In Vitro Digestibilities of Six Rumen Protected Fat-Protein Supplement Formulas

L Hartati[1)*], A Agus[2)], BP Widyobroto[2)] and LM Yusiati[2)]

[1)]Faculty of Agriculture, Lambung Mangkurat University, Jl. A. Yani km 36, Banjarbaru, South Kalimantan, Indonesia
[2)]Faculty of Animal Science, Gadjah Mada University, Jl. Fauna 3, Bulaksumur, Yogyakarta 55281, Indonesia
***Corresponding author e-mail: hartati_gk74@yahoo.com**

Abstract. The aim of the research was to evaluate the efficacy of protection method of rumen protected fat-protein supplements. In vitro digestibility test was carried out to examine nutrients digestibility of different supplement formula based on the sources of protein and oil. The research used two sources of fat namely crude palm oil (CPO) and fish oil (FO) and three sources of protein namely milk skim, soy flour, and soybean meal. Thus there were 6 combinations that subjected in the in vitro digestibility test. The observed variables were the digestibility of dry matter (DM), organic matter (OM), crude fat (CF), and crude protein (CP). Results indicated that the method for protecting protein and fat was effective. This was showed by low nutrients digestibility in the rumen and high nutrients digestibility in the post rumen. In conclusion the combination between skim milk and CPO gave the best results among the other supplement formula.

Keywords: rumen protected nutrient, fat-protein supplement, rumen digestibility, in vitro

Abstrak. Tujuan penelitian ini adalah untuk mengevaluasi metode proteksi dari suplemen protein lemak terproteksi. Kecernaan nutrien dari beberapa kombinasi protein dan lemak diuji secara in vitro. Penelitian ini menggunakan dua sumber lemak berupa minyak sawit mentah dan minyak ikan, serta tiga sumber protein yaitu susu skim bubuk, tepung kedelai dan bungkil kedelai. Ada enam kombinasi yang diuji kecernaannya secara in vitro. Variabel yang diamati adalah kecernaan bahan kering, kecernaan bahan organik, kecernaan lemak kasar dan kecernaan protein kasar. Metode yang digunakan untuk proteksi protein dan lemak cukup efektif, dengan rendahnya kecernaan nutrien di pencernaan rumen tetapi tinggi kecernaannya di pencernaan pasca rumen. Kombinasi antara susu skim dan minyak sawit mentah memberikan hasil yang paling baik dibandingkan kombinasi yang lainnya.

Kata kunci: nutrien terproteksi dalam rumen, suplemen protein-lemak, kecernaan rumen, in vitro

Introduction

The energy density of a ruminant ration can be enhanced by incorporating fermentable carbohydrates such as cereal grains or fats. However there is a limitation to use high levels of cereal grains in the ration since it reduces rumen pH which can cause rumen acidosis. On the other hand, negative impact of high level of fat supplementation on rumen metabolism can be avoided by protection method (Naik et al., 2009). The need for protecting fat sources in ruminant feed can be explained by the chemical structure of free fatty acids. The antimicrobial effect of lipids in the rumen greatly resembles the cytotoxic effect of fatty acids on the membrane function of eukaryotic cells. Long-chain fatty acids readily attach to lipid bilayers in biological membranes (Febel et al., 2002).

Crude palm oil and fish oil contain high level of poly unsaturated fatty acid (PUFA) (Gurr, 1984; Saify et al., 2003). In order to increase supply of dietary PUFA concentration as precursor for the synthesis of milk PUFA, the dietary PUFA should be protected from hydrolysis and bio hydrogenation processes in the rumen. Several methods have been developed to protect PUFA from rumen bio hydrogenation, and one of the effective methods is oil capsulation using protein matrix

which is protected by formaldehyde (Doreau et al., 1991; Gulati et al., 2005). Formaldehyde will make a cross link with amino acid in the protein, so called methylene bridge ($–CH_2-$), and result on protein resistance from microbial degradation (Kiernan, 2000).

