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Degradability of Rumen-Protected Soybean Meal with Different Temperatures and Heating Times in Bali Cattle

Wulandari[1*], Rinanti Eka Aldis[2], Dananto Ramadhan[2], Wulanningtyas[3], Andriyani Astuti[2], Adiarto[4], Lies Mira Yusiati[2], Cuk Tri Noviandi[2], Budi Prasetyo Widyobroto[4], and Ali Agus[2]

[1]Research Center for Animal Husbandry, National Research and Innovation Agency, Bogor, West Java, 16915, Indonesia
[2]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[3]Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[4]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

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* Corresponding author:
Telp. +62 822 2734 6063
E-mail: wulandari.1@brin.go.id

ABSTRACT

The goal of this study was to ascertain the impact of soybean meal's temperature and heating duration as undegraded protein (UDP) on Bali cattle's dry matter (DM) and organic matter (OM) degradation kinetics. Soybean meal is a feed ingredient with high protein content, which is about 48%, and is rapidly degraded in the rumen. In this investigation, a 4 × 4 factorial design was employed with the first factor being temperature (60, 80, 100, and 120°C), and the second factor being heating time (10, 20, 30, and 40 min). Protected soybean meal was tested for degradation using the in sacco technique on the rumen of fistulous Bali cattle. A sample of 5.0 g was put into a nylon bag and then for 0, 2, 4, 8, 16, 24, and 48 h in the rumen, then analyzed for feed residues for DM and OM. The results showed that soybean meal protected by the heating method could reduce the degradation of DM and OM in the rumen ($p<0.05$). Heating at 120°C for 40 min showed the lowest DM and OM degradations in this study.

Keywords: Heat treatment, *In sacco,* Protection, Rumen undegradable protein

Introduction

Microbial protein can meet the basic needs of amino acids for the maintenance of the livestock body, but under certain conditions it becomes insufficient. Body tissues may manufacture non-essential amino acids at a pace that is sufficient to meet production needs, but to reach maximal production levels, more necessary amino acids must be added to the diet. Even if the availability of essential amino acids from rumen bacteria is adequate for survival and satisfying the needs of low levels of production, supplementation of essential amino acids is still required. This is needed to achieve the highest average level of animal body weight gain during the growth period of young cattle or commercially achieved milk production (Owens *et al*., 2014). Bali cattle are one of the original Indonesian cattle that are widely kept by farmers and are included as a commodity for beef cattle (Baliarti *et al.,* 2021; Kusumastuti *et al.,* 2021). Protein is one of the nutrients that play a role in body weight gain, so this study used Bali cattle with rumen fistula.

By being digested into peptides and amino acids by rumen microorganisms, feed protein can then be further broken down into organic acids like NH_3 and CO_2. Rumen organisms employ ammonia, which is synthesized along with a number of peptides and free amino acids, to synthesize microbial proteins. The small intestine directly absorbs feed protein that is not digested in the rumen. When protein synthesis lags behind protein decomposition, more NH_3 than is ideal accumulates in the rumen fluid. The blood carries NH_3 to the liver where it is processed into urea. Although saliva can help some of this urea return to the rumen, most of it is expelled in the urine and squandered (McDonald *et al*., 2011).

High-quality feed protein can be converted by rumen microorganisms into NH_3, but they can also lose energy during fermentation in the form of CO_2 and CH_4, which lowers the biological value of the protein. Rumen microbes can also transform low-quality protein into high-quality protein (Cheeke, 2005). Although soybean meal is a concentrated source of high-quality protein, ruminants destroy 80–90% of the protein in the rumen (Widyobroto *et al.,* 1998). Protein protection techniques are one of the feed manipulation strategies needed to prevent rumen bacteria from degrading high-quality feed protein.

