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The Effect of Soybean Meal Heating Time on the *in vitro* Digestibility and Ruminal Fermentation Profile

Research Article

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ABSTRACT

The aim of this study was to determine the most optimal heating time in protection of protein rich feedstuff on digestibility and *in vitro* ruminal fermentation profile. Proteinous feedstuffs used in this study is soybean meal (*Glycine max*). This study is designed using one way ANOVA, with five treatments of heating time (T0 (control)= unheated, T1= 10 min, T2= 20 min, T3= 30 min, and T4= 40 min) at 120 ˚C and 6 replications. All the treatment samples then incubated for 48 h according to the 2-stage *in vitro* technique. The results showed that protecting soybean meal through heating decreased the dry matter (DM), organic matter (OM) digestibility, NH_3 concentration and acetic acid:propionate ratio (A:P) ($P<0.05$) compared with the control group. In general, there were no significant effects on ruminal pH, total and proportion of volatile fatty acids (VFA), and microbial protein. A decrease in NH_3 concentration and A:P ratio was seen in T2 (49.05 mg/100 mL and 1.52, respectively). It can be concluded that protein protection in soybean meal through heat treatment can decrease rumen degradation. The best heating time for protecting soybean meal was found at 20 minutes.

KEY WORDS degradation, *in vitro*, protection, rumen, soybean meal.

INTRODUCTION

Ruminant needs a special way to supply its protein requirement. Some of the proteins in the rumen will be denatured into amino acids and followed by deamination into ammonia (NH_3), while some are not degraded by microbial rumen, which is categorized as rumen undegradable protein (RUP) (Nedelkov, 2019). The proteins available for livestock production are largely originated from microbial proteins in the rumen. It is estimated that 60-90% of the nitrogen consumed by livestock is converted to ammonia by rumen microorganisms, with around 50-70% of the nitrogen was used for microbial synthesis (Millen *et al.* 2016). The deamination of protein into ammonia (NH_3) is sometimes more than required for the microbial protein synthesis, thus resulted in an excess of NH_3, which will be excreted through urine in the form of urea. On the other hand, high-quality protein is required to provide amino acids to support ruminant productivity, thus feeding protein to ruminants should consider its fermentability and resistance towards rumen degradation. Protein in feed is known to have different rumen degradation level. Soybean meal is one of protein-rich feed with high nutritional value, but it has a rumen degradation rate at 71-79% (Stern *et al.* 2006), which means that most of the protein in soybean meal protein will be degraded in the rumen.

Protection of protein in feedstuff is important, to inhibit protein degradation in the rumen. Nothing that protein degradation will eliminate the function of the protein-rich feed to supply amino acids needed by ruminant (Haryanto,

2012). Moreover, protection of protein will increase the amount of digested protein in the intestinal tract, which is often regarded as "rumen undegraded protein" (Boucher *et al.* 2009).

A relatively easy method to protect protein in feed is by heating. The heating of protein in feedstuff can reduce proteolysis by inhibiting the proteolytic microbial enzymes, which then will reduce the rate of protein degradation in the rumen. Protection of protein by heating can be done through several methods, which were by heating with oven, toaster, or autoclave. Heating will induce Maillard reaction on the proteins, which will stop the protein degradation in rumen.

Heating the feedstuff at 100 to 150 ˚C has been shown to reduce the rumen digestibility (Haraki *et al.* 2018). In addition, heating the hempseed cake at 130 ˚C for 30 minutes can increase the amount of undegraded protein in the rumen, from 25.9 to 62.9% (Karlsson *et al.* 2012). However, overheating will result in loss of flavor and decrease the nutrient value. In this study, protein protection was carried out through heating in an oven at 120 ˚C with regards to the heating time. This research was conducted to obtain the optimal heating time to suppress soybean meal digestibility in rumen.