Laboratory in vitromethod was conducted to evaluate the digestibility of certain feedstuffs. Feedstuffs are digested by preparations of microorganisms or of enzymes which are similar in function to those present in the digestive tract of the ruminant . The present research was aimed to evaluate the nutrients digestibility of rumen protected fat-protein (RPFP) supplements which formulated in combinations of different sources of fat and protein.

Materials and Methods

Three protein sources and 2 fat sources were used in this research. The sources of protein were skim milk, soy flour, and soybean meal. The sources of fat were crude palm oil (CPO) and fish oil. The procedure in the capsulation of fat with protein matrix was based on the method from our previous research i.e. such amount of fat and protein (1:3) was mixed homogeneous and then sprayed with formaldehyde (37% of formaldehyde solution) in amount to find the final dosage of formaldehyde in the mixture was 1.5%.

There were 6 combinations of rumen protected fat-protein (RPFP) formula, evaluated with two stages in vitro Tilley and Terry method (1963), namely: C1 (skim milk and CPO); C2 (skim milk and fish oil); C3 (soy flour and CPO); C4 (soy flour and fish oil); C5 (soybean meal and CPO); and C6 (soybean meal and fish oil).

Rumen fluid from two fistulate dairy cows was collected before morning feeding. Rumen fluid was filtered through 4 layers of linen cloth and stored in anaerobic jar at 38.5^{o}C before serving as in vitro solution. In order to compare rumen digestibility and post rumen digestibility, two groups of RPFP combination were prepared, those were (1) one stage in vitro Tilley and Terry Method (IVT) and (2) two stage IVT. After 48 hours of incubation (for one stage IVT) or 96 hours of incubation (for two stage IVT) the in vitro solution was filtered out from fermentation tube and the substrate was analyzed for contents of dry matter (DM), organic matter (OM), crude fat (CF), and crude protein (CP) using AOAC method (AOAC, 1995) to calculate their digestibility. Data were analyzed using the general linear model of SPSS 16.0 for windows.

Results and Discussion

Dry matter digestibility of rumen protected fat-protein supplements is presented in Table 1. Rumen protected fat-protein formula significantly affected the dry matter digestibility in 0rumen and post rumen digestion. In rumen digestion (one stage IVT), combinations of skim milk + CPO and combination of skim milk + fish oil showed the highest digestibility value while combination of soy flour + fish oil showed the lowest digestibility value ($P<0.05$). In post rumen digestion (two stage IVT), combinations of skim milk + CPO and skim milk and fish oil resulted in higher value of dry matter digestibility ($P<0.05$) than the combinations of soy flour + CPO, soy flour + fish oil, soybean meal + CPO, and soybean meal + fish oil. These results indicated that due to substrate protection from rumen microbial fermentation, dry matter digestibility was higher in one stage IVT than in two stages IVT.

Addition of essential oil reduced dry matter digestibility. Their result suggested that there was an inhibition of the overall rumen fermentation process by oil addition in the feed. Reduction of dry matter digestibility in the rumen was caused by high level of fat content in the feed; that would adversely affect the microbial fermentation process (Benchaar et al., 2007). Varying of full fat canola seeds had no different effect on in vitro digestibility of dry

matter (Kilic and Garipoglu, 2009). Evaluated in vitro digestibility of protected fat on different level on ration (Naik et al., 2009). Varying sources of fat supplement result in variation in dry matter degradation (Febel et al., 2002).

Organic matter digestibility of rumen protected fat-protein supplements is presented in Table 1. The combination significantly affected organic matter digestibility in rumen and post rumen digestion. Combination of skim milk + CPO showed the highest digestibility value whilst combination of soy flour + crude palm oil, soy flour + fish oil, and soybean meal + fish oil had the lowest digestibility value on one-stage IVT ($P<0.05$). These results were also similar to two-stage IVT ($P<0.05$). The organic matter digestibility in two-stage IVT was higher than in one-stage IVT. The explanation for these results was similar to the dry matter digestibility result in that the protection method successfully protected feed substrates from rumen microbial fermentation. Kilic and Garipoglu (2009) found that varying of full fat canola seed in rations resulted similar organic matter digestibility of rations.