Ingredients for feed, like grains and soybeans, typically employ heat treatment to protect proteins. Heating proteins will have greater resistance to enzymatic hydrolysis. However, the Maillard reaction between sugar and amino acids is triggered by the high temperature and prolonged heating, which damages important amino acids including lysine, methionine, and cystine. The amount of some amino acids and the small intestine's ability to digest protein can both be affected by damage to these amino acids (Kamalak *et al.,* 2005). Protein breakdown in the rumen is reported to be decreased by soybean meal heated to 120°C using an autoclave in non-lactating dairy cows of the Norwegian Cattle breed (Ljùkjel *et al.*, 2000). Furthermore, heating the hempseed cake for 30 min at 130°C may raise the quantity of protein that isn't broken down in the rumen from 25.9% to 62.9% (Karlsson *et al*., 2012). Referring to this, it is hoped that heating at a certain temperature and time can reduce the level of nutrient degradation of soybean meal by rumen microbes. The goal of this study was to ascertain how heating time and temperature affected the rate at which dry matter and organic matter degraded in the rumen.

Materials and Methods

The soybean meal utilized in this research was obtained from the Indonesian feed mill PT Sari Rosa Asih in Yogyakarta. Two Bali cattle (*Bos javanicus*) approximately 243 to 327 kg that had permanent rumen cannulas were used to collect rumen fluid. Every proximate analysis adhered to the AOAC's protocol (AOAC, 2005). Nylon bags, an analytical balance with an accuracy of 0.0001 (Ohaus, NJ, USA), a grinder (Thomas Willey Laboratory Mill, Philadelphia, USA), an oven (Memmert, Schwabach, Germany), and a digital balance with an accuracy of 0.1 (Shanghai Yamato, Shanghai, China) were among the tools utilized.

Sample analysis and protected preparation. Samples of soybean meal were dried in a 55°C oven to a consistent weight before being processed in a Willey mill fitted with a 1 mm sieve. According to the AOAC method, samples were examined for their chemical composition (dry matter (DM), ash, crude protein (CP), crude fat (extracted ether, EE), and crude fiber (CF)) (AOAC, 2005). Soybean meal is heated in an oven at temperatures of 60, 80, 100, and 120°C for 10, 20, 30, and 40 min, respectively, to achieve protection by heating.

***In sacco* measurements.** Two Bali cattle were subjected to nylon bag rumen degradation measurements following a one-week adaptation period. Balinese cattle weigh approximately 223-316 kg live weight and are equipped with a permanent rumen cannula. Livestock was fed twice daily at 7:00 am and 2:00 pm with a concentrated diet (80:20 ratio), a maintenance diet, and water ad libitum. Evaluation of diet components *in Sacco* using Ørskov *et al*. (1980), Widyobroto *et al.* (1995), and Soejono *et al.* (1998) developed a method. The in sacco method uses nylon/polyester bags (Shabi *et al.*, 1998) with a porosity of 45 µm and a size of 6 × 11 cm, which are fixed on all three sides and left open on one side before, the pouches were appropriately labeled/marked for handling, incubation times and replication. The bags were then dried in an oven at 55°C for 6 h and tared. A 5 g sample was placed in a nylon bag, folded, and pressed against the fourth side. A nylon bag filled with a diet sample is attached to a chrome-plated iron ring with a rope, and the ring is tied with a 40-60 cm long plastic rope. Samples were cultured in ruminant fistula cattle for different time intervals of 0, 2, 4, 8, 16, 24, and 48 h. Each incubation point was repeated 6 times. Each sample at the incubation point is tied with a different colored rope to facilitate collection at a specific point.

In addition, nylon bag samples taken from the rumen according to the incubation time were either stored in a freezer at a temperature of −15°C or immediately washed in a washing machine under running water for 6 min. Cleans feed particles and germs adhering to the nylon bag, and rumen bacteria bound to the feed particles remaining in the bag. The bags were dried in an oven at 55°C. for 72 h to constant weight and then the residue was weighed. DM, and OM were analyzed and the disappearance value was calculated as the difference in nutrient weight before and after incubation for each sample. Degradability data obtained for DM, and OM for each diet were fitted to the equation.

$$P = a + b(1 - e^{-ct})$$

where p = potential for degradation, t = incubation time, a = rapidly soluble fraction, b = potentially degradable fraction, c = degradation rate of b fraction (Ørskov *et al.*, 1980).

Statistical analysis. The generalized linear model (GLM) technique in SAS (version 9.1.3, SAS Institute Inc., Carry, NC, 2008) was used to evaluate the results as a completely randomized design with a 4×4 factorial pattern. The significance level was set at $p<0.05$. The new multiple range test developed by Duncan was used to assess differences between means (Steel *et al*., 1997).

