

Fermentability and Digestibility of Dairy Cattle Ration Containing Corn-NFC, Protected Soybean and Sulfur Supplementation

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Abstract. Protein is an important nutrient for dairy cattle to produce milk. Ration with protected soybeans by autoclave, non-fiber carbohydrate (NFC) from corn, and sulfur supplementation can maximize protein availability for ruminants. This study aimed to evaluate the effect of a dairy ration containing corn NFC, protected soybean, and sulfur supplementation on fermentability and digestibility of the ration. This study used a randomized block design consisting of five treatments namely rations without corn and soybeans (R1), rations with corn without soybeans (R2), rations with corn and soybeans (R3), rations with corn and protected soybeans by autoclave (R4), and rations with corn, protected soybeans by autoclave, and sulfur supplementation (R5). The treatments were repeated four times as replications. The data were analyzed using the ANOVA and followed by an orthogonal contrast test. The results showed pH, protozoal number, dry matter, and organic matter digestibility were not affected by the treatment. Although treatment R3 produced significantly ($P < 0.05$) higher ammonia concentration than R4 and R5 but R5 produced the highest VFA and total bacteria. It can be concluded that supplementation of sulfur to the corn-NFC, and protected soybeans dairy ration improved organic matter fermentability by bacterial activity.

Keywords: autoclave, corn, non-fiber carbohydrate, rumen degradable protein, sulfur, soybean.

1. Introduction

Protein is an essential nutrient for dairy cattle to produce milk, but some protein can be degraded in the rumen. Rumen microbes will utilize the results of protein degradation (ammonia) to synthesize microbial protein [1]. The use of feedstuff high in RDP becomes inefficient because ammonia will be metabolized in the liver and then excreted through urine [2]. The selection of feed ingredients with high RDP content needs to be considered so that they are not wasted.

Soybeans have a high protein content which correlated with high RDP level (86.21%) [3, 4], so adding soybeans to dairy cow rations becomes inefficient. One way to reduce the high RDP level in soybeans is to provide physical treatment to soybeans. Physical treatments such as moist heating can reduce RDP in feed ingredients through the Maillard reaction [5]. Heating with autoclave significantly changed protein profile, protein subfraction, rumen degradability, and protein molecular structure compared to dry heating [6]. According to Doiron et al. [7], using an autoclave at flaxseed can reduce the RDP value and digestibility, indicating undigested RUP.

Rumen microbes require both ammonia and amino acids, including sulfuric amino acids. Al-Rabbat et al. [8] reported that 61% of microbial nitrogen was driven from ammonia, and 39% came from amino acids and peptides. High-producing dairy cows require amino acids that are high in sulfur. A good source of sulfur minerals used in the dairy ration is inorganic sulfur, such as Na_2SO_4 , which can be more easily absorbed in the digestive tract of ruminants compared to organic sulfur, such as Methionine Hydroxyl Analog [9]. Rosmalia et al. [10] revealed that sulfur supplementation from Na_2SO_4 increased the digestibility of dry matter and organic matter. In addition, sulfur plays a role in forming sulfuric amino acids such as cysteine and methionine by rumen microbes so that the availability of amino acids for livestock becomes available.

Optimizing the availability of protein for dairy cattle can also be done by increasing microbial protein that has high biological value. Rumen microbes require available energy to synthesize microbial protein. Availability of energy for rumen microbes can be obtained from non-fiber carbohydrates (NFC) as a direct energy source. NFC provides sufficient energy for maximal microbial protein synthesis [11]. Corn has 70.89% NFC [12], with carbohydrates in the form of starch with an amylose content of 27% and amylopectin of 73% [13]. Protein availability for dairy cattle can be maximized by compiling rations with soybean protein protected by autoclave, corn NFC, and sulfur supplementation, protein availability for dairy cattle is maximized. This study aimed to evaluate the fermentability and digestibility of dairy cattle rations containing NFC corn, protected soybeans, and sulfur supplementation.

2. Materials and Methods

2.1. Experimental diet

The experimental diets consisted of elephant grass, corn, rice bran, pollard, molasses, copra meal, palm kernel meal, soybean, protected soybean, corn gluten meal (CGM), corn gluten feed (CGF), corn distillers dried grains with solubles (DDGS), dicalcium phosphate (DCP), CaCO_3 , and Na_2SO_4 . The forage-to-concentrate ratio in the ration was 50:50 (dry matter basis) and the ratio of RDP:RUP was 60:40. The experimental diets were formulated into five types of dairy rations, namely rations without corn and soybeans (R1), rations with corn without soybeans (R2), rations with corn and soybeans (R3), rations with autoclave protected corn and soybeans (R4), and rations with corn, soybeans protected by autoclave, and supplemented with sulfur (R5). The ration composition and the nutrient content of the rations in 100% dry matter can be seen in Tables 1 and 2.

