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# Research Article

# Supplementation of Minerals (Phosphorus and Sulfur) and *Saccharomyces cerevisiae* in a Sheep Diet Based on a Cocoa By-product

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## Abstract

**Objective:** The objective of this research was to determine the productivity of sheep that were fed a complete diet consisting of ammoniated cocoa waste (leaves and pod husks) supplemented with minerals (phosphorus and sulfur) and *Saccharomyces cerevisiae* (*S. cerevisiae*). **Methodology:** Four local sheep aged 10 months to 1 year were used in this study and were divided into 4 feed treatment groups (four sheeps per/treatment) with four replications for each treatment as follows: Treatment A served as the control and sheep were fed a complete diet that consisted of cocoa waste (fiber source)+concentrate, treatment B consisted of treatment A+0.4% phosphorous (P) and 0.3% sulfur (S), treatment C consisted of treatment B+1% *S. cerevisiae* and treatment D consisted of treatment B+2% *S. cerevisiae*. Each treatment had a fiber source: concentrate ratio of 40:60. The data collected included feed intake, nutrient digestibility, average daily weight gain and feed efficiency. **Results:** Treatment C had highly significant effects (p<0.01) on dry matter intake, organic matter intake, crude protein intake, digestibility, crude fiber intake, average daily gain and feed efficiency. Thus, the complete diet consisting of ammoniated cocoa waste increased feed intake, digestibility, daily weight gain and feed efficiency. The use of ammoniated cocoa waste-based complete feed with minerals (P and S) and 1% *S. cerevisiae* yielded 130 g/day of average daily weight gain. **Conclusion:** The diet consisting of ammoniated cocoa waste supplemented with minerals (P and S) and 1% *Saccharomyces cerevisiae* can be effectively used for fattening sheep.

Key words: Cocoa waste, digestibility, *Saccharomyces cerevisiae*, phosphorus, sulfur, sheep, daily gain

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The leaves and pod husks of cocoa plants are plantation by-products that have poor nutritional value due to their high fiber content[1]. This waste is highly available in Indonesia. Indonesia is one of the largest cocoa producers worldwide with a production capacity of 760,429 t from a total area of 1,7222,315 ha[2]. Cocoa has the potential to be used as an alternative to grass feed, which has begun to diminish. Lignin is a polymer compound that binds to cellulose and hemicellulose in plant tissues. High lignin content in cocoa waste limits digestibility[3]. Ammoniation treatment is one way to loosen lingo-cellulose and lingo-hemicellulose that exist in the cocoa waste, thereby making it easier for rumen bacteria to digest fiber and supply nitrogen for growth[4]. Zain[3] reported that ammoniated pod husks changes 100% native grass into sheep rations. The combination of 75% ammoniated cocoa leaves+25% ammoniated cocoa pod husks can be used as a fiber source for ruminant rations[1]. The supplementation of rumen microbial growth factors such as minerals (P and S) and direct-fed microbials is an alternative to improve nutrient digestibility of cocoa waste. Phosphorus is important for the metabolism and maintenance of the cell wall and cell membrane and it is a component of nucleic acids and high energy molecules (ATP, ADP and AMP), sulfur supports amino acid and microbial protein synthesis[5-7]. Supplementation of the minerals P and S into cocoa waste-based ruminant rations increases the digestibility of nutrients *in vitro*[8,9]. Direct-fed microbial products, such as a *S. cerevisiae*, have been used to improve fiber digestibility[10] and animal production[11]. The beneficial effects of these microbial compounds are associated with an increase in cellulolytic bacteria[12] and they have been considered as a potential feed to improve nutrient digestibility in goats[13]. Therefore, the objective of the present study was to determine the productivity of sheep fed a complete diet consisting of ammoniated cocoa waste (leaves and pod husks) supplemented with minerals (phosphorus and sulfur) and *S. cescerevisiae*.

## MATERIALS AND METHODS

This study was conducted using experimental sheep cages at the Laboratory Nutrition of Animal Food Science, Faculty of Animal Science, Andalas University.