Results and Discussion

Protection of soybean meal using the heating method with variations in temperature and length of heating time against DM and OM degradation *in sacco* is presented in Tables 1 and 2. Parameters observed included estimation of the rapidly soluble fraction (a value), the potentially degradable fraction (b value), the rate of degradation of fraction b (c value), and effective degradability (ED).

Based on the soluble fraction (a-value) results presented in Tables 1 and 2, it is shown that there is a significant difference ($p<0.05$) due to variations in temperature and heating time and the interaction of the two factors. Heat-protected

soybean meal reduced DM and OM degradations scores compared to control/unprotected soybean meal. The heating temperature of 120°C shows the lowest value and is significantly different from other temperature variations, while for the variation of heating time, 40 min show the lowest value but is not significantly different from the heating time of 20 and 30 min. The results of the variance indicate an interaction between temperature and heating time. The lowest value was found in the combination of heating temperature treatment of 120°C with a long heating time of 40 min, namely 30.27% (DM degradation) and 24.91% (OM degradation). Overall the combination of heating protection treatment has a DM degradation a value range of 30.27% to 34.66%, this result is below the value of unprotected soybean meal, which is 34.8%, but still higher when compared to extruded soybean meal, namely 27.9% (Griffiths, 2004).

Potentially degraded fractions (b value) DM and OM in this study showed significantly different results due to variations in temperature, length of heating time, and the interaction between the two ($p<0.05$). Soybean meal protected by heating increased the value of b when compared to unprotected soybean meal. The increase in the value of b in the treatment compared to the control can be assumed to be less successful in the protection of the soybean meal heating method so

Table 1. Dry matter degradation characteristics and effective degradability of soybean meal protected

Parameters	Temperature (°C)	Heating time (min) 10	20	30	40	Average
a (%)	60	31.93±0.83^{b}	31.78±1.17^{b}	31.80±1.63^{b}	31.09±0.62^{bc}	32.25±1.63^{q}
	80	31.78±0.56^{b}	31.77±0.66^{b}	31.99±0.15^{b}	31.43±0.65^{b}	32.33±1.36^{q}
	100	31.58±0.40^{b}	31.66±0.99^{b}	31.99±0.39^{b}	31.42±0.70^{b}	32.27±1.43^{q}
	120	31.58±0.39^{b}	31.18±0.51^{bc}	30.79±0.63^{bc}	30.27±0.53^{c}	31.69±1.69^{r}
	Average	32.30±1.37^{y}	32.21±1.53^{yz}	32.25±1.76^{yz}	31.77±1.85^{z}	
b (%)	60	73.24±1.92^{a}	71.77±3.12^{ab}	70.30±3.26^{bcd}	71.12±3.87^{abc}	70.32±3.92^{p}
	80	73.27±1.46^{a}	71.24±1.14^{abc}	69.22±0.60^{bcd}	69.27±1.46^{bcd}	69.64±3.05^{pq}
	100	71.54±2.28^{ab}	70.48±1.50^{bcd}	67.74±0.90^{de}	68.47±0.81^{cd}	68.68±2.73^{qr}
	120	70.22±1.99^{bcd}	69.07±1.22^{bcd}	68.51±1.49^{cd}	68.08±0.64^{d}	68.21±2.27^{r}
	Average	70.69±3.55^{w}	69.55±3.03 ^{x}	68.19±2.75^{y}	68.42±3.03^{y}	
c (%/h)	60	0.07±0.01^{d}	0.08±0.02^{bcd}	0.09±0.02^{b}	0.09±0.03^{bc}	0.10±0.04^{q}
	80	0.07±0.01^{d}	0.08±0.01^{bcd}	0.09±0.00^{bc}	0.09±0.00^{bcd}	0.10±0.04^{q}
	100	0.07±0.01^{cd}	0.08±0.01^{bcd}	0.09±0.00^{bc}	0.08±0.00^{bc}	0.10±0.04^{q}
	120	0.07±0.01^{bcd}	0.08±0.00^{bcd}	0.07±0.01^{bcd}	0.08±0.01^{bcd}	0.10±0.04^{q}
	Average	0.09±0.04^{z}	0.09±0.04^{yz}	0.10±0.04^{y}	0.10±0.04^{y}	
ED (%)	60	73.39±1.67^{defg}	75.05±2.75^{bcde}	76.83±2.69^{b}	76.07±2.78^{bc}	77.10±2.81^{q}
	80	73.19±1.09^{efg}	74.37±0.64^{cdef}	76.56±0.71^{b}	75.59±0.35^{bc}	76.78±2.13^{q}
	100	72.74±0.50^{fg}	74.15±0.57^{cdef}	75.98±0.57^{bc}	75.23±0.63^{bcd}	76.45±1.82^{q}
	120	72.09±0.51^{gh}	73.07±1.21^{fg}	71.83±0.84^{gh}	70.84±0.89^{h}	74.40±1.75^{r}
	Average	75.12±2.58^{z}	76.16±2.04^{y}	77.08±1.76^{x}	76.38±2.28^{xy}	