2.2. In vitro study

In vitro method is used in this study to determine fermentability and digestibility. The method used is a two-stage Tilley and Terry method which refers to Rosmalia et al. [14]. The fermentability level was measured by incubating the sample with rumen fluid (10 ml) and McDougall's saliva buffer (40 ml) for 4 hours. The total bacterial and protozoa populations were calculated using the Ogimoto and Imai method [14]. The supernatant from the incubated sample was used to measure the pH value, ammonia, and total VFA concentration [14]. Digestibility was measured by incubating the sample in rumen fluid for 48 hours. Then the sample was re-incubated in pepsin-HCl enzyme for 48 hours to imitate the

enzymatic process. The residue was filtered to obtain dry matter digestibility and organic matter digestibility.

2.3. Moist heating protected protein method

Five hundred grams of soybean were put in a hollow aluminum container. The top of the aluminum container is closed using aluminum foil given several holes. Next, the hollow aluminum container containing soybean was inserted into the autoclave. The temperature used is 120°C for 60 minutes. After heating, the soybean was left at room temperature and then ground.

Table 1. The ration ingredients used

Feed	R1	R2	R3	R4	R1
	%				
Elephant Grass	50.00	50.00	50.00	50.00	50.00
Corn	0.00	6.25	6.25	6.25	6.25
Rice bran	6.00	3.70	4.70	4.70	3.78
Pollard	9.33	6.91	5.05	5.05	4.80
Molasses	6.00	4.00	4.00	4.00	4.00
Copra meal	8.23	9.15	9.00	9.00	9.15
Palm kernel meal	5.70	6.00	9.00	9.00	9.00
Soybean	0.00	0.00	5.00	0.00	0.00
Protected soybean	0.00	0.00	0.00	5.00	5.00
CGM	2.25	1.79	1.55	1.55	1.55
CGF	6.13	6.30	2.60	2.60	2.60
DDGS	5.28	4.80	1.75	1.75	2.35
DCP	0.10	0.10	0.10	0.10	0.10
CaCO ₃	1.00	1.00	1.00	1.00	1.00
Na ₂ SO ₄	0.00	0.00	0.00	0.00	0.43

R1 = ration without corn and soybeans; R2 = ration of corn without soybeans; R3 = ration of corn and soybeans; R4 = ration of corn and soybean protected by autoclave; R5 = ration of corn and soybean protected by autoclave and sulfur supplementation; CGM= corn gluten meal; CGF= corn gluten feed; DDGS= corn distillers dried grains with solubles; DCP= dicalcium phosphate.

Table 2. The nutrient content of ration tested (%DM)

Feed	R1	R2	R3	R4	R5
	%				
Dry matter	90.96	91.77	91.89	91.36	91.69
Ash	12.15	12.03	12.06	11.37	11.83
Ether extract	2.60	3.29	4.20	3.76	4.15
Crude protein	13.79	13.91	13.28	13.83	14.44
Crude fiber	19.99	20.89	19.39	20.15	19.50
Total digestible nutrient*	59.24	59.04	61.28	60.46	61.73

R1 = ration without corn and soybeans; R2 = ration of corn without soybeans; R3 = ration of corn and soybeans; R4 = ration of corn and soybean protected by autoclave; R5 = ration of corn and soybean protected by autoclave and sulfur supplementation; *according to Sutardi [15].

2.4. Statistical analysis

The experimental design used in this study was a Randomized Block Design consisting of five treatments and four groups. The data were analyzed using the Analysis of Variance (ANOVA) method using SPSS IBM vers. 20 (IBM SPSS Statistics, USA). The significant differences between each treatment were further tested with an orthogonal contrast test. The following orthogonal contrast test was used: R1 vs R2, R2 vs. R3, R3 vs. R4 and R5, R4 vs. R5.

3. Results and Discussion

3.1. *In vitro* fermentation characteristics

The characteristics of ration fermentation *in vitro* can be seen through the rumen pH value and the concentration of the primary fermentation products, such as ammonia and total VFA concentrations. The treatment had no significant effect ($P>0.05$) on pH, but the treatment had a significant effect ($P<0.05$) on the concentration of ammonia and volatile fatty acid (VFA). The effect of treatment rations on rumen fermentability is shown in Table 3.