**Experimental design:** Four local sheep aged 10 months to 1 year were used in this research. Sheep were divided into feed treatment groups with four replications for each treatment as follows: Treatment A served as the control and consisted of a complete diet of cocoa waste [fiber source+concentrate], treatment B consisted of treatment A+0.4% phosphorous (P) and 0.3% sulfur (S), treatment C consisted of treatment B+1% *Saccharomyces* sp. and treatment D consisted of treatment B+2% *Saccharomyces*. Each treatment had a fiber source:concentrate ratio of 40:60. The composition of feed including the concentrate is shown in Table 1.

Table 1: Ingredient composition and nutrition of experimental diet (% DM)

| | Diet (% DM) | | | |
|---|---|---|---|---|
| Items | A | B | C | D |
| Cacao leaves ammonited | 30 | 29.79 | 29.49 | 29.19 |
| Cacao pod husk ammoniated | 10 | 9.93 | 9.83 | 9.73 |
| Rice brand | 32 | 31.78 | 31.46 | 31.14 |
| Corn | 15 | 14.90 | 14.75 | 14.60 |
| Coconut cake | 10 | 9.93 | 9.83 | 9.73 |
| Salt | 1 | 0.99 | 0.98 | 0.97 |
| Cattle Mix | 2 | 1.99 | 1.97 | 1.95 |
| P | | 0.40 | 0.40 | 0.40 |
| S | | 0.30 | 0.30 | 0.30 |
| *S. cerevisiae* | | | 1 | 2 |
| Total | 100 | 100 | 100 | 100 |
| **Nutrition (%)** | | | | |
| Protein | 12.78 | 12.78 | 12.86 | 12.93 |
| TDN | 63.73 | 63.73 | 63.73 | 63.73 |
| Ether extract | 4.78 | 4.78 | 4.78 | 4.78 |

Digestibility trials were conducted using four animals for each treatment and animals were separated in individual pens. Sheep were fed *ad libitum* during the adaption period (7 days). During the last 14 days of each preliminary period, animals were equipped with fitted bags for the total collection. During the collection period, accurate records were kept for each individual intake. The total fecal excretion was collected once daily and 10% representative samples were dried at 60°C overnight and kept in sealed bags until the analysis. Dry matter, organic matter, crude protein and crude fiber were analyzed by standard AOAC methods[14]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by standard methods according to Van Soest *et al.*[15].

**Statistical analysis:** The data were analyzed using a one-way analysis of variance (ANOVA) for a 4x4 Latin square design and Duncan's Multiple Range Test (DMRT)[16] was used to test the differences between treatments with confidence intervals of 1%.

## RESULTS AND DISCUSSION

The effect of the minerals (P and S) and *S. cerevisiae* on the feed intake, nutrient digestibility of

Table 2: Feed intake of sheep with experimental diet

| | Treatments | | | |
|---|---|---|---|---|
| Parameters (g/day) | A | B | C | D |
| Dry matter intake (DMI) | 436.74$^{a}$ | 497.39$^{b}$ | 574.09$^{c}$ | 555.51$^{c}$ |
| Dry matter intake (% BW) | 2.72$^{a}$ | 3.09$^{b}$ | 3.44$^{c}$ | 3.32$^{bc}$ |
| Organic matter intake (OMI) | 374.71$^{a}$ | 429.05$^{b}$ | 492.75$^{c}$ | 476.82$^{c}$ |
| Crude protein intake (CPI) | 55.52$^{a}$ | 63.57$^{b}$ | 73.00$^{c}$ | 70.64$^{c}$ |

$^{a-c}$Means in the same row with different letters are significantly different (p<0.01)

Table 3: Nutrient digestibility of sheep with experimental diet

| | Treatments | | | |
|---|---|---|---|---|
| Parameters (%) | A | B | C | D |
| Dry matter | 43.01$^{a}$ | 52.68$^{b}$ | 59.75$^{c}$ | 36.65$^{c}$ |
| Organic matter | 44.32$^{a}$ | 53.05$^{b}$ | 62.78$^{c}$ | 44.80$^{c}$ |
| Crude protein | 42.80$^{a}$ | 52.60$^{b}$ | 60.03$^{c}$ | 57.28$^{c}$ |
| Crude fiber | 36.65$^{a}$ | 44.80$^{b}$ | 57.28$^{c}$ | 55.74$^{c}$ |