MATERIALS AND METHODS

The soybean meals were obtained from PT Sari Rosa Asih Feedmill located in Yogyakarta, Indonesia. The instruments used for proximate analysis are following AOAC (2005). Other instruments used were analytical scales (Ohaus, New Jersey, the USA with precision 0.0001), digital scales (Shanghai Yamato, Shanghai, China with precision 0.1), oven (Memmert, Schwabach, Germany), muffle furnace (Advantec, Tokyo, Japan), water bath, spectrophotometer (Genesys 20, Swedesboro, USA), micropipette, and Wiley mill (Thomas Willey Laboratory Meal, Philadelphia, USA).

Ruminal fluid preparation

The ruminal fluid used to observe the digestibility and *in vitro* fermentation was derived from two male Bali cattle (weighted approximately 223 to 316 kg). The feed adaptation was given twice a day at 7:00 a.m. and 14:00 p.m. with the ratio of forage and concentrates at 80:20 and given free access to water. The feed for adaptation contained 12% crude protein (CP) and 68% total digestible nutrient (TDN). King grass was given as a forage, while the concentrate has consisted of soybean meal, rice bran, and wheat pollard. A period of adaptation was carried out a week before the research. The ruminal fluid collection was carried out in the morning before the cattle were fed.

Soybean meal preparation and analysis

The soybean meals were dried (at 55 ˚C) with an oven for one day and grounded with Willey mill through 1 mm sieve. The samples were then analyzed for its composition (dry matter (DM), ash, crude protein (CP), crude fat, and crude fiber) by following AOAC method (AOAC, 2005).

Soybean meal heating

The protection of soybean meal protein was done through heating method. The heating was carried out at 120 ˚C, for 10, 20, 30, and 40 minutes respectively, with one-way ANOVA design.

Fermentation parameters and *in vitro* digestibility analysis

The digestibility was analyzed *in vitro* by following Tilley and Terry (1963) method.

Artificial saliva preparation

The materials used for one liter of artificial saliva were 9.8 g $NaHCO_3$, 9.3 g $Na_2HPO_4.12H_2O$, 0.47 g NaCl, 0.57 g KCl, 0.04 g $CaCl_2$ and, 0.06 g $MgCl_2$ anhyd. An addition of 0.1 mL $CaCl_2$ anhyd per liter of saliva was done when saliva will be used. All of ingredients were mixed in 1.5 liters Erlenmeyer except for the $CaCl_2$ anhyd. The ingredients are then dissolved with distilled water until reached 1 liter and then stored in a bottle at 38-39 ˚C.

Preparation before incubation

A total of 0.50 g (dry weight) sample was put in 80-90 mL *in vitro* tubes. The sample-filled tubes, container bottles containing artificial saliva and the tools used for testing were then placed into a water bath at 39 ˚C.

In vitro digestibility analysis

The *in vitro* incubation was performed the next day. Initially, a thermos bottle was filled with warm water with a temperature of ± 39 ˚C and previously been flowed with CO_2 gas before the ruminal fluid was poured. An initial pH measurement was done before collected. The ruminal fluid with pH at 6.2-6.8 was used for the research. If the pH was suitable, the water in the thermos bottle was then removed and substituted with the collected ruminal fluid directly. To remove the filtrate, the ruminal fluid was screened with PeCap screen (Noviandi *et al.* 2014) before poured on the thermos bottle. The ruminal fluid was then mixed with artificial saliva (Mc Dougall's solution) at the ratio of 1:4. The mixture of rumen fluid and artificial saliva as much as 50 mL was then put into a sample tube, flowed with CO_2 gas and sealed with a rubber cork equipped with a gas release valve.

The tubes were incubated for 48 h and shook for every 8 hours. After 48 hours of incubation, the solution was filtered in a glass wool contained crucible with the help of warm water. Thereafter, the filtered sample was dried in the oven at 55 ˚C for 2 days and weighed. The sample was then analyzed for *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) as described in Tilley and Terry (1963). The remaining ruminal fluid was used for pH measurement with pH meter, ammonia rumen measurement following Chaney and Marbach (1962), microbial proteins synthesis measurement following Plummer (1971), and volatile fatty acid (VFA) measurement following Filipek and Dvorak (2009) with gas chromatography technique.

