In Vitro Degradation and Rumen Fermentation Characteristics of Soybean Meal Protected with Different Levels of Formaldehyde

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ABSTRACT

The objective of this study was to determine the *in vitro* degradation and rumen fermentation characteristics of soybean meal that were protected with different levels of formaldehyde. In a completely randomized design experiment, 4 levels of formaldehyde (0, 0.6, 0.8, 1.0, and 1.2%; volume/weight) were applied on soybean meal. Protected soybean meals were incubated for 48 h using a 2-stage *in vitro* technique. The results showed that protecting soybean meal with formaldehyde decreased the dry matter and organic matter digestibilities ($P<0.05$) with the lowest on 0.8 to 1.2% formaldehyde treatments. Although there was no significant effects on rumen culture pH, total and proportion of VFA, a significant decrease in NH_3-N concentration and slightly decrease of microbial protein concentration were noticed due to formaldehyde treatments ($P<0.05$). Low NH_3-N and microbial protein concentrations was detected on 0.8, 1.0, and 1.2% formaldehyde treatments 16.3, 15.0, and 10.5 mg/100 mL, respectively, for NH_3-N and 8.32, 7.05, and 6.35 mg/mL, respectively, for microbial protein. It can be concluded that protecting soybean meal with formaldehyde can decrease rumen degradation and NH_3-N concentration with the cost of microbial protein synthesis. The best concentration of formaldehyde for protecting soybean meal was at 0.8%.

Keywords: Formaldehyde, Soybean meal, *In vitro*, Rumen degradation, Rumen fermentation characteristics

INTRODUCTION

Ruminant has microbes in the rumen, including bacteria, protozoa, and fungi, which have important functions in the process of feed degradation. Rumen microbes degrade dietary protein; approximately 60% of feed protein to ammonia (NH_3) (Wina and Abdurrohman, 2005), and cause the loss of energy during the fermentation process in the form of gas (CO_2 and CH_4). It caused a decline in the biological value of high quality feed protein (Cheeke, 2005). It also produced amino acids and then incorporate these products into microbial protein.

Soybean meal is a major protein source for animals. It contains about 48% protein and high biological value (Sitompul, 2004). When good quality protein is fed to ruminant, it is subject to extensive microbial fermentation. The high level of degradation in the rumen caused a decrease in the efficiency of feed protein usage by ruminant. One way to improve absorption of feed protein in the intestine is to protect dietary protein from microbial degradation. Formaldehyde (HCHO) treatment is known to protect feed protein from rumen

microbial degradation (Varvikko *et al.,* 1983). The aim of this study was to determine the *in vitro* degradation and rumen fermentation characteristics of soybean meal that had been protected with different levels of formaldehyde.

MATERIALS AND METHODS

Sample preparation and chemical analysis

Samples of soybean meal were obtained from one of the feed mill in Yogyakarta. The samples were oven dried (55°C) for one day and ground with willey mill through 1 mm sieve. The sample was analyzed for chemicals composition (dry matter, ash, crude protein, crude fat, and crude fiber) by AOAC (2005) method.

Treatment of soybean meal with formaldehyde

Protected soybean meal was carried out through a process of coating the soybean meal. Four levels of formaldehyde (0, 0.6, 0.8, 1.0, and 1.2%; volume/weight) were sprayed on soybean meal. After thorough mixing the material was kept overnight (about 12 hours), then dried 2 to 3 days (11-15% moisture content).

Animals and diet

Rumen fluid from two cannulated male Bali cattle (approximately 223 to 316 kg live weight) were used. The cattle was fed twice a day, at 07.00 and 14.00 h with maintenance diet of forage:concentrates (80:20) and free access to water. The adaptation period preceeding the experiment was one week.

In vitro degradation

Protected soybean meal was tested using a modified 2-stage *in vitro* Tilley and Terry method, but in this study only used a single stage assumed digestion in the rumen. Sample of oven-dried (0.5 g) were weighed into 80-90 ml glass centrifuge tubes, this capacity allowed space for formation of foam and prevented losses during shaking. Rumen liquor and buffer (1:4) were added, the mixture was stirred, gassed with CO_2, and 50 ml were added to each tube. The space above the liquid in each tube was thoroughly fluhed out with CO_2 gas, and the tube was then sealed with a rubber cork fitted with a gas release valve. After sealing, the tubes were incubated at 38°C in waterbath for 48 h, being shaken every 8 h. The parameters observed were digesbility (dry matter and organic matter), and rumen fermentation characteristics consists of rumen microbial protein using Lowry method, pH meter measurement, ammonia, and volatile fatty acid (VFA) with gas chromatography technique.