$^{a-c}$Means in the same row with different letters are significantly different (p<0.01)

Table 4: Average daily gain and feed efficiency of sheep with experimental diet

| | Treatments | | | |
|---|---|---|---|---|
| Parameters | A | B | C | D |
| Average daily gain (g/day) | 75.01$^{a}$ | 100.04$^{b}$ | 130.00$^{c}$ | 125.03$^{c}$ |
| Feed efficiency (%) | 17.02$^{a}$ | 20.53$^{b}$ | 22.64$^{c}$ | 22.52$^{c}$ |

$^{a-c}$Means in the same row with different letters are significantly different (p<0.01)

cocoa waste and average daily weight gain of sheep are presented in Table 2-4.

**Feed intake of ammoniated cocoa waste:** The palatability of a feed material can be accessed from the level of feed intake by livestock. The higher the value of feed intake indicates the ration has good palatability. The statistical analysis showed that treatment C had highly significant effects (p<0.01) on DMI, OMI and CPI, suggesting that supplementation of minerals (P and S) and 1% *S. cerevisiae* with ammoniated cocoa waste can increase the intake of dry matter, organic matter and crude protein. The significant difference (p<0.01) in treatment C was due to increased feed intake resulting from increased digestibility attributed to the combination of mineral supplementation (P and S) and 1% *S. cerevisiae*. This result is consistent with the opinion of Febrina *et al.*[17], who showed that the level of feed intake is influenced by the digestibility of the feed.

The addition of P and S minerals to treatment C increased the feed intake of dry matter rations. Feed intake will increase if the digestibility increases and if the rumen fermentation process is optimal. Increased feed intake in treatment C was due to P and S mineral supplementation causing increased digestibility, resulting in faster rumen content emptying, causing the livestock to consume more quickly. Microbial growth and metabolism in the rumen was optimal because P is an important component for microbial growth[18,19]. The S is an important component of rumen bacteria for microbial protein synthesis because the digestibility of fibrous feed is highly dependent on the enzymes produced by the microbes. According to Komisarczuk and Durand[20], the main function of S is to support the formation of amino acids containing S and microbial protein synthesis and S is also important for the synthesis of some vitamins (thiamin and biotin) and coenzymes. The increasing population of microbes, especially cellulolytic microbes, requires increasing amounts of the resulting enzymes. Increased microbial activity causes optimal fermentation processes to occur in the rumen. For example, Zain *et al.*[5] stated that P and S mineral supplementation in fiber feed rations improves fiber digestibility.

The combination of minerals (P and S) and the addition of 1% *S. cerevisiae* can improve the palatability and digestibility of feed to allow faster emptying of the rumen content, which causes cattle to consume more feed. Yoon and Stern[12] claimed that *S. cerevisiae* in the rumen utilize oxygen to ensure anaerobic conditions for rumen bacteria and to stimulate a population of certain rumen bacteria, which is followed by increased utilization of ammonia and lactic acid, resulting in a stable pH of the rumen. Anaerobic conditions and stable pH in the rumen allow for better microbial protein synthesis, which increases the total population of rumen bacteria and the digestibility of crude fiber. Increased digestibility of crude fiber also increases feed intake and the supply of nutrients to the intestines, which ultimately improves the overall production response. For example, Paryad and Rashidi[13] demonstrated that digestibility of goat livestock rations supplemented with *S. cerevisiae* is higher than non-supplemented rations and Dann *et al.*[21] reported an increase in the intake of dry matter ration in dairy cattle due to the addition of *S. cerevisiae*.

Treatment D was not significantly different from treatment C but was lower than treatment C, suggesting that addition of 1% *S. cerevisiae* of the dry matter (DM) ration was optimum for the growth of rumen microbes. This result is due to the fact that the more *S. cerevisiae* inoculum is added, the more nutrients are required. When nutrients are not sufficient, then *S. cerevisiae* cannot multiply, thus inhibiting its metabolic activity and resulting in the death of *S. cerevisiae*. This is in accordance with research conducted by Sukma *et al.*[22], who found a decrease in the fermentation of *Aspergilus terreus* A with increasing doses of inoculum.