Table 3. *In vitro* fermentation characteristics

Treatment	pH	NH ₃ (mM)	Total VFA (mM)	Protozoa count (log cell ml ⁻¹)	Rumen Bacteria count (log CFU ml ⁻¹)
R1	6.57 ± 0.21	4.03 ± 0.70 ^c	106.11 ± 12.41 ^b	6.43 ± 0.17	11.83 ± 0.26 ^b
R2	6.49 ± 0.27	4.37 ± 1.04 ^c	102.64 ± 3.56 ^b	6.50 ± 0.07	11.99 ± 0.09 ^b
R3	6.59 ± 0.24	6.41 ± 0.80 ^a	104.44 ± 9.33 ^b	6.39 ± 0.10	11.66 ± 0.09 ^b
R4	6.67 ± 0.30	5.32 ± 0.57 ^b	94.78 ± 7.90 ^b	6.48 ± 0.14	11.52 ± 0.31 ^b
R5	6.68 ± 0.15	5.15 ± 0.82 ^b	116.19 ± 5.75 ^a	6.55 ± 0.09	12.21 ± 0.07 ^a
P-value	0.072	0.013	0.040	0.430	0.023

^{abc}Means in the same column with different superscripts are significantly different ($p<0.05$). R1 = ration without corn and soybeans; R2 = ration of corn without soybeans; R3 = ration of corn and soybeans; R4 = ration of corn and soybean protected by autoclave; R5 = ration of corn and soybean protected by autoclave and sulfur supplementation

The rumen pH value describes the rumen's environmental conditions that affect the fermentation process. Some rumen bacteria cannot grow well if the rumen pH is too low or too high, so the fermentation process is disrupted. Changes in the rumen pH value can be caused by the feed consumed by livestock. The high content of NFC in the feed causes a decrease in the pH value, resulting in a reduction in fiber digestibility [16]. Adding corn with a high NFC content can affect the rumen pH value. According to Huo et al. [17], adding corn in the ration up to 25% can reduce the rumen pH value to 5.93, and adding corn by 50% in the ration can reduce the rumen pH up to 5.65. The decrease in pH value due to the addition of corn in the ration is due to the readily fermentable corn carbohydrates that can increase the number of lactic acid bacteria so that the rumen pH can decrease rapidly [18]. Based on the analysis of variance in this study, the treatment ration had no significant effect ($P>0.05$) on changes in pH values, indicating that the NFC content in the ration is good and does not interfere with the rumen environment. The pH value in this study ranged from 6.49 to 6.68. Based on the research results of Poulsen et al. [18], the pH value of 6-7 did not interfere with rumen microbial activity, meaning that this range was the normal pH range in the rumen.

Ammonia is a product of the fermentation of feed protein. The higher the protein content of the feed, the higher the concentration of ammonia produced. The treatment significantly affected ammonia concentration ($P<0.05$). Based on research by Rosmalia et al. [14], the RDP level in the ration at 60%

resulted in the highest ammonia concentration compared to the lower RDP level. The protein type influences the degradation level of RDP, its amino acid characteristics, and the method is taken from the feed ingredients [19]. Based on the results of the orthogonal contrast further test, the R2 ration with the R3 ration gave a significant difference. The difference between the two rations is in the constituent feed ingredients, soybeans. The ammonia concentration in the R3 treatment had the highest value 6.41 mM due to the easily degradable of soybean. Soybean has an RDP of 86.21% [3]. High RDP in soybeans causes high concentrations of ammonia. The results of the orthogonal contrast further test also showed a significant difference between the R3 treatment and the R4 and R5 treatment. This difference was caused by using autoclave-protected soybeans in the R4 and R5 treatments. Protected soybeans run into a Maillard reaction, and there is a change in the level of degradation in the rumen. Samadi and Yu [3] showed that heating using an autoclave could reduce the RDP value of soybean seeds from 86.21% to 66.35% and change the protein structure in soybean seeds. Ammonia concentration in the R4 and R5 treatments had the same value, meaning sulfur supplementation in the R5 treatment did not affect the ammonia concentration. This is in line with Rosmalia et al. [10] that sulfur supplementation did not affect ammonia concentration. The minimum ammonia concentration to synthesize microbial protein is 3.6 mM [20]. The concentration of ammonia in this study ranged from 4.03 mM to 6.41 mM, meaning that the treatment provided could meet the needs of microbial protein synthesis.

The balance of nitrogen and energy available in the form of NFC in the rumen can maximize rumen microbial activity so that the fermentation product becomes good. The concentration of Volatile Fatty Acid results from carbohydrate fermentation by rumen microbes which can be utilized as host energy. The treatment ration in this study significantly ($P < 0.05$) increased total VFA concentration in the sulfur supplemented ration. Total VFA concentration in R2 treatment decreased compared to R1 treatment due to corn inclusion as an NFC source in the ration. Corn is a feedstuff with a low level of degradation (slow degradation) and can only be degraded in the rumen about 40%-60% [12]. The orthogonal contrast test results showed that total VFA concentration at R3 and R4 treatments were in the same value indicating the inclusion of protected soybean seeds in the ration did not interfere total VFA concentration. In contrast, Wulandari et al. [21] reported that the concentration of VFA will decrease with the length of heating time for feed ingredients due to the Maillard reaction.