Statistical analysis

The data were analyzed by one-way ANOVA (5 treatments and 6 replications), the means and standard deviation were calculated for each group, and the significance was set at $P < 0.05$. The differences between means were analyzed by using Duncan's new multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Soybean meal as the main ingredient in this study was analyzed by proximate before the heating treatment. The results proximate analysis showed that soybean meal had high crude protein content (CP), while other nutrient compositions were low (Table 1). The high CP content in soybean meal caused the feedstuff to be classified as a protein source, thus should be protected from ruminal degradation.

The results of *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of soybean meal after heated at 120 ˚C for 0, 10, 20, 30, and 40 minutes in the rumen were presented in Table 2. The dry matter (DM) and organic matter (OM) digestibility of soybean meal protected with different heating time showed a significant effect ($P<0.05$) compared with the unprotected soybean meal but showed no significant effect between the four different heating time.

The effect of heating soybean meal at 120 ˚C for 10, 20, 30, and 40 minutes on ruminal fermentation profile was shown in Table 3. The results showed no significant difference in the pH of ruminal fluid, total VFA, the VFA proportion and microbial protein between five treatments. Moreover, there is a significant effect ($P<0.05$) on the ammonia in 5 treatments. The lowest ammonia concentration was found at 20 minutes heating time. The low NH_3 production was due to the protection of protein, which inhibits microbial rumen to deaminates protein the soybean meal.

The acetic acid:propionate (A:P) ratio showed different effects ($P<0.05$) between 5 treatments. Soybean meal protection by heating for 30 minutes resulted in the lowest A:P ratio.

Chemical analysis

The high CP content of soybean meal (Table 1) showed that the feedstuff is classified in protein source (Scanes, 2010). Hartadi *et al.* (1980) reported that feed ingredients classified as protein sources are materials containing 20% or more crude protein derived from animals or cakes, bran, and other feed ingredients.

In addition, soybean meal is a byproduct of extraction soybean oil. Soybean meal contains 49.4-54% protein with 89% DM (Agus, 2012).

The most of amino acids compositions and crude protein are contained in soybean seeds compared to other legume seeds. Compared with other feed protein sources, soybean meal has relatively lower crude fibre (CF) content. A little starch and a lot of pectin and hemicellulose are contained in soybean meal. Soy protein characterized by high quantity of tryptophan, lysine, isoleucine, threonine, and valine, but less sulfate amino acid. Furthermore, the highest protein digestibility in soybean is lysine and methionine (Banaszkiewicz, 2011).

Aside from being the source for amino acids, soybean meal is also often used in rations to help reduce methane produced by livestock. This is shown by the research of Wiryawan *et al.* (2017) which showed that complete ration containing soybean and soy pod pods at 15% and 30% did not affect the protozoa population, ammonia concentration and total VFA production compared to rations that were 100% native grass. On the contrary, the use of soybean pods and soybean by-products in concentrate rations or complete rations can reduce the proportion of acetate and increase the proportion of butyrate compared to livestock that has just native grass. Concentrate ration or complete ration containing 15% soybean pod can increase the methane emission, however it decreased when 30% soybean pod level was given.

On the other hand, ruminal microbes can convert low-quality proteins into high-quality proteins and also break down high-quality feed proteins into ammonia that resulted in energy loss during fermentation processes by forming CO_2 and CH_4 gas, which reduced the nutritional value of high-quality feed proteins (Cheeke, 2005). Soybean meal is one of the high-quality protein concentrate feed but in ruminant livestock, most of the protein fraction (around 91.9%) is potentially degraded within the rumen (Mjoun *et al.* 2010). Accordingly, feed manipulation should be done to avoid excess of degradation by ruminal microbes.