Statistical analyses

Results were analyzed as a one way completely randomized design by analysis of variance. Differences between means were tested for significance using Duncan's multiple range test (Astuti, 1981).

RESULTS AND DISCUSSION

Chemical composition of soybean meal

Soybean meal nutrient composition was presented in Table 1. The results showed that soybean meal has nutrient characteristics with high protein content.

Table 1. Chemical compotition of soybean meal

Chemical compotition	Soybean meal
Dry matter (DM) %	88.11
Crude protein (CP) %	49.13
Ether extract (EE) %	1.20
Crude fiber (CF) %	4.92
Ash (%)	7.77
Total digestible nutrients (TDN)	77.30

The amount of crude protein allowed the soybean meal was classified in feed ingredients of protein source or fifth-grade feed ingredients. Soybean meal was a by-product of soybean oil extraction. Soybean meal contains crude protein up to 49.4 until 54%, with dry matter reaching 89% and TDN 84 to 87% (Agus, 2008). Soybean meal was one of the high quality protein concentrate, but in most ruminant around 80-90% protein was degraded in the rumen (Widyobroto *et al.,* 1998).

In vitro degradation and rumen fermentation characteristics

Digestibility of dry matter, organic matter, and fermentation characteristics of soybean meal protected with different levels of formaldehyde were presented in Table 2.

***In vitro* degradation.** Digestibility of DM and OM on soybean meal protected with different treatments showed significant effect (P<0.05) when compared with unprotected soybean meal. Levels of 0.8 to 1.2% formaldehyde treatments showed lower digestibility than 0.6%, but there was no significant effects between the three treatments. Kamalak *et al.* (2005) reported that soybean meal with formaldehyde was resistant to rumen degradation, but it was digestible in the lower tract. The HCHO protein reaction may become very stable with time and result in a bond that is not susceptible to enzymatic digestion. Suhartanto *et al.* (2014) explained that the use of 1% formaldehyde in the treatment of undegraded protein (UDP) of soybean meal added in ration was able to protect the protein from rumen microbial degradation, as reflected by the decrease of IVDMD and IVOMD compared to the levels of formaldehyde (0, 0.5, 1.5, and 2.0%).

Table 2. Digestibility and fermentation characteristics of soybean meal protected

Parameter	Level of formaldehyde (%)				
	0	0,6	0,8	1,0	1,2
% IVDMD	94,60±1,60^{c}	55,15±6.50^{b}	49,53±0,70^{a}	48,88±2,61^{a}	47,97±1,75^{a}
% IVOMD	94,11±1,77^{c}	47,02±7,35^{b}	39,03±1,52^{a}	38,40±3,02^{a}	36,66±1,82^{a}
NH_3 (mg/100ml)	73,43±7,59^{c}	24,79±5,74^{b}	16,29±4,52^{a}	14,95±6,50^{a}	10,54±5,99^{a}
Microbial protein (mg/ml)	14,89±0,77^{c}	12,15±1,14^{b}	8,32±1,57^{a}	7,05±1,25^{a}	6,35±2,71^{a}
pH^{ns}	7,28±0,12	7,18±0,10	7,25±0,05	7,25±0,08	7,30±0,10
Total VFA^{ns} (ml mol/l)	51.83±19.10	30.78±3.81	37.99±3.42	46.10±2.63	43.93±1.96
Asetat (C_2)^{ns} m*M*	29.99±11.51	18.00±1.66	21.79±2.12	27.04±1.75	24.21±1.22
Propionat (C_3)^{ns} m*M*	16.75±5.87	9.79±1.84	12.76±1.40	15.59±0.78	15.89±0.65
Butirat (C_4)^{ns} m*M*	5.09±1.79	2.99±032	3.44±0.08	3.97±0.42	3.83±0.26
C_2:C_3 ratio	1.78±0.16^{b}	1.86±0.16^{b}	1.71±0.05^{ab}	1.73±0.04^{b}	1.52±0.05^{a}

$^{a, b, c}$ Different superscripts on the same line show a significant effect (P<0.05)
IVDMD = In vitro dry matter degradability; IVOMD = In vitro organic matter degradability.

