Characteristics of Polyunsaturated Fatty Acids and Nutrient Digestibility Feed Cattle of the Fermented Rumen Fluid by One and Two Stage in Vitro

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ABSTRACT: The objective of this research was to determine the effect of supplementation with polyunsaturated fatty acids (PUFA) protected in the feeding beef cattle based on the content of PUFA and the nutrients digestibility parameters by in vitro one-stage and two-stage. Fatty acid supplement derived from soya and lemuru fish oil. Soya groats and lemuru fish oil (SoyLem) both mixed with a ratio of 4: 1 are protected by an aldehyde of the formaldehyde concentration 37% as much as 2% of the dry matter. Fermentation was observed at one stage (48 hours) and two stage (96 hours) after incubation by in vitro. Rumen fluid were taken from the Ongole crossbred cow fistulated. Feed treatments including T1 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem unprotected, T2 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 7.5% SoyLem unprotected + 7.5% SoyLem protected, and T3 = = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem protected. The results of this study were the rumen fermentation in vitro showed palmitic C16:0), linoleat (C18:2), linolenat (C18:3), gama linolenat (C20:3) and arachidonat (C20:4) fatty acid content significantly different (P <0.05) at one stage of the incubation while gama linolenat (C20:3) dan arachidonat (C20:4) fatty acid content significantly different (P <0.05) at two stage of the incubation. Digestibility of dry matter, organic matter and crude protein were significantly different (P<0.05) in both the incubation one stage and two stages. The conclusion of research is a mixture of soya groats and lemuru fish oil (4: 1) protected aldehydes can be used as a supplement of up to 15% in cattle feed rich in fatty acids and without disturbing the digestibility by in vitro one-stage and two-stage.

Keywords: polyunsaturated fatty acids (PUFA), degestibilty, protected, one stage invitro, two stage invitro

INTRODUCTION

Soybean groats and lemuru fish oil are both rich in linoleic acids. Both of fatty acids should be provided from the feed. Linoleic fatty acids undergo elongation and desaturation during biosynthesis. Linoleic fatty acid produces PGF2 in soybean groats through Arachidonic acid. PUFA that escape from ruminal hydrogenation enters circulatory system and subsequently stimulates ovarian cyclicity and corpus lueteum function, therefore contributes to the estrous cycles, ovulation, and fertility (Dirandaeh *et al.,* 2013). During the digestive process in the rumen, PUFAs undergo hydrogenation by rumen bacteria to become saturated fatty acid, thus affect the post-ruminal PUFA availability that will be absorbed by circulatory system (Mahadevan *et al.,* 1983). For the PUFA to escape from ruminal hydrogenation process, protection is necessary in the rumen. Protection can be conducted, among other, by encapsulation of fat using protein-bond that have been protected from formaldehyde using aldehyde (Lourence *et al.,* 2010). The present study employs encapsulation protection method that is conducted by mixing soybean groats and lemuru fish oil and blending them evenly until they form capsules and then added with formaldehyde. Encapsulation protection is intended to escape PUFA from hydrogenation and feed protein in the rumen.

MATERIALS AND METHODS

The research is conducted at Laboratory of Nutritional Biochemistry Faculty of Animal Science of Gadjah Mada University. Rumen fluid were taken from the Ongole crossbred cow fistulated. Three ruminally fistulated Ongole crossbred cow were employed in this study as the ruminal fluid donors. The three of them were fed with 60% forages and 40% basal concentrate. Forage is composed of 30% elephant grass and 30% rice straw. The basal concentrate is composed of 10% rice bran, 5% wheat bran, 3.5% coffee husks, 5% soybean meal, 1% minerals-vitamins, and 0.5% salts. Soya groats and lemuru fish oil (SoyLem) both mixed with a ratio of 4: 1 are protected by an aldehyde of the formaldehyde concentration 37% as much as 2% of the dry matter

Feed treatments including T1 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem unprotected, T2 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 7.5% SoyLem unprotected + 7.5% SoyLem protected, and T3 = = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem protected. Experimental design used was randomized. If the variance of the test results mean different then the average difference was tested using the Duncan multiple range test (DMRT).