^{w,x,y,z} Different superscripts at the same row showed significant effects ($p<0.05$).
^{p,q,r,s} Different superscripts at the same column showed significant effects ($p<0.05$).
$^{a-i}$ Different superscripts at the same row and column showed significant effects ($p<0.05$) in the treatment mean showed an interaction between temperature and heating time.
ED = effective degradability, a = the rapidly soluble fraction, b = the potentially degradable fraction, c = the rate of degradation of fraction b.

Table 2. Organic matter degradation characteristics and effective degradability of soybean meal protected

Parameters	Temperature (°C)	Heating time (min) 10	20	30	40	Average
a (%)	60	24.56±1.12^{bcd}	25.60±1.27^{bcd}	25.95±1.79^{b}	25.31±1.26^{bcd}	26.23±2.26^{y}
	80	24.17±0.96^{d}	25.66±0.42^{bcd}	26.05±0.30^{b}	25.66±0.58^{bcd}	26.25±2.06^{y}
	100	24.35±0.38^{cd}	25.66±0.51^{bcd}	25.83±0.45^{bc}	25.54±0.59^{bcd}	26.22±2.01^{y}
	120	24.55±0.38^{bcd}	25.16±0.55^{bcd}	25.13±0.31^{bcd}	24.91±0.51^{bcd}	25.90±2.10^{y}
	Average	25.47±2.36^{r}	26.36±1.95^{q}	26.54±2.16^{q}	26.23±2.23^{q}	
b (%)	60	82.73±3.56^{ab}	78.77±4.71^{cd}	75.95±4.22^{defg}	76.67±6.05^{cdef}	76.98±5.69^{x}
	80	83.38±1.22^{a}	77.30±0.35^{cde}	74.29±0.91^{efghi}	73.20±0.95^{fghi}	75.79±4.61^{x}
	100	79.91±1.09^{bc}	76.08±1.42^{defg}	72.41±1.50^{h}i	72.73±0.70^{ghi}	74.38±3.64^{y}
	120	77.14±1.06^{cde}	74.70±1.21^{efgh}	74.79±2.06^{efgh}	74.23±1.01^{efghi}	74.33±2.63^{y}
	Average	78.79±5.07^{p}	75.53±3.66^{q}	73.64±3.33^{r}	73.52^{r}±3.83^{r}	
c (%/h)	60	0.06 ±0.00	0.07 ±0.02	0.08 ±0.02	0.08 ±0.02	0.09 ±0.04
	80	0.06 ±0.00	0.07 ±0.00	0.08 ±0.00	0.08 ±0.01	0.09 ±0.03
	100	0.06 ±0.00	0.07 ±0.00	0.08 ±0.00	0.08 ±0.00	0.09 ±0.03
	120	0.06 ±0.00	0.07 ±0.00	0.07 ±0.00	0.07 ±0.01	0.08 ±0.03
	Average	0.08±0.04^{r}	0.09±0.03^{qr}	0.09±0.04^{q}	0.09±0.04^{q}	
ED (%)	60	69.47±1.97^{bcde}	70.68±3.03^{bcd}	71.44±3.23^{b}	70.73±3.26^{bcd}	72.92±5.43^{y}
	80	68.99±0.93^{cde}	70.23±0.32^{bcd}	71.19±0.61^{bc}	70.10±0.90^{bcd}	72.56±5.08^{y}
	100	68.70±0.99^{def}	69.73±0.75^{bcde}	70.70±0.57^{bcd}	69.78±1.09^{bcde}	72.24±5.25^{y}
	120	67.60±0.64^{ef}	68.66±0.78^{def}	67.75±0.41^{ef}	66.84±1.25^{f}	70.62±6.04^{z}
	Average	71.41±5.71^{r}	72.32±5.34^{q}	72.67±5.89^{q}	71.95±6.30^{qr}	