The result of IVDMD and IVOMD, soybean meal protected as ruminant feed could be used with the 0.8% formaldehyde, because there was no significant effect between 1.0 and 1.2% formaldehyde. Increasing the concentration of formaldehyde was not necessary given that formaldehyde is a harmful chemical, if present in greater amounts, can decrease microbial activity in the rumen and pollute the environment. The European Food Safety Authority stated that formaldehyde in animal feed at 68-680 ppm was rapidly absorbed in the gastrointestinal tract and joined to formaldehyde pool in the body. The formaldehyde was rapidly oxidized to formic acid and then CO_2 and water (Adiveter, 2014).

Rumen fermentation characteristics. The ammonia values of formaldehyde treatment showed different effects ($P<0.05$) compared with control, but there was no difference between 0.8 to 1.2% formaldehyde. The low production of NH_3 in the treatment was due protection of protein so the rumen microbial can not degraded to ammonia. McDonald (2002) suggested that concentration of NH_3 in the rumen was range from 8.5 to 30 mg/100 ml, an excess of NH_3 will not be used for microbial protein formation and will be wasted. Ammonia in the rumen depends on the rough protein of the feed. Higher protein content of feed will be produced high concentration of NH_3. Most of the protein hydrolysis will occur catabolism (deamination) to NH_3. Ammonia derived from the feed protein catabolism was very large contribution to the synthesis of microbial protein. The amount of NH_3 in this study were still in the normal range of fermentation in the rumen or exceeds the minimum NH_3 concentration required for optimum growth of the rumen microbial by 5 mg/100 ml (Satter and Slyter, 1974).

Microbial protein values in this study were consistent with the change in NH_3 levels. Microbial protein synthesis requires the supply of energy and nitrogen to achieve optimal production. Dewhurst *et al*. (2000) suggested that microbial protein is an important source of protein source for ruminants. Microbial proteins meet the protein requirement reaching $^1/_3$ to ½ of total livestock demand. Microbial proteins are affected by the availability of energy and nitrogen in the form of NH_3. Increased efficiency of microbial protein synthesis can be done by manipulating feed protein content. Bacteria can utilize non-protein nitrogen (NPN) such as urea as a source of NH_3, while protozoa can not utilize NPN. Protozoa build their bodies through the degradation of feed proteins and bacteria protein, thereby decreasing the number of protozoa can improve the efficiency of protein utilization and the synthesis of microbial proteins in the rumen.

The in vitro of protected soybean meal with various levels of formaldehyde showed no significant difference in pH ruminal fluid value ($P>0.05$). This result indicated that the treatments did not affect the rumen microbial activity. Owens and Zinn (1988) stated that the normal pH range for rumen microbial activity in fermentation process and degradation of feed is 5.5 to 7.6, while microbial activity can take place optimally in the pH range of 6.7 to 7.0. McDonald *et al*. (2002) explained that if the pH value is below the normal range, the phosphate and bicarbonate present in the saliva react to buffer to help balance the pH. The pH value of the rumen may affect the production of NH_3 and VFA in the rumen because the microbial activity in the rumen was influenced by pH (Russell and Wilson, 1996).

The total VFA as well as the proportion of VFA (acetate, propionate, and butyrate) of formaldehyde treatment showed no significant difference ($P>0.05$), but there was a marked difference in the C_2:C_3 ratio between the controls with the 1.2% level of formaldehyde ($P<0.05$). The proportion of VFA in rumen fluid varies depending on the type of feed, forage and concentrate, and it is distribution (McDonald *et al*., 2002). Based on this study, the concentration of VFA is not influenced by the level of formaldehyde, this is because only a small part of UDP was degraded. On the one hand, the presence of UDP will increase the substrate, but on the other hand will decrease the activity of microoganism in the rumen, so

that the concentration of VFA is relatively the same at various treatments (Suhartanto *et al*., 2014). The proportion of acetate and propionate is affected by the level of formaldehyde in protected soybean meal. Usually in supplemented feeds with concentrates such as soybean meal, the acetate-propionate ratio falls (Vermorel, 1988). If the C_2 and C_3 ratios are lower, then C_3 formation increases and C_2 decreases, so methane production will decrease.

CONCLUSIONS

It can be concluded that the use of 0.8% formaldehyde in the protection of soybean meal was able to protect the protein against rumen microbial degradation. This is indicated by decreasing degradation of dry matter, organic matter, ammonia, microbial protein and acetate-propionate ratio. However, soybean meal protected did not affect the pH of rumen fluids, total VFA and VFA proportions. Soybean meal protected can improve the efficiency of the beneficiaries of good quality feed without affecting the environmental conditions of the rumen.

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