The effect of fermented cocoa pod (Theobroma cacao) husk supplemented with mineral on in vitro digestibility, rumen bacteria population and rumen liquid characteristics

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Abstract. This study aimed to determine the effect of mineral supplementation, such as S, P and Zn on the nutrients digestibility of fermented cocoa pod husk, the population of rumen bacteria and rumen liquid characteristics in vitro. The study used a randomized block design with 5 treatments and 4 replicates. The treatments tested were: T0 = without minerals; T1 = 0.2% S mineral; T2 = 0.27% P mineral; T3 = S and P; and T4 = S, P and Zn at 50 ppm. Parameters measured were: (1) digestibility of dry matter and organic matter; (2) rumen bacterial and cellulolytic bacterial populations; (3) characteristics of rumen liquid in vitro. The results of the study showed that mineral supplementation significantly (P <0.05) improved dry matter and organic matter digestibility. Mineral supplementation had no effect on the total population of rumen bacteria and cellulolytic rumen bacterial populations. The characteristics of rumen liquid such pH, VFA and NH₃ were in optimal condition. In conclusion supplementation of S, P and Zn simultaneously gave the best results to improve the digestibility of dry matter and organic matter and to maintain rumen liquid characteristics under optimal conditions for growth and microbial activity

1. Introduction

Cocoa (Theobroma cacao) is primarily grown for its bean to be further processed by the chocolate industry. The bean itself is located inside the cocoa fruit. When the bean is removed from its fruit, the leftover is called the pod and may constitute about 75% of the weight of the whole fruit [1]. Although other by-products from cocoa processing such as cocoa shell and cocoa dust exist and may also be used as animal feeds, the pod is the predominant cocoa by-product [2].

Indonesia is the second largest producer of cocoa in terms of total production after the Ivory Coast. Therefore, quite an amount of cocoa by-product is available in the country, especially in the areas of cocoa plantations or where cocoa processing plants are located. Unfortunately, despite its abundant quantity, so far cocoa pod has not been optimally utilized by farmers. Usually the pod is left to rot on the plantation area or is used as mulching material. If the cocoa pod could be fed to animals, its economic value would be improved [3]. In general, a main obstacle of utilizing cocoa pod as an animal feed is its high fiber and low protein contents. It also contains a considerable amount of lignin,
i.e. between 12% to 19% dry matter (DM), and such value is 2 to 3 times higher than that of rice straw [4]. Studies using high inclusion levels of untreated cocoa pod in diets have resulted in lower digestibility and animal performance [5], confirming its low nutritional quality. Two main nutritional strategies have been proposed to overcome such limitation of cocoa pod, i.e. either by mixing with a more fermentable or digestible feedstuff [6].

Pod cocoa fermentation with local microorganisms of rumen contents proved to increase dry matter digestibility of 30.07% [7], but still needed mineral supplementation to improve rumen bacterial populations to digest higher. S and P minerals are essential for rumen microbes digesting fiber. S mineral needs from 0.14 to 0.26% (average 0.2%) and P 0.16 to 0.38% (average 0.27%) of dry matter [8]. The mineral is often deficient in feed materials derived from agricultural waste, and become a limiting factor for the growth of rumen microbes. This is due to the feed in the tropics and feed derived from agricultural waste are often deficient in essential minerals for the growth of microbes, such as S and P [9]. In addition biolavability of mineral is also low. This will negatively affect the synthesis of microbial protein and digestibility of nutrients. S and P supplementation on palm leaves ammoniation improve in vitro digestibility of 36.68% from 34.67% to 47.39% [10]. Furthermore, the in vivo digestibility of ammoniation palm leaf supplemented with mineral S and P was increased from 51.5% to 56.0% in sheep [11]. In cattle, supplementation of S and P on ammoniation palm oil frond improve feed digestibility, and give similar body weight gain to natural grass [12]. The present study was conducted to investigate the effect of supplementation of S, P and Zn in fermented cocoa pod on dry matter, organic matter digestibility, rumen bacterial populations and characteristics of rumen fluid in vitro.

2. Materials and Methods
This research material was fermented cocoa pod, sulfur as a source of S, TSP fertilizer as a source of mineral P, and ZnCl2 as a source of Zn, Mc Doughall’s solution as a buffer and as a donor microbial rumen fluid. The tools used are in-vitro devices, digital pH meter to measure the pH of rumen fluid, and a set of laboratory equipment for analysis Proximate Van Soest, VFA and NH3-N. The study used a randomized block design with 5 treatments and 4 replicates. The treatments were supplementation of S, P and Zn in fermented cocoa pod as follows: T0 = without mineral supplementation (control); T1 = 0.2% S mineral supplementation; T2 = supplementation of P at 0.27%; T3 = supplementation of S and P; and T4 = supplementation of S, P and Zn 50ppm. The amount of supplementation of S and P were calculated based on the content of S and P in the material so that the amount be the same as the standard [8], whereas Zn supplementation of 50 mg/kg ration [13]. Data were analyzed using analysis of variance according to R G D Steel, and J H Torrie [14]. The difference between treatments was tested by Duncan's Multiple Range Test (DMRT).

2.1. Laboratory analysis
This study used an in vitro method as described by J M A Tilley and Terry[15]. Samples of fermented cocoa pod before used they were dried and finely ground, as well as sulfur and TSP. They were inserted into the erlenmeyer and added minerals in accordance with each treatment, and mineral and sample mix evenly. They then were given the buffer solution Mc Doughall's and rumen fluid with a ratio of 4: 1, the CO2 gas flowed for 30 seconds so that the anaerobic conditions and incubated for 2x24 hours in a water bath shaker. After fermentation ends, all the samples were centrifuged at 1200 rpm for 15 minutes to separate the filtrate and precipitate. The filtrate was used for the measurement of rumen fluid characteristics (pH, NH3-N and VFA) and analysis of rumen bacteria, whereas in vitro fermented esidues were dried to analyze content of nutrients. Parameter observed was the digestibility of dry matter and organic matter, the total population of rumen bacteria and cellulolytic rumen bacterial populations, and the characteristics of rumen fluid such as pH, levels of VFA and steam distillation, and the content of NH3-N.
3. Results and Discussion

Effect of mineral supplementation on dry matter (DM) and organic matter digestibility of fermented cocoa pod are presented in Table 1.

### Table 1. Dry matter and organic matter digestibility

| Treatments | DM (%) | OM (%)  |
|------------|--------|---------|
| T₀         | 28.62ᵃ | 23.88ᵃ  |
| T₁         | 30.23ᵇ| 27.09ᵇ  |
| T₂         | 29.29ᵃ | 25.11ᵇ  |
| T₃         | 29.06ᵃ | 26.26ᵇ  |
| T₄         | 30.52ᵇ | 27.04ᵇ  |

Notes: Different superscripts in the same column shows significantly different (P<0.05)

Data on Table 1 showed that the mineral supplementation significantly affect digestibility of dry matter and organic matter of fermented cocoa pod. Dry matter ranged from 28.62% to 30.52% and organic matter digestibility ranged from 23.88% to 27.04%. Supplementation of S, P plus Zn also showed the highest dry matter and organic matter contents. S supplementation improved the digestibