Fermentation parameters and total gas production of some rumen protected fat-protein formulation[1]

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ABSTRACT: The aim of this study was to evaluate some formulation of rumen protected fat-protein (RPFP) based on the fermentation parameters and total gas production in the in vitro gas production test technique. Two sources of fat, i.e. crude palm oil and fish oil and three sources of protein i.e. skim milk, soybean and soybean meal were used in the formulation of RPFP, and thus there were 6 treatment combinations. Fermentation parameters were measured by incubating the sample in a rumen liquor buffer taken from a rumen fistulated dairy cow. The measurements were done at 3, 6, 9, 12, 24, 48 and 72 h after starting the incubation. The pH, NH_3 and VFA of fermentation medium were measured after 72 h of incubation. Result showed that the highest pH (6.77) was produced from combination of soybean meal and fish oil, while the lowest pH (6.59) was produced from the combination of skim milk and crude palm oil. Similarly, the highest NH_3 content was found on the combination of soybean meal and fish oil (10.64 mg/100ml) and the lowest content was produced on the combination of skim milk and fish oil (7.90 mg/100 ml). The highest total gas production after 72 hours of incubation was reached by the combination of skim milk and fish oil (27.52 ml/200 mg DM) and the lowest production was found on the combination of soybean meal and fish oil (14.56 ml/200 mg DM).

Key words: rumen protected fat-protein, gas production test, the parameter of fermentation

INTRODUCTION

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 bio hydrogenation process 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 (Gulati et al., 2005). Formaldehyde will make a cross link with amino acid in the protein, so called methylene bridge (–CH2-), and result on protein resistance from microbial degradation (Kiernan, 2000). Study by Soliva et al. (2005) showed that different sources of protein effects on the different result of fermentation parameters of protein.

The in vitro gas production technique (GPT) is a relatively simple method for evaluating feeds, as large numbers of samples can be incubated and analyzed at the same time. This method has been successfully employed for a variety of purpose in feed evaluation, such as to calculate the organic matter digestibility, to analyse the metabolizable energy of feeds and the kinetics of their fermentation, and to determine how feed value is affected by fat addition, anti nutritive factors, or rumen modifiers (Getachew et al. 2004).

The aim of this research was to evaluate the differences of various sources of fat and protein in the formulation of rumen protected fat-protein (RPFP) based on the fermentation parameters and total gas production using in vitro gas production test technique (GPT).

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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) were homogeneously mixed and then sprayed with 37%-formaldehyde solution to find the final dosage of 1.5%-formaldehyde in the mixture. There were 6 combinations of RPFP formula which were evaluated using GPT technique, namely: P1 (skim milk and CPO), P2 (skim milk and fish oil), P3 (soy flour and CPO), P4 (soy flour and fish oil), P5 (soybean meal and CPO) and P6 (soybean meal and fish oil).

The protocol of the GPT technique was conducted using the following procedure. The samples were incubated in a rumen liquor buffer (1:2) for 72 hours in 39^0C. Three hundred and twenty two mg of sample was incubated in 40 ml mixture of rumen fluid, buffer, main mineral solution, trace mineral solution, and reductor solution. Rumen fluid was collected before morning feeding from two fistulated dairy cows. Buffer solution consisted of $NaHCO_3$ and $(NH_4)_2HCO_3$. Main mineral solution consisted of Na_2HPO_4. $2H_2O$, KH_2PO_4 and $MgSO_4.7H_2O$. The trace mineral solution consisted of $CaCL_2.2H_2O$, $MnCl_2$. $4H_2O$, $CoCl_2.6H_2O$ and $FeCl_3.6H_2O$. The reductor solution consisted of NaOH and $Na_2S.7H_2O$. The volume of gas production was observed at 3, 6, 9, 12, 24, 48 and 72 h after the incubation started. After 72 h, filtrate of in vitro solution was taken out from fermentation bottle and measured for pH, NH_3 and VFA.

RESULTS AND DISCUSSION

Different formulations resulted in different chemical compositions (Tabel 1). Formulas P5 and P6 resulted in the highest crude protein content, while formulas P1 and P2 resulted in the highest crude fat content.

