

Evaluation of Protein Protected in the Cow Beef Cattle Rations Base-on the Fermentation and Microbia Activities Ruments by *In Vitro*

Riyanto, J¹, E. Baliarti², T. Hartatik², D.T. Widayati² and L. M. Yusiati²

¹Animal Husbandry Department, Faculty of Agriculture, Sebelas Maret University, Indonesia

²Faculty of Animal Science, Gadjah Mada University, Indonesia

Corresponding email: ¹ jokoriyanto@staff.uns.ac.id

ABSTRACT

The purpose of this study was to evaluate the effect of protein protection in the rations of beef cattle on rumen fermentation and microbial activities *in vitro*. Soybean groats as a source of protein. Protection using 37% formaldehyde at 2% dry matter of feed mixture of soybean groats and lemuru fish oil was conducted. Rumen fluid were taken from the Ongole crossbred cow fistulated. Rumen fermentation was observed 48h of incubation in rumen fluid by *in vitro*. Feed treatments including T0 (30% fermented straw+30% elephant grass+40% control concentrate), T1 (30% fermented straw+30% elephant grass+32.5% control concentrate+7,5% protein unprotected), and T2 (30% fermented straw+30% elephant grass+32.5% control concentrate+7,5% protein protected). The results of the rumen fermentation by *in vitro* evaluation showed that pH, ammonia and VFA levels were not significantly affected by the treatment. The microbial activity by *in vitro* evaluation showed that microbial protein synthesis and CMC-ase were not significantly different ($p>0.05$) but protozoa population significantly reduced ($p<0.05$). The number of protozoa in the 7.5% of protected proteins in the cow beef cattle rations was lower than in other treatments *in vitro*. It was concluded that are rumen fermentation and rumen microbial activity by *in vitro* evaluation were not affected by the formaldehyde treatment. Formaldehyde treatment at 7.5% resulted in lower number of protozoa but this did not interfere with the continuity of *in vitro* rumen fermentation.

Keywords: Protein protected, The cow beef cattle rations, Fermentation ruments, Microbiaactivities ruments, *In vitro*

INTRODUCTION

High protein feed sources for ruminants can be obtained from soybean cultivation. In the processing of soybean grains, soybean groats is the fraction of soybeans seeds after the removal of the hull so that the nutrient content in it is the same as soybean (Lukito, 2010). Feed ingredients of high-protein sources have high degradation rates in the rumen, which are influenced by the ability of rumen microbes to degrade proteins, ammonia concentrations and their solubility rates in the rumen (Puastuti *et al.*, 2006).

Soybean groats as a protein source needs to be protected to reduce the rate of degradation by microbial populations in the rumen. One method of protecting protein from fermentation and degradation in the rumen is the use in the treatment with formaldehyde (Riyanto *et al.*, 2013). The protein-aldehyde bond that is formed during the treatment can protect the proteins from the rumen microbes, hence by-passing the rumen and eventually

digested and absorbed in the small intestines. Formaldehyde protection can increase the fraction of non-degraded proteins by 50-80% but does not decrease the digestibility in the small intestine.

The primary role of microbes in the rumen is in the digestion of crude fibre in the feed and at the same time synthesize proteins. Microbial population in the rumen are also important in the process of digestion of complex proteins with the help of enzymes. The digestive end result by microbes are volatile fatty acids (VFA) and ammonia compounds that can be utilized by ruminants as a source of energy for ruminants (Filipek and Dvorak, 2009). Rumen requires an optimum condition so that microbial rumen can perform fermentation activities well and will improve the digestibility, of both fibrous and non-fibrous compounds in the rations that are consumed. This condition is expected to produce VFA in the normal amount. Based on the above thinking, it is necessary to do research to determine the effect of the use of protected proteins on the fermentation characteristics in vitro.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Nutritional Biochemistry Faculty of Animal Science, Gadjah Mada University in Indonesia. Rumen fluid was taken from a fistulated Ongole crossbred cow. Rumen fermentation was observed 48h of incubation in rumen fluid by in vitro. Protection of soybeans groat was conducted as follows: soybean meal flour was first mixed with lemuru fish oil with a ratio of 4: 1. The mixture was then sprayed with 37% formaldehyde solution at 2% dry matter and allowed to stand for 24 hours in a closed state.