Parameters of rumen fermentation includes Saturated and unsaturated fatty acid composition is determined using Gas Chromatography (Plummer, 1987). Digestibility test include dry matter content, organic matter, crude fat, and crude protein using methods Tilley and Terry (1963) fermentation was observed at one stage (48 hours) and two stage (96 hours) after incubation by in vitro.

RESULTS AND DISCUSSION

Fatty acid level in parent beef cattle feeds that contain soybean groats and lemuru fish oil (at a 4:1 ratio), protected and unprotected from formaldehyde, respectively. Fermented ruminal fluid (in vitro) of one-stage (48 hours) and two-stages (96 hours) can be seen in Table 1.

Table 1. Fatty acid level (mg/100g) in cattle beef feeds contain protected and unprotected soybean groats and lemuru fish oil resulted from fermented ruminal fluid in vitro one-stage (48 hours) and two-stages (96 hours).

Fatty acids (mg/100g)	Treatment		
	T1	T2	T3
in vitro one-stage (48 hours):			
Laurat C12:0^{ns}	1.9918±1.20	3.9506±2.11	2.6352±0.92
Miristat C14:0^{ns}	0.0503±0.01	0.0788±0.04	0.1622±0.09
Pentadecanoat C15:0^{ns}	0.1174±0.07	0.2417±0.16	0.1210±0.04
Palmitat C16:0	2.1783±1.23^{a}	4.0725±2.38^{b}	1.8125±0.56^{a}
Heptadecanoat C17:0^{ns}	0.1100±0.08	0.0821±0.07	0.0612±0.01
Stearat C18:0^{ns}	0.0489±0.03	0.0341±0.03	0.0189±0.00
Behenat C22:0^{ns}	0.0171±0.01	0.0181±0.02	0.0024±0.00
Lignocerat C24:0^{ns}	0.0259±0.01	0.0352±0.03	0.0290±0.01
Palmitoleat C16:1^{ns}	0.0377±0.02	0.0753±0.03	0.0356±0.01
Oleat C18:1^{ns}	1.1305±0.69	1.5844±0.99	2.2212±0.84
Erusat C22:1^{ns}	0.0348±0.02	0.0182±0.01	0.0156±0.01
Nervonat C24:1^{ns}	0.0244±0.01	0.0543±0.06	0.0086±0.00

Linoleat C18:2	0.0259±0.01[a]	0.0382±0.02[a]	0.1112±0.02[b]
Linolenat C18:3[ns]	0.0306±0.03	0.0418±0.02	0.0226±0.01
Gama Linolenat C20:3	0.0373±0.01[a]	0.0980±0.08[a]	0.2246±0.04[b]
Arachidonat C20:4	0.0420±0.05[a]	0.0504±0.03[ab]	0.1692±0.02[b]
Eicosapentanoic C20:5[ns]	0.0054±0.00	0.0111±0.01	0.0036±0.00
in vitro one-stage (96 hours)			
Laurat C12:0[ns]	2.6187±1.55	3.077±1.45	3.2966±0.60
Miristat C14:0[ns]	0.1785±0.03	0.1825±0.11	0.2838±0.08
Pentadecanoat C15:0[ns]	0.1634±0.05	0.2038±0.08	0.2527±0.07
Palmitat C16:0[ns]	1.9381±0.23	1.4675±0.83	2.8529±0.64
Heptadecanoat C17:0[ns]	0.0766±0.05	0.1050±0.04	0.1540±0.01
Stearat C18:0[ns]	0.0299±0.00	0.0210±0.00	0.0288±0.00
Behenat C22:0[ns]	0.0170±0.00	0.0614±0.00	0.0707±0.03
Lignocerat C24:0[ns]	0.0116±0.00	0.0323±0.01	0.0406±0.01
Palmitoleat C16:1[ns]	0.0900±0.02	0.0483±0.01	0.1006±0.03
Oleat C18:1[ns]	2.3958±0.38	2.8937±1.24	4.1782±0.02
Eurat C22:1[ns]	0.0110±0.00	0.0418±0.01	0.0433±0.02
Nervonat C24:1[ns]	0.0148±0.00	0.0266±0.01	0.0233±0.01
Linoleat C18:2[ns]	0.0659±0.03	0.0551±0.01	0.1319±0.11
Linolenat C18:3[ns]	0.0346±0.01	0.0649±0.02	0.0343±0.00
Gama Linolenat C20:3	0.0547±0.06[a]	0.0643±0.04[a]	0.2280±0.08[b]
Arachidonat C20:4	0.0325±0.01[a]	0.0735±0.03[a]	0.1783±0.02[b]
Eicosapentanoic C20:5[ns]	0.0185±0.00	0.0237±0.01	0.0658±0.04