Table 1 The content of soybean meal nutrients as feed ingredient of protected protein source

Nutrient content	Value
Dry matter (DM) %	88.11
Organic matter (OM) %	92.23
Crude protein (CP) %	49.13
Ether extract (EE) %	1.20
Crude fiber (CF) %	4.92
Total digestible nutrients (TDN)	77.30
Nitrogen-free extract (NFE)	37.08

Table 2 *In vitro* degradability of protected soybean meal in the rumen

Variable	Length of heating (minute)				
	0	10	20	30	40
% IVDMD	94.60±1.60^{b}	87.81±5.36^{a}	86.24±5.72^{a}	85.29±3.00^{a}	84.70±4.13^{a}
% IVOMD	94.11±1.77^{b}	87.94±5.88^{a}	86.42±5.60^{a}	88.17±2.65^{a}	83.13±2.91^{a}

IVDMD: *in vitro* dry matter degradability and IVOMD: *in vitro* organic matter degradability.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Characteristics of rumen fermentation on protected soybean meal with different length of the heating

Variable	Length of heating (minute)				
	0	10	20	30	40
pH	7.28±0.12	7.36±0.14	7.42±0.16	7.18±0.04	7.42±0.21
Total volatile fatty acid (m*M*)	71.98±22.24	67.59±1.57	68.12±3.81	62.02±4.85	59.88±19.18
Acetatet (m*M*)	41.35±11.77	37.11±1.28	36.59±1.90	31.72±2.25	33.45±12.31
Propionate (m*M*)	23.45±7.94	23.26±0.48	24.06±1.35	22.49±3.32	19.36±5.48
Butirat (m*M*)	7.18±2.56	7.22±0.18	7.46±0.63	7.81±1.18	7.06±1.42
A:P ratio	1.80±0.11^{c}	1.59±0.02^{ab}	1.52±0.02^{ab}	1.44±0.23^{a}	1.69±0.14^{bc}
NH_3 (mg/100 mL)	75.58±5.47^{b}	74.98±7.42^{b}	49.05±6.13^{a}	72.16±3.38^{b}	86.63±1.01^{c}
Microbial protein (mg/100 mL)	14.89±0.078	14.67±0.16	15.29±0.15	15.76±0.15	13.89±0.26

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Mjoun *et al*. (2010) showed that soybean meal also contains an essential amino acid of 442.0 grams/kg which amino acids include Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val.

Effect of protected soybean meal on *in vitro* digestibility

This showed that the protection of soybean meal at 120 ˚C for 10 minutes has been able to reduce IVDMD and IVOMD; an increased heating time up to 20, 30, and 40 minutes was no longer effective in decreasing IVDMD and IVOMD. This may due to changing a protein structure that can decrease the degradation in the rumen (Table 2). Protection of protein in feed can be done through several methods which involve Maillard reaction, such as extrusion, roasting, expeller, and lignosulfonate. The key to successful protection of soybean or soybean meal protein is to optimize the healing process including temperature and heating time. Optimal heating for feed protection can also be affected by carbohydrate and protein content. The Maillard reaction or non-enzymatic reaction is a reaction which involves both the amino group and the sugar residue, which creat the amino-sugar complex bond, which had permanently digested bond.

Heating significantly increased the α-helix to β-sheet ratio structure and changed its chemical profile. This structural change caused a decrease in the protein and overall dry matter (DM) degradability (Murray *et al*. 2009; Lin and Kung, 2015).

Extruded soybean meal (ESBM) had slower protein degradation rate and lower rumen digestibility compared with control / solvent soybean meal without extruder (SSBM). The ESBM diets tend to increase the C_{18} fatty acid and decrease most of the milk fatty acid with chain length up to C_{17} in milk. Overall, the researchers suggest that substituting SSBM with ESBM in rations will have a positive effect on feed intake and milk yield in dairy cows (Giallongo *et al*. 2015).

The results of digestibility in this study are in accordance with the results of El-Waziry *et al*. (2007), which showed that the presence of a protective protein treatment on soybean meal through heating by autoclave at 121 ˚C for 30 minutes can decrease IVOMD in rumen when compared with unprotected soybean meal from 82.97 to 49.88%. Nobar (2011) stated in his research that soybean meal protected by 6% of black liquor with microwave radiation for 4 minutes showed lowering the value of rapidly degraded

fraction (a) was 40% (9.35 g/kg) compared to unprotected soybean meal (15.13 g/kg).