Ration of beef cattle was prepared containing 60% forage and 40% of basal concentrate. The forage consisted of 30% fermented rice straw and 30% elephant grass. The basal concentrate composed of 16% rice bran, 8% wheat bran, 5.6% coffee husks, 8% soybean meal, 1.6% vitamin and Minerals and 0.8% salt.

The dietary treatments were: T0 (30% fermented straw+30% elephant grass+40% control concentrate), T1 (30% fermented straw+30% elephant grass+32.5% control concentrate+7.5% protein un protected), T2 (30% fermented straw+30% elephant grass+32.5% control concentrate+7.5% protein protected).

Experimental design used was a completely randomized design. When there was significant difference among the means difference was tested using the Duncan multiple range test (DMRT). Parameters of rumen fermentation by in vitro evaluation measured were pH, ammonia and VFA. Parameter of rumen microbial activity by in vitro evaluation were microbial protein, CMC-ase, and protozoa number.

RESULTS AND DISCUSSION

Rumen Fermentation

The mean pH, ammonia (NH₃), and volatile fatty acids (VFA) on the in vitro rumen fermentation parameters of various treatments are shown in Table 1.

The mean pH of rumen fluid in each treatment were not significantly different ($P > 0.05$). This indicates that the dietary treatment did not affect the pH of the rumen fluid. The mean pH of rumen fluid in each treatment was in the range of microbial activity. Johnson (1966) suggests that the optimal pH range for microbial activity works well is 6.7 - 7. The results agree with the findings of Yulianto (2011) who suggested that adequate proportion of forage in the ration is important so that the pH of rumen does not decrease and increase dramatically.

Table 1. Rumen fermentation (pH, ammonia and VFA) by in vitro

Parameter	Treatment			P Value
	T0	T1	T2	
pH	7,03	7,03	7,07	0,11
NH ₃ (mg/100ml)	20,01	18,86	16,07	0,42
VFA (mM)	59,09	54,00	48,88	0,76

T0 (30% fermented straw+30% elephant grass+ 40% control concentrate), T1 (30% fermented straw+30% elephant grass+32.5% control concentrate+7,5% protein unprotected), and T2 (30% fermented straw+30% elephant grass+32.5% control concentrate+7,5% protein protected).

The NH₃ production of rumen fluid in each treatment was not significantly different ($P>0.05$). This shows that dietary treatment of T0, T1 and T2 did not affect NH₃ production. The NH₃ concentration values are strongly influenced by the rumen microbial ability to degrade the ration protein and show the amount of protein present in the rumen. Soybean groats which is a source of protein that has high degradation properties allow to produce a high enough NH₃ as well. The NH₃ value in this study was in the range of 16.07 - 20.01 mg / 100ml. McDonald *et al.* (2002) pointed out that the NH₃ concentration in cow rumen ranged from 85 - 300 mg / l (8.5 - 30 mg / 100ml). This result suggest that the treatments used provided NH₃ in sufficient levels for microbial growth. The NH₃ production in the rumen was supported by an optimum rumen pH fluid environment for microbes to work optimally. Microbial activity was not disturbed because the resulting NH₃ is at an amount sufficient for microbial requirements.

The production of VFA of rumen fluid in each treatment were not significantly different ($P>0.05$). Rumen requires optimum conditions for bacteria to perform fermentation activities well. VFA is utilized by host animal through the rumen wall as a digestible energy to meet its energy needs by 60% (Fathul and Wajizah, 2010). The results of this study indicate that the VFA production in the range 48.88 - 59.09 mM was lower than the range recommended by McDonald *et al.* (2002) where the optimal VFA concentration required for microbial growth ranges at 80-160 mM. The low VFA concentrations in this study was probably due to the low protein available as a result of protein protection. Harwanto (2013) showed similar results with the addition of cinnamaldehyde containing aldehyde compounds. Protein protection causes the reduction of the degraded protein to the resulting amino acid as one of the VFA-formers, resulting in reduced VFA production.