The results of the further contrast test also showed a significant effect ($P < 0.05$) on the R4 vs. R5 treatments. The difference between the two rations was in the addition of sulfur supplementation in the R5 treatment. Sulfur is an essential mineral that has a role in rumen microbes' growth and fermentability activity [22]. According to Rosmalia et al. [10], there was an increase in total VFA concentration in the diet supplemented with Na_2SO_4 . When energy was adequate, it will be utilized by rumen microbial to grow and ferment the feed. In addition, sulfur supplementation can improve rumen microbial growth by helping to synthesize sulfur amino acids. Zhao et al. [22] revealed that sulfur supplementation increases total VFA concentration due to the increasing cellulolytic and amylolytic bacteria population. Corn with a high NFC content becomes a direct energy provider for microbes. The addition of corn as a source of NFC and sulfur supplementation increased total VFA concentration due to the high bacterial population. The range of total VFA concentration in this study ranged from 94.78 mM to 119.87 mM, meaning that the VFA concentration in this study was considered normal. This result is also in line with Dieho et al. [23], who stated that the total VFA concentration in forage-based rations and concentrates ranged from 77 mM to 120 mM.

RDP-based rations, NFC from corn, and sulfur supplementation had a significant effect ($P < 0.05$) on the increase in the total bacterial population. Based on the orthogonal contrast test results, there was a significant effect on the increase in the total bacterial population in the R4 ration with the R5 ration. The difference between the two rations is in the mineral sulfur supplementation. Sulfur supplementation increased the number of ruminal bacteria [22]. The increasing rumen bacteria in R4

and R5 treatments were also in line with the increasing total VFA. Zhao et al. [22] reported that Na₂SO₄ supplementation improved the number of *Ruminococcaceae*, *Ruminococcus*, *Butyrivibrio*, and *Pseudobutyrvibrio* bacteria, which led to an increase in total VFA. Sulfur is one of the substrates to form microbial protein, and many *Ruminococcus* bacteria grow on sulfur media [24].

Although there was an increase in the parameters of the total bacterial population, the treatment in this study had no significant effect ($P>0.05$) on the protozoa population. This study's range of protozoa population values ranged from 6.39 to 6.65 ml⁻¹. The number of these protozoa is still in the normal range. The average protozoa population ranges from 5-6 log cell ml⁻¹ [25]. Protozoa act as starch digesters so that bacteria will compete with protozoa for their substrates, but protozoa have slower activity than bacteria [26].

3.2. In vitro digestibility

Dry matter digestibility (DMD) describes the value of all feed nutrients digested by livestock. While organic matter digestibility (OMD) describes all organic matter except minerals in a feed that livestock can digest. The results of dry matter and organic matter digestibility are presented in Table 4.

Table 4. The DMD and OMD value

Treatment	Perlakuan					P- Value
	R1	R2	R3	R4	R5	
DMD (%)	60.73 ± 1.17	62.71 ± 2.05	62.35 ± 1.38	62.08 ± 1.63	62.85 ± 1.78	0.211
OMD (%)	64.01 ± 1.18	65.54 ± 2.36	65.38 ± 1.58	65.06 ± 1.81	65.54 ± 1.97	0.454

^{abc}Means in the same column with different superscripts are significantly different ($p<0.05$). R1 = ration without corn and soybeans; R2 = ration of corn without soybeans; R3 = ration of corn and soybeans; R4 = ration of corn and soybean protected by autoclave; R5 = ration of corn and soybean protected by autoclave and sulfur supplementation

The treatments did not have a significant effect ($P>0.05$) on dry matter digestibility and organic matter digestibility. Afzalzadeh et al. [27] reported the ration containing NFC and RDP did not affect DMD and OMD values. In contrast Rosmalia et al. [14] stated that the NFC affected the DMD and OMD values. The insignificant effect in this study is due to adding protected soybeans in the ration is quite good because it will not affect the decrease in digestibility. However, soybean protection using an autoclave can change the chemical structure of the soybean constituents. Samadi and Yu et al. [3] reported that heating in an autoclave can reduce the level of digestibility of a material.

Soybeans that were protected by autoclave at 120°C for 60 minutes had a digestibility rate of RUP in the small intestine of 89.79% [3]. The high absorption rate of RUP in the small intestine indicates that the protected protein can be well absorbed in the form of amino acids in the small intestine. This indicates that the addition of autoclave-protected soybeans in the diet can be digested well, which is supported by the data in this study that DMD and OMD with protected soybean rations ranged from 62.08-62.85% and 65.06-65.54% compared to the control soybean ration with a dry matter and organic matter digestibility of 62.35% and 65.38%, respectively.

4. Conclusions

It can be concluded that supplementation of sulfur to the corn-NFC, and protected soybeans dairy ration

improved organic matter fermentability by bacterial activity.

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