Effect of protected soybean meal on ruminal fermentation profile

The ruminal fluid pH in this study was in the normal range of ruminal pH and no significant difference was shown among five treatments (Table 3). The ideal pH value of protected and unprotected soybean meal is 7.18-7.42, which is similar to the normal ruminal fluid pH. Similarly, Mudita *et al*. (2016) showed that ruminal fluid pH of Bali cattle supplemented with biosuplemen ranged from 7.04 to 7.34 resulted in a not significantly different between each treatment. This can be interpreted that the soybean meal protection treatment at 120 ˚C for 10, 20, 30, and 40 minutes has no effect on the microbial rumen environment, so it is expected that microbial rumen performance will not be disturbed in degrading the feed. The variety of ruminal fluid pH will affect the digestibility of feed fibers and production of NH_3 and VFA in the rumen because the microbial activity in the rumen is affected by pH (Schmidt and Zsedely, 2011). McDonald *et al.* (2011) reported that phosphate and bicarbonate in the saliva will react to buffer to maintain pH balance.

Volatile Fatty Acid concentrations were not significantly different from the ruminal fluid pH; this indicates that the VFA production was low, so it did not reduce the ruminal fluid pH. The same results were reported by Chen *et al.* (2002) that heating the soybean meal with at 141 ˚C did not affect the total VFA content in ruminal fluid. The consumed protein content in the feed is closely related to the VFA production when soybean meal protein was protected causing the carbohydrates contained therein also protected. This is because the proteins contained in soybean meal each other binds to another group of carbohydrates. Owens and Basalan (2016) reported that the fermentation of crude proteins yielded VFA, whereas the fermentation of true proteins resulting NH_3 and VFA. In addition, proteins can be the source for VFA formation when the feed material contained high rumen degradable protein (RDP) (Pilachai *et al.* 2011).

In this study, the result indicates that a 20-minute heating treatment can protect more proteins thereby decreasing NH_3 formation. Chen *et al.* (2002) reported that the protection of protein in soybean meal with heating at 141 ˚C gave a significant effect on reducing NH_3 concentration in the rumen when compared with unheated soybean meal. The ammonia concentration in each treatment is still above the normal range. Research showed that the ammonia concentration in Bali cattle rumen is ranged from 9.82 mM/dL to 40.8 mM/dL (Mudita *et al*. 2016) of the high ammonia levels in this study is caused by the single feed material with high protein levels was used, thus resulted in high deamination. Dourado *et al*. (2011) stated that soybean meal contains 47% CP. Jeong *et al*. (2015) reported that NH_3 concentrations produced by ruminal fermentation of soybean meal without additional feed ingredients were higher compared with soybean meal mixtures and other feed ingredients. Vanegas *et al*. (2016) stated that the protein protection on sunflower seeds and sunflower seed meals through heating and malic acid addition would show decrease the NH_3 concentration in the rumen.

Protein protection by heating for 20 minutes yields the lowest ammonia levels. However, no significant effect on the microbial rumen concentrations. The microbial concentration in the ruminal fluid found in this study was lower compared to the microbial protein concentrations reported by Suhartanto *et al*. (2014), which is 32.1 mg/100 mL. The synthesis of microbial proteins requires carbohydrates as energy and nitrogen in the form of NH_3. The low concentration of microbial protein is due to low carbohydrate content in soybean meal, which is 4.66-7.00% (Banaszkiewicz, 2011), thus the synthesis of microbial rumen protein becomes obstructed.

CONCLUSION

The soybean meal protection through heating at 120 ˚C for 20 minutes showed the best protein protection against rumen degradation. Furthermore, the 20 minutes heating at 120 ˚C decreased the dry matter digestibility, organic matter digestibility, NH_3 concentration, and acetate:propionate ratio in soybean meal, but showed no effect on the fermentation process.

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