Microbial Activity

The average microbial protein, the number of protozoa and CMCase on the parameters of microbial activity of in vitro assay are shown in Table 2.

The microbial protein content of rumen fluid in each treatment was not significantly different ($P>0.05$). Microbial proteins are closely related to the availability of VFA and NH₃. Harwanto (2013) stated that the addition of cinnamaldehyde which was one of the aldehyde compounds in the ration did not affect the total microbial protein. The results of this study showed that the amount of microbial protein produced was related to the VFA and NH₃ production. These results showed that the formaldehyde treatment did not affect microbial proteins production. It is probable that the treatment may result in unbalanced availability of VFA and NH₃. Setyawati (2008) states that if the source of protein in the supply of nitrogen (N) and energy sources in providing unbalanced carbon framework can cause microbes not able to form their body protein properly.

Table 2. Microbial activity (microbial proteins, Protozoa Number and CMC-ase) by in vitro

Parameter	Treatment			P Value
	T0	T1	T2	
Microbial activity (mg/100ml)	63,27	71,50	63,77	0,54
Protozoa number (x 10 ³ sel/ml)	27,33 ^a	21,80 ^a	12,47 ^b	0,03
CMC-Ase (U/g)	5,97	6,64	6,83	0,91

^{ab} different superscripts in the same row showed significant differences ($P < 0.05$). T0 (30% fermented straw+30% elephant grass+40% control concentrate), T1 (30% fermented straw+30% elephant grass+32.5% control concentrate+7,5% protein unprotected), and T2 (30% fermented straw+30% elephant grass+32.5% control concentrate+7,5% protein protected).

The formaldehyde treatment of soybean significantly ($P < 0.05$) affected the number of rumen protozoa. Soybean groats protection decreased the amount of protozoa, suggesting that the availability of feed proteins in the rumen is reduced as a result of protein protection. Undegraded proteins in large quantities can cause a reduction in the availability of proteins for protozoa, hence a reduction in protozoa population. Dewhurst *et al.* (2000) states that the number of protozoa is influenced by protein levels and changes in the number of protozoa that are predatory will provide opportunities for bacteria to thrive. Protozoa form the body through the degradation of protein feed and protein from bacteria.

The method of protein protection is performed by utilizing the protein matrix to bind to the aldehyde of formaldehyde, so that the outer matrix of proteins is protected. According to Kiernan (2000) the protection of proteins by using formaldehyde leads to the formation of cross-links with proteins that enclose the outer layer of protein matrix. The rumen microbes including the protozoa can not degrade the protein as a result of the protection. Decreasing the number of protozoa in the rumen does not interfere with the VFA concentration, and the decline in the number of protozoa still considered normal. Arora (1989) stated that protozoa is important because it can stabilize the pH during fermentation so that it can function as a buffer.

Protection of soybeans groats in feed ration did not significantly affect CMC-ase activity. Weimer (1996), showed that the onset of CMC-ase enzyme activity can occur due to fibers derived from forage fermented substances for the growth of CMC-ase-producing bacteria which act to degrade cellulose as feed fiber component. Similarly, Harwanto (2013) showed that the use of aldehyde from cinnamaldehyde did not affect the activity of CMC-ase. This is in line with the results of this study that the administration of formaldehyde protection had no significant effect on the activity of CMC-ase. The activity of CMC-ase was not affected by the treatment. CMC-ase activity is associated with cellulolytic bacteria. Cellulose can only be broken down by cellulase enzymes that can only be secreted by cellulolytic microbes. Cellulolytic bacteria can survive by utilizing NH₃ rumen concentrations. According to Erwanto (1995) about 82% of rumen microbial species utilize nitrogen sources in the form of ammonia (NH₃) for protein synthesis. This study showed that CMC-ase was not affected by protected soybean in rations, therefore the decrease in NH₃ resulting from protected proteins did not affect the growth of rumen microbes. Protozoa have a relatively large role in degrading fibers from bacteria, so decreasing the number of protozoa will reduce fiber degradation, and the decrease in CMC-ase activity does not interfere with partial VFA concentrations.