Table 2 shows that the feed intake of organic matter has a positive correlation with the feed intake of dry matter, which is due to the fact that substances contained in organic matter are part of the dry matter. The amount of dry matter intake will affect the intake of organic matter. Thus, increased dry matter intake results in increased feed intake of organic material and vice versa. The mean feed intake of crude protein in this study ranged from 55.22-73.00 g/day, showing that it met the standard estimate of the adequacy requirement of crude protein based on body weight to achieve a daily body weight gain of 100-56-58 g/day[23]. Thus, protein feed intake in this study is sufficient for basic living needs and growth because protein feed intake is closely related to the increase in body weight of livestock. The value of rough protein intake in treatment C (73.00 g day$^{-1}$) in this study was similar to that of Suparjo *et al.*[23], who used cocoa pod-based rations fermented with *Phytophthora palmivora* bacteria and reported a protein intake of 72 g/day.

**Nutrient digestibility of ammoniated cocoa waste:** The digestibility of food substances from the ration determines the quality of the ration because a higher digestibility rate of a ration means the ration is a good quality for livestock feed intake and is used for the metabolism process of the body. Table 3 shows that the highest digestibility values of dry matter, organic matter, crude protein and crude fiber ($p<0.01$) were present in treatment C. The addition of P and S minerals to treatment C enhanced digestibility. Microbial growth and metabolism in the rumen were optimal because P is an enzyme activator and is involved in reactions related to energy liberation to form ATP. Jamarun *et al.*[19] stated that microbial populations and microbial protein synthesis will increase with the addition of mineral doses of P. S is an important component for rumen bacteria for microbial protein synthesis because the digestibility of fiber feed is highly dependent on the enzymes produced by these microbes. Large microbial populations, especially cellulolytic microbes, can produce more enzymes. According to Komisarczuk and Durand[20], the main function of S is to support the formation of amino acids containing S and microbial protein synthesis and S is also important for the synthesis of some vitamins (thiamin and biotin) and coenzymes. Increased microbial activity causes an optimal fermentation process in the rumen, which is in line with research conducted by Jamarun *et al.*[24], who reported that *in vitro* P supplementation enhanced the digestibility of fiber fractions (NDF, ADF, cellulose and hemicellulose) in fermented palm fronds. Additionally, a study conducted by Zain *et al.*[7] proved that the addition of S minerals is able to improve the digestibility of the amber rice straw.

The protein digestibility value is related to the condition of rumen fluid populations, especially the proteolytic population. The proteolytic proportions of each treatment were also different. The presence of a combination of P and S minerals improved the activity of proteolytic bacteria. The combination of 0.4% P and 0.3% S supplementation in ammoniated cocoa waste has also been shown to increase the digestibility of dry matter, organic matter and crude protein *in vitro*[9]. Supplementation of P and S minerals is indispensable because the feed ingredients from agricultural/plantation waste in Indonesia are deficient for these important minerals and their supplementation produces positive results in cattle performance[5].

*Saccharomyces cerevisiae* reduces methane production by increasing the digestibility of organic matter. Carbohydrates are the most influential component of organic matter in the determination of organic matter digestibility because carbohydrates as energy producers (volatile fatty acid) are the largest components in feed. By increasing the proportion of propionate, *S. cerevisiae* can reduce methane. This result is consistent with the results of a study by Lync and Martin[25], who found increased propionate production, thereby reducing methane production in yeast-supplemented rations. Propionate is a glucogenic VFA because it can be catabolized into glucose, whereas acetate and butyrate are non-glucogenic (ketogenic) VFAs. The rumen fermentation system that leads to propionate will result in lower methane production, resulting in more VFA produced as a major energy source for ruminants and for the synthesis of microbial proteins[26]. Low methane production will increase the value of hexose conversion as less energy is wasted in methane. This is in accordance with the opinion of Krehbiel *et al.*[27], who stated that the rumen fermentation system that leads to the synthesis of propionic acid is more advantageous because the energy wasted as methane gas is reduced.