Crude fat digestibility of rumen protected fat-protein supplements is presented in Table 1. In rumen digestion, combination of soyflour + fish oil resulted in the highest crude fat digestibility value, whilst the lowest value was resulted by combination of skim milk + CPO and skim milk + fish oil ($P<0.05$). In post rumen digestion, the highest crude fat digestibility was resulted from combination of skim milk +CPO and the lowest value was of soy flour + fish oil ($P<0.05$). In this protection method, oil was capsulated by protein substrate, i.e. skim milk, which was protected from rumen microbial digestion using formaldehyde solution. Therefore, in rumen digestion (one stage IVT), crude fat had very low digestibility while in post rumen digestion (two stages IVT), crude fat was more digestible due to degradation of protein capsule by acidic and enzymatic protein digestions. Study by Rossi et al. (2005) revealed that different sources of protected fat would result in different fat digestibility.

Crude protein digestibility of rumen protected fat-protein supplements is presented in Table 1. In rumen digestion, all combinations showed a very low crude protein digestibility. This result indicated a strong formaldehyde protection to the protein capsule against microbial rumen digestion. In the post rumen

Table 1. Average value of in vitro dry matter, organic matter, crude fat, and crude protein digestibility of six rumen protected fat-protein supplement formulas (%)

In vitro Tilley and Terry method	Formula					
	C1	C2	C3	C4	C5	C6
Dry matter digestibility						
One stage	55.19^{a}	53.32^{a}	28.47^{c}	26.25^{d}	32.33^{b}	30.55^{b}
Two stages	86.42^{a}	84.34^{a}	72.28^{b}	73.48^{b}	77.48^{b}	73.65^{b}
Organic matter digestibility						
One stage	52.59^{a}	48.64^{b}	31.24^{d}	33.88^{cd}	36.19^{c}	33.33^{d}
Two stages	85.27^{a}	83.02^{b}	69.42^{d}	69.33^{d}	72.38^{c}	70.33^{cd}
Crude fat digestibility						
One stage	8.67^{e}	8.20^{e}	17.05^{d}	25.79^{a}	19.82^{c}	22.36^{b}
Two stages	82.97^{a}	78.83^{ab}	71.13^{bc}	69.81^{c}	73.46^{bc}	74.89^{abc}
Crude protein digestibility						
One stage	0	0	0	0	0	0
Two stages	67.62^{b}	55.61^{c}	80.60^{a}	76.38^{a}	75.37^{a}	78.04^{a}

Values bearing different superscript at the same row differ significantly ($P<0.05$); C1 (skim milk and CPO); C2 (skim milk and fish oil); C3 (soy flour and CPO); C4 (soy flour and fish oil); C5 (soybean meal and CPO); and C6 (soybean meal and fish oil).

digestion, combination of soy flour + CPO resulted in the highest crude protein digestibility value which was not significantly different among the combinations of soy flour + fish oil, soybean meal + CPO, and soybean meal + fish oil. The lowest crude protein digestibility value was shown by the combination of skim milk + CPO and skim milk + fish oil combination ($P<0.05$).

Different protein sources resulted in different in vitro crude protein digestibility (Febel et al., 2002). Formaldehyde effectively protects protein from microbial activity and affects on the decrease of crude protein digestibility in the rumen. However, protein treated by formaldehyde is digestible in the lower tract, and the amount of N digested depends on the length of time after formaldehyde treatment (Phillips, 1981).

The protection of proteins by formaldehyde might make more protein or amino acids available for the host animal but it might reduce the synthesis of microbial biomass as well (Kamalak et al., 2005). Formaldehyde is more effective than tannin for protein by pass rumen technique. This is due to formation of a cross link between amino acid in the protein, so called Methylene Bridge, by formaldehyde effect (Kiernan, 2000).