CONCLUSIONS

In conclusion, rumen fermentation and rumen microbial activity were not affected by either unprotected and protected protein sources. The dietary treatment with 7.5% protected proteins resulted in lower number of protozoa but did not interfere with the continuity of rumen fermentation and rumen microbial activity invitro.

REFERENCES

- Arora, S. P. 1989. Digestion of Microbes in Ruminants. Gadjah Mada University Press, Yogyakarta.
- Dewhurst, R. J., D. R. Davies, and R. J. Merry. 2000. Microbial protein supply from the rumen. *Anim. Feed Sci. Technol.* 85: 1-21.
- Erwanto. 1995. Optimization of Rumen Fermentation System through Sulfur Supplementation, Defnuation, Reduction, Methane Emissions and Stimulation of Microbial Growth in Ruminant Livestock. Post Graduate Program. Bogor Agricultural Institute.
- Fathul, F. and S. Wajizah. 2010. The addition of Mn and Cu microminerals in ration to rumen sheep biofermentation activity in vitro. *J. Anim. Veterinary Sci.* 15: 9-15.
- Filipek, J. and R. Dvorak. 2009. Determination of the volatile acid content in the rumen liquid: comparison of gas chromatograpy and capillary isotachophoresis. *Acta Veterinaria Brno.* 78: 627-633.
- Harwanto. 2013. The Influence of Addition of Cinnamon (*Cinnamomum Burmanni* Ness ex BI) as Sinamaldehyd Source in the Feed to Methane Production and Thin Tail Sheep Performance. Thesis. Universitas Gadjah Mada. Yogyakarta.
- Johnson, R. R. 1966. Tecchniques and procedures for in vitro and in vivo rumen studies. *J. Anim. Sci.* 25: 855-875.
- Kiernan, J. A. 2000. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Article published in *Microscopy Today* 00-1 pp. 8-12.
- Lukito, V. A. 2010. Effect of Use of Soybean Cherry, Fish Meal and Oil Palm Cake Protected Against pH, NH₃ Concentration and Microbial Protein Rumen Beef Peranakan Ongole Berfistula. Essay. Sebelas Maret University, Surakarta.
- Mc Donald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 2002. *Animal Nutrition*. 5th ed. Longman Scientific and Technical, New York.
- Puastuti, W., I. W. Mathius, and D. Yulisiani. 2006. Soybean meal protected by liquid banana stem as feed of sheep stock: in sacco and in vivo. *J. Anim. Veterinary Sci.* 11: 106-115.
- Riyanto, J., S. D. Widyawati, W. P. Suprayogi, A. P. Astuti, and T. R. Setiadi. 2013. In vivo degradation of fish meal, soybean cake and protected palm oil meal in beef cattle rations. In: *Proceedings of National Seminar on Sustainable Livestock 5*. Jatinangor. pp. 247-251.
- Setyawati, R. 2008. Effect of Oil Palm Oil Protection Using Formaldehyde To The Number Of Protozoa and Micro Rumen In Vitro Rumen Fermentation Products. Essay. Universitas Gadjah Mada. Yogyakarta.

- Weimer, P. J. 1996. Why don't ruminal bakteri digest cellulose faster?. J. Dairy Sci. 79: 1496-1502.
- Yulianto, A. 2011. Effect of Urea Coating In Ration on pH, NH₃ and VFA Concentration on Local Lambs. Essay. Sebelas Maret University, Surakarta.