**Average daily gain and feed efficiency of ammoniated cocoa waste:** The increase in body weight is a benchmark of growth rate. Table 4 shows the average daily weight gain for the experimental animals ranging from 75.01-130 g. The highest daily weight gain ($p<0.01$) was in treatment C. The body weight increases with increasing digestibility due to increasing intake of dry matter, which directly affects the intake of organic matter and the digestibility of food substances. Differences in daily weight gain associated with digestibility and the amount of digestible food substances are further utilized for the growth of body tissues. Growth is related to the feed consumed because the level of feed intake directly affects muscle growth, especially crude protein and energy. Increased daily gain in treatment C was due to mineral supplementation (P and S) and *S. cerevisiae*. This supplementation increased the growth of rumen microbes, especially cellulolytic bacteria.

The P minerals are required for the degradation of cell wall fractions and the cellulolytic bacteria requirement of these P minerals is higher than hemicellulolytic or amylolytic bacteria[6]. S is indispensable in rumen microbes for the formation of amino acids containing sulfur. According to Komisarczuk and Durand[20], the main function of S is to support the formation of amino acids containing S and microbial protein synthesis and it is also important for the synthesis of some vitamins (thiamin and biotin) and coenzymes. Metabolic processes involving growth, weight gain and enzyme or hormone activity are largely determined by the availability of the essential amino acid methionine. Methionine is an amino acid containing S.

Increasing the microbial population by P and S mineral supplementation causes increased digestibility and nutrient availability for growth, which also increases the body weight gain of livestock. This is consistent with Febrina *et al.*[17,18], who reported that the addition of minerals both *in vivo* and *in vitro* has a positive effect on rumen microbial activity. In the case of mineral deficiency status, the microbial fermentation activity in the rumen does not optimally occur, which will lead to low ration feeding efficiency and will eventually decrease the growth of livestock.

*Saccharomyces cerevisiae* stimulates the growth of rumen bacteria, especially cellulolytic bacteria, which increases digestibility and feed intake. Callaway and Martin[28] demonstrated that the increased feed intake of dry matter and ration organic ingredients also increases the feed intake of nutrients, which increases the nutrients available in the body and eventually increases weight gain. Wallace and Newbold[29] suggested that one of the benefits of direct-fed microbials to ruminants, especially in adult cattle, is to increase meat production.

Feed efficiency is influenced by feed intake of dry matter/day and weight gain/day. Table 4 shows that the average feed efficiency ranges from 17-22.65%. The highest ration efficiency was found in treatment C (22.64%) and the lowest efficiency was found in treatment A (17.02%). A ration will be more efficiently used if the ration is consumed in small quantities and is able to provide large daily body weight replacement. The average daily weight gain for treatment C (130 g/day) was attributed to the protein intake that is sufficient for livestock needs for basic life and growth. The mean protein feed intake during the study ranged from 55.22-73.00 g/day. The amount of protein intake met the estimated raw protein adequacy standard based on body weight to reach 100 g/day. For example, Suparjo *et al.*[23] fed fermented cocoa pods to goats and these rations were 56-58 g day$^{-1}$ of protein feed intake to reach the 100 g day$^{-1}$ body weight.

The ration efficiency value in this study was higher than that in the study by Suparjo[30], who obtained a ration efficiency value of 18% for a fermented pod-based diet fed to goats. The ration efficiency in the present study was better in the treatment with ammonia cocoa waste supplemented with *S. cerevisiae* and minerals (P and S) because the *Saccharomyces cerevisiae* supplementation improved the level of feed intake and digestibility of food substances, resulting in better livestock growth.

## CONCLUSION

According to the results of this experiment, the supplementation of minerals (P and S) and *S. cerevisiae* into ammoniated cocoa waste-based rations could improve feed intake, nutrient digestibility and sheep performance.

## SIGNIFICANCE STATEMENT

This study demonstrates that supplementation with direct-fed microbials, namely, *S. cerevisiae* and minerals (P and S) can increase the growth performance, nutrient digestibility and feed efficiency of sheep. This study will enable researchers to further investigate the effects of *S. cerevisiae* and the minerals P and S on sheep that are fed an ammoniated cocoa waste-based diet, which was previously unexplored. Thus, a new hypothesis regarding the effects of supplementation with *S. cerevisiae* and the minerals P and S may be developed.

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