[ab] different superscripts in the same row showed highly significant differences (P <0,01), [ns] not significantly different (P> 0.05), T1 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem unprotected, T2 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 7.5% SoyLem unprotected + 7.5% SoyLem protected, and T3 = = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem protected.

The result indicates that cattle feeds containing 15% protected polyunsaturated fatty acid (PUFA), especially arachidonic and linoleic fatty acids in hinger level than that of other treatment, both in one-stage (48 hours) fermentation and two-stages (96 hours) incubation of invitro fermentation. Both fatty acids have be proven to escape biohydrogenation by ruminal bacteria, therefore available post-ruminally. Protection is conducted by encapsulation of fat using matrix protein protected by aldehyde of formaldehyde. Matrix protein serves to bind aldehyde in formaldehyde in order for PUFA to escape ruminal hydrogenation so that PUFA can still be obtained post-ruminally and absorbed by small intestine as PUFA source. Linoleic fatty acids underwent elongation and desaturation in the process of biosynthesis. Linoleic fatty acid in soybean groats generates PGF2 using arachidonic acids. PUFA that escapes ruminal hydrogenation enters into circulatory system and subsequently stimulates ovarium cyclicity and corpus luteum function, therefore contributes to estrous cycle, ovulation, and fertility. Formaldehyde protection in fat using aldehyde binding with matrix can escape fatty acids from ruminal metabolism by up to 90% through the changes in three dimensional structure of aldehyde (Emanuele and Putnam, 2006). Protected fish oil supplements as sources of fatty acid can improve the duodenal conjugated linoleic acids (CLA) flow (Duckett and Gilis, 2010). Supplementation of fish oil, soya oil, and fish oil: soya oil (1:1) affects the differences in concentration of plasma glucose, triglyceride, and total cholesterol (Ghasemzadeh-Nava *et al.,* 2011). Protective treatment in soybean meal can protect ruminal microbial fermentation, reducing digestibility of organic matters in vitro and decreasing gas production during incubation significantly lower than that of protected fat supplements (Palizdar *et al.,* 2012). Feed with higher level of linoleic acid affect the reproduction of beef cattle, feed rich in linoleic acids (C18:2) increase arachidonic acid concentration (C20:4) in the tissue and feed rich in linoleic acids (C18:3) increase the concentration and constitute a competitive inhibitor of enzyme complex involved in prostaglandin synthesis of arachidonic acids (C20:4) (Scholljegerdes *et al,* 2004).

Dry matter, organic matter and crude protein digestibility of beef cattle feeds contain soybean groats:lemuru fish oil (4:1) protected and unprotected against formaldehyde produced by ruminal liquid in vitro in single phase (48 hours) and two phases (96 hours) are presented in Table 2.