Conclusions

All rumen protected fat-protein supplements formulas in the research showed a low nutrient digestibility in rumen digestion but high in post rumen digestion. Different sources of fat and protein resulted in different in vitro nutrient digestibility of the supplements. The combination between skim milk and CPO showed the best digestibility on dry matter, organic matter and crude fat because the effective protection method was low in rumen digestion and high in post rumen digestion. Therefore, it was recommended to make fat-protein formula.

Acknowledgement

This researh was funded by DGHE of National Education Ministry.

References

AOAC. 1995. Official Methods of Analysis, 16th Ed., AOAC, Washington DC, USA.

Benchaar C, AV Chaves, GR Fraser, Y Wang, KA Beauchemin and TA Mc Allister. 2007. Effects of essential oils and their components on in vitro rumen microbial fermentation. Can. J. Anim. Sci. 87:413–419.

Doreau M, Y Chilliard, D Bauchart and B Michalet-Doreau. 1991. Influence of different fat supplements on digestibility and ruminal digestion in cows. Annu. Zootech. 40: 19-30.

Fébel H, F Husvéth, T Veresegyházy, E Andrásofszky, I Várhegyi and S Huszár. 2002. Effect of different fat sources on in vitro degradation of nutrients and certain blood parameters in sheep. Acta Vet. Hung. 50(2):217–229.

Getachew G, EJ De Peters and PH Robinson. 2004. In vitro gas production provides effective method for assessing ruminant feeds. Calif. Agric. 58(1):54-58.

Getachew G, M Blumel, HPS Makkar and K Becker. 1998. In vitro gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Tech. 72:261-281.

Gulati SK, MR Garg and TW Scott. 2005. Rumen protected protein and fat produced from oilseed and/or meals by formaldehyde treatment; their role in ruminant production and product quality: a review. Aust. J. Exp. Agric. 45:1189-1816.

Gurr MI. 1984. The Chemistry and Biochemistry of Plant Fats and Their Nutritional Importance. In: Fats in Animal Nutrition. Edited by J. Wiseman. Butterworths. London.

Hamid P, T Akbar, J Hossein and MG Ali. 2007. Nutrient digestibility and gas production of some tropical feeds used in ruminant diets estimated by the in vivo and in vitro gas production techniques. Am. J. Anim. Vet. 2(4):108-113.

Kamalak A, O Canbolat, Y Gurbuz and O Ozay. 2005. Protected protein and amino acids in ruminant nutrition. J. Sci. Eng. 8(2):84-88.

Kiernan JA. 2000. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Microscopy Today. 1:8-12.

Kilic U and AV Garipoglu. 2009. In situ rumen degradability, in vitro digestibility and in vitro gas production of full fat canola seeds. Asian J. Anim and Vet. Advances 4(4):200-208.

Kumeno F, BA Dehority and RR Johnson. 1967. Development of an in vitro fermentation technique for estimating the nutritive value of high energy mixed rations for ruminants. J. Anim. Sci. 26:867-871.

Philips WA. 1981. In vitro digestion of soybean meal treated with formaldehyde. J. Anim. Sci. 53(6):1616-1622.

Naik PK, S Saijpaul and N Rani. 2009. Effect of ruminally protected fat on in vitro fermentation and apparent nutrient digestibility in buffaloes (*Bubalus bubalis*). Anim. Feed Sci. Tech. 153:68–76.

Rossi F, AM Pulimeno and F Masoero. 2005. In situ and in vitro nutritional evaluation of rumen-protected lipids. Italian J. Anim. Sci. 4:156-158.

Saify ZS, S Akhtar, KM Khan, S Perveen, SAM Ayattollahi, S Hassan, M Arif, SM Haider, F Ahmad, S Siddiqui and MZ Khan. 2003. A Study on the fatty acid composition of fish liver oil from two marine fish, *Euspyra blochii* and *Carcharhinus bleekeri*. Turkey J. Chemistry. 27:251-258.

Soliva CR, M Kreuzer, N Foidl, G Foidl, A Machmuller and HD Hess. 2005. Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation in vitro. Anim. Feed Sci. Tech. 118:47-62.