^{x,y,z} Different superscripts at the same row showed significant effects ($p<0.05$).
^{p,q,r,s} Different superscripts at the same column showed significant effects ($p<0.05$).
$^{a-i}$ Different superscripts at the same row and column showed significant effects ($p<0.05$) in the treatment mean showed an interaction between temperature and heating time.
ED = effective degradability, a = the rapidly soluble fraction, b = the potentially degradable fraction, c = the rate of degradation of fraction b.

that the potentially degraded fraction still has a high percentage. The range of b values in this study is 65.19% to 73.24%, this is still too high when compared to the b value of soybean meal without protection, which is 70.10% (Islam *et al*., 2002).

The rate of degradation (c value) on DM degradation due to differences in heating temperature and heating time showed significantly different results ($p<0.05$). The temperature variation treatment showed a different decrease in the value of c when compared to the control but did not show a significant difference between temperatures from 60°C to 120°C. The same thing also happened to the treatment of variations in the length of heating time. Different heating times showed a decrease in the value of c when compared to the control. Based on the results of the variance, it shows that there is an interaction between temperature and the length of heating time so it shows a significant difference in the value of c. The lowest value of c is found in the combination of heating temperature treatment of 120°C with a heating time of 40 min. The decrease in the value of c in all treatment groups when compared with the control can be assumed that the heating of soybean meal causes a decrease in the rate of degradation (c value) of feed ingredients in the rumen. The value of c in this study ranged from 0.07% to 0.16% per hour. This result is still quite high when compared to the c value of unprotected soybean meal, which is 0.07% per hour (Islam *et al*., 2002), and the c value of extruded soybean meal, which is 0.012% per hour (Griffiths, 2004).

Based on the result data in Tables 1 and 2, variations in temperature, length of heating time, and the interaction of the two gave significantly different results to the EDDM and EDOM values ($p<0.05$). The lowest ED value was found at the treatment temperature of 120°C when compared to other temperatures and controls. In the treatment, the heating time of 40 min showed the lowest ED value when compared to other times, but did not show a significant difference with the heating time of 20 and 30 min. The combination of 120°C heating treatment with 40 min of heating showed the lowest ED value (70.84%) compared to other combinations, but not significantly different at 120°C temperature combination for 10 and 30 min (72.09 and 71.83%). The decrease in DM and OM degradation by heating at 120°C is also following the results of in vitro research that soybean meal heated at 120°C for 10, 20, 30, and 40 min reduces the digestibility of DM and OM by 7-10% compared to unprotected soybean meal (Wulandari *et al.,* 2020). Overall, the ED value in the soybean meal protection treatment by heating still shows great results when compared to the ED value in the soybean meal protection treatment with formaldehyde, which is 47-52% (Wulandari *et al*., 2022), so it can be assumed that the protection by the heating method is less. effective when compared to the formaldehyde method.

The decrease in the digestibility value of DM and OM soybean meal was caused by the formation of complex bonds between protein and carbohydrates due to heating. Heating the protein causes the Maillard reaction. The Maillard reaction is a reaction that occurs between the aldehyde group of carbohydrates and the amine group of proteins. The reaction that occurs between the two groups will form a cross-link. The cross-links formed will cause a decrease in digestibility due to inhibition of enzyme penetration into the protein substrate or due to the closure of protein sites that can be attacked by enzymes (Palupi *et al.,* 2007), thus decreasing protein solubility and increasing the rumen bypass protein (Reddy *et al*., 1993; Dhiman *et al*., 1997; Rafiee-Yarandi *et al*., 2016).

Conclusions

In conclusion, this study showed that protection of soybean meal by the heating method can reduce the degradation of DM and OM in the rumen, thereby increasing the efficiency of animal feed. Heating soybean meal at a temperature of 120°C for 40 min showed the lowest DM and OM degradation values compared to other treatments in this study.

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