 1994), and degree of resistancy to antinutrients (curcin and saponin) which may be greater in microbes from buffalo than cattle; however, these still needs a further clarification.

Differences in rumen fluid affected DM and OM digestibilities ($P<0.01$) with greater result was obtained from the buffalo (Table 1). DM digestibility was comparable to those found by Hakim (2002) for ration containing grass and concentrate (38-46%). The greater microbial population and its ability to ferment nutrients from rumen fluid of buffalo provided more nutrients that were more easily digested by enzymes in the post ruminal organ.

Conclusion

Addition of JCSM crude curcin extracts up to 3% (v/w) did not produce negative effects on ration fermentability and digestibility, except for protozoal population. The greater numbers of bacteria in rumen fluid of buffalo than cattle caused higher DM and OM digestibilities.

Acknowledgment

Thanks are due to Department of Animal Nutrition & Feed Technology, Faculty of Animal Science, Bogor Agricultural University, for research funding (Due-*like* Project 2006).

References

Ahmed, O. M. M., & S. E. I. Adam. 1979. Effects of *Jatropha curcas* on calves. Veterinary Pathology 16 : 476-482. http://www.vetpathology.org/misc/terms. [17 Mei 2008].

Aregheore, E. M., K. Becker & H. P. S. Makkar. 2003. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. J. South Pacific Nat. Sci. 21:50-60.

Bhatia, S. K., K. Pradhan & R. Singh. 1980. Ammonia anabolizing enzymes in cattle and buffalo fed varied non protein nitrogen and carbohydrates. J. Dairy Sci. 63: 104-1108.

Brodjonegoro, T. P., I. K. Reksowardjojo & T. H. Soerawidjaja. 2005. Jarak pagar, sang primadona. http://www.pikiran-rakyat.com/cetak/2005/1005/13/cakrawala/tama02.htm [2Desember 2007].

de Oliviera, A. S., M. R. C. Oliviera, J. M. S. Campos, R. P. Lana, O. L. T. Machado, C. A. Retamal, E. Detmann, & S. C. Valadares Filho. 2010. *in vitro* ruminal degradation of ricin and its effect on microbial growth. Anim. Feed Sci. and

Technol. 157:41 54.
Fardiaz, S. 1992. Mikrobiologi Pangan. PT. Gramedia Pustaka Utama. Jakarta.
General Laboratory Procedures. 1966. Department of Dairy Science. University of Wisconsin. Madison.
Hakim, R. S. 2002. Evaluasi *in vitro* respons mikroba rumen ternak ruminasia terhadap penambahan DABA (2,4-diaminobutyric acid) dan lamtoro merah (*Acacia villosa*) dalam ransum. Skripsi. Fakultas Peternakan. IPB. Bogor.
Juan, L., C. Yu, X. Ying, Y. Fang, T. Lin & C. Fang. 2002. Cloning and expression of curcin, a ribosome-inactivating protein from the seeds of *Jatropha curcas*. http://www.bioline.org.br. [2 Desember 2007].
Makkar, H. P. S., A. O. Aderibigbe & K. Becker. 1998. Comparative evaluation of non toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. J. Food Chem. 62:31-36.
Makkar, H. P. S., & K. Becker. 1997. *Jatropha curcas* toxicity : identification of toxic principles. 5th International Symposium on poisonous plants. May 19-32. San Angelo.
Makkar, H. P. S., & K. Becker. 2004. Nutritional studies on rats and fish carp (*Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non toxic provenance. J. Chem. and Mat. Sci. 52:183-192.
Makkar, H. P. S., & K. Becker. 2010. Are *Jatropha curcas* phorbolesters degraded by rumen microbes. J. Sci. in Food and Agric. 90:1562-1565.
Makkar, H. P. S, K. Becker, F. Sporer & M. Wink. 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J. Agric. Food and Chem. 45:3152-3157.
Martinez-Herrera, J., G. Davila-Ortiz, G. Francis, P. Siddhuraju & K. Becker. 2006. Chemical composition, toxin/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chem. 96:80-89.
McDonald, P. R., A. Edwards, J. F. D. Greenhalg & C. A. Morgan. 2002. Animal Nutrition 6th Ed. Pearson Education Ltd. Harlow.
Ogimoto, K, & S. Imai. 1981. Atlas of Rumen Microbiology. Japan Science Society Press. Tokyo.
Pradhan, K. 1994. Rumen ecosystem in relation to cattle and buffalo nutrition. Proceeding 1st Asian Buffalo Association Congress.
Steel, R. G. D., & J. H. Torrie. 1993. Prinsip dan Prosedur Statistik suatu Pendekatan Biometrik. Terjemahan. Gramedia. Jakarta.
Stirpe, F., A. Pession-Brizzi, E. Lorenzoni, P. Strocchi, L. Montanaro & S. Sperti. 1976. Studies on the proteins from the seeds of *Croton tiglium* and *Jatropha curcas*. J. Biochem. 156:1-6
Sutardi, T. 1979. Ketahanan protein dalam makanan terhadap degradasi oleh mikroba rumen dan manfaatnya bagi peningkatan produktivitas ternak. Prosiding

Seminar Penelitian dan Penunjang Pengembangan Peternakan. LPP. Balitbangtan. Departemen Pertanian Republik Indonesia. Bogor.

Tilley, J. M. A., & R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Bri. Grassland Soc. 18:104-111.

Tjakradidjaja, A. S., I. K. Amrullah & H. Bulwafa. 2011. Toleransi mikroba rumen kambing dan domba terhadap penambahan ekstrak kasar antinutrien bungkil biji jarak pagar (*Jatropha curcas* L.) ke dalam ransum berdasarkan fermentabilitas dan kecernaan *in vitro*. Prosiding Seminar Nasional Peternakan Berkelanjutan III. Fakultas Peternakan. Universitas Padjadjaran. 2-3 November 2011. Jatinangor.

Tjakradidjaja, A. S., Suryahadi & Adriani. 2007. Fermentasi bungkil biji jarak pagar (*Jatropha curcas* L.) dengan berbagai kapang. Proceeding Konferensi Jarak Pagar - Menuju Bisnis Jarak Pagar yang Feasible -2007. SBRC - IPB. Bogor.

Tjakradidjaja, A. S., K. G. Wiryawan & A. Ulya. 2008. Rumen microbial tolerance to the use of *Jatropha curcas* seed meal fermented by rumen microbes from several ruminants. (Paper) International Jatropha Conference 2008. SBRC - IPB. 24-25 June 2008. Bogor.

Tjakradidjaja, A. S., K. G. Wiryawan & G. S. Dewi. 2010. *in vitro* evaluation of *Jatropha curcas* seed meal fermented by rumen microbes from several ruminants. (Paper) International Seminar of Indonesian Society for Microbiology. 4-7 October 2010. Bogor.