Table 1. The chemical composition of RPFP sample

Formula	Dry matter, %	Organic matter, %	Crude protein, %	Crude fiber, %
P1	91.83	98.19	8.14	40.04
P2	91.11	98.24	7.94	40.03
P3	88.07	96.89	25.53	34.74
P4	86.02	97.09	24.29	35.69
P5	85.84	93.14	34.52	18.6
P6	86.18	93.12	34.4	20.05

Total gas production describes the amount of feed that has been degraded and fermented by rumen microbe. The volume of gas production has positive correlation with the amount of feed which is degraded and fermented by rumen microbe. Table 2 shows that formula P6 and P2 produced the lowest (P<0.05) and highest (P<0.05) total gas production, respectively.. High content of protein in the P6 apparently related with low gas production. According to Kiernan (2000) formaldehyde builds methylene bridge which protect protein from microbe activities (Kiernan, 2000). Comparing to other studies, our study resulted lower total gas production. Hamid et al. (2007) used soybean meal as sample and produced total gas of 63.8 ml/200mg DM at 72 h of incubation.

Basically, gas production in a rumen solution buffer resulted from fermentation of carbohydrates and mainly composed of acetate, propionate and butyrate. Volume of gas production from protein fermentation is relatively low as compared to that from carbohydrate fermentation (Getachew et al., 1998). However, total gas production will be varied among feedstuffs which is containing different concentrations of crude protein (Soliva et al., 2005). According to Taghizadeh et al. (2008), variation in the chemical composition of feedstuff will result in the variation of gas production.

The NH_3 concentration in the medium of fermentation describes the degradation of feed protein. Soliva et al. (2005) stated that the NH_3 concentration was clearly affected by the individual protein sources. Therefore, the differences in protein content among feedstuff result in the differences in NH_3 concentration. According to Hristov et al. (2008), the effect of essential oil on NH_3 concentration

varied from has no effect until moderate effect. In this research, formula P6 resulted on the highest of NH_3 content whereas formula P2 produced the lowest NH_3 content. P5 and P6 had higher values of crude protein than other formulas.

Table 2. Total gas production, NH_3 and pH after 72 h of incubation

	Formula					
	P1	P2	P3	P4	P5	P6
Gas production after 72 h, ml/200mg DM sample	29.64[a]	30.92[a]	19.11[b]	18.76[b]	19.93[b]	14.56[c]
NH_3, mg/100ml	7.94[c]	7.90[c]	9.20[b]	9.11[b]	9.72[b]	10.64[a]
pH	6.59[c]	6.60[c]	6.69[b]	6.66[b]	6.70[b]	6.77[a]

[abc] Within a row, means without a common superscript differ (P<0.05).

Different formula affected (P<0.05) the pH of in vitro medium after 72 h of fermentation. However, these pHs were ranging in relatively normal values. Slight differences in pH value between formulas were apparently due to the differences of feedstuffs for the formulation. Similar to the result for NH_3 concentration, the highest pH value was found to be in P6 formula. These results confirmed that concentration of NH_3 in the fermentation medium will increase the pH value. Hristov et al. (2008) reported that essential oils had negligible effects on the pH of fermentation medium.

Table 3 shows that P1 and P5 formula resulted in the highest and lowest (P<0.05) production of acetate, respectively. Production of acetate, propionate and butyrate in this research tend to be lower than other research. Formaldehyde protection to the substrate apparently reduced rumen microbe attachment to the feed particles.

Table 3. The average of acetate, propionate and butyrate concentration of in vitro medium after 72 hours of incubation

	Formulation					
	P1	P2	P3	P4	P5	P6
Acetate, mmol	18.22[a]	15.97[ab]	13.42[b]	13.06[b]	12.74[b]	13.75[ab]
Propionate, mmol	6.59[a]	5.60[ab]	4.82[ab]	4.48[ab]	3.49[c]	3.92[c]
Butirate, mmol	2.56	2.60	1.62	2.84	1.84	2.02

[abc] Within a row, means without a common superscript differ (P<0.05).

Total VFA productions were not significantly different among formulas since the DM were similar. Dry matter content of P1formula was higher than those of other formulas, accordingly with the VFA production. According to Hamid et al. (2007), variation in the VFA production is depending on the type of substrate or feedstuff.

CONCLUSIONS

Evaluation some rumen protected fat-protein (RPFP) by in vitro gas production gave different result on total gas production, pH, NH_3, acetate production and propionate production, but not for butyrate production.

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