Table 2. Dry matter, organic matter and crude protein digestibility of beef cattle feeds contain soybean groats, protected and unprotected lemuru fish oil produced from ruminal liquid fermentation in vitro in singe phase (48 hours) and two phases (96 hours)

Parameter	Treatment		
	T1	T2	T3
in vitro one-stage (48 hours):			
dry matter digestibility (%)	30.73±2.17[a]	26.11±3.97[ab]	23.34±0.79[b]
organic matter digestibility (%)	29.69 ±3.90[a]	25.46 ±2.40[ab]	21.56 ±2.74[b]
crude protein digestibility (%)	40.67±4.64[a]	48.32±10.32[a]	28.87±6.29[b]
in vitro one-stage (96 hours)			
dry matter digestibility (%)	37.29±3.56[a]	33.42±1.77[ab]	29.98±2.29[b]
organic matter digestibility (%)	50.68 ±6.23[a]	35.61 ±10.77[ab]	27.78 ±9.51[b]
crude protein digestibility (%)	57.91±7.66[a]	71.67±6.82[b]	71.25±1.20[b]

[ab] different superscripts in the same row showed significant differences (P<0,05), T1 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem unprotected, T2 = 30% elephant grass + 30% rice straw + 25% basal concentrate + 7.5% SoyLem unprotected + 7.5% SoyLem protected, and T3 = = 30% elephant grass + 30% rice straw + 25% basal concentrate + 15% SoyLem protected.

The results of the study indicate that differences in the treatment of soybean groats and lemuru fish oil supplements protected and unprotected against formaldehyde affect the digestibility of dry matter, organic matter, and raw protein (P<0.05) generated from ruminal liquid fermentation in vitro in single phase (48 hours) and two phases (96 hours). Cattle feed supplemented with soybean groats and lemuru fish oil by 15% protected against formaldehyde have lower digestibility of dry matter, organic matter, and raw protein than those unprotected (P<0.05) resulted from ruminal liquid fermentation in vitro in one phase (48 hours) and two phases (96 hours). Table 1 indicates that the digestibility of dry matter, organic matter, and raw protein generated from ruminal liquid fermentation in vitro in one phase (48 hours) of the three is lower than that in two phases (96 hours). Decreased dry matter digestibility is due to the strength of protection so that ruminal digestive microbes and enzymes are not strong enough to digest dry matters during in the rumen, therefore ruminal microbes activities run optimally and improve the whole process of fermentation in the rumen. Riyanto *et al*., (2011) notes that dry matter digestibility affects organic matter digestibility. The digestibility of organic matter is proportional to that of dry matter as the former is the constituent of the latter. Cattle feed with protein level that provides sufficient nitrogen such as NH3 for microorganism and energy source that also sufficient for ruminal microbes will help organic matter digestion run normally. According to Stanton *et al*., (1983), soybean meal protected against 0.3% formaldehyde indicates decrease in ruminal nitrogen digestibility compared to the control, 0.2% and 0.6%. The use of formaldehyde has decreased raw protein digestibility, which is proportional to the decrease in dry matter digestibility. Proteins that have not been degraded in the rumen are those that escape the 48 hours incubated ruminal degradation. Increased protein digestibility post-ruminally, more precisely in the abomasums with 96 hours incubation can be caused by protein bounded to formaldehyde that previously degraded in the rumen, finally can be degraded post-ruminally. Maynard and Loosly (1979) note that digestion coefficients are not similar for each food or cattle beef as they are influenced by several factors: chemical composition, food processing, amount of feed and the animal breed. Jayanegara *et al.,* (2006) elucidate that concentrate rich in raw protein will activate ruminal microbes, therefore increasing the number of proteolitic bacteria and increased deaminase that increase the digestibility value of organic matter. Feed rich in linoleic acids (C18:2) will increase the arachidonic acid concentration (C20:4) in the tissue and feed rich in linoleic acid (C18:3) increases the concentration and is the competitive inhibitor of enzyme complex involved in prostaglandin synthesis from arachidonic acid (C20:4) (Scholljegerdes *et al.,* 2004).

CONCLUSIONS

The conclusion of research is a mixture of soya groats and lemuru fish oil (4: 1) protected aldehydes can be used as a supplement of up to 15% in cattle feed rich in fatty acids and without disturbing the digestibility by in vitro one-stage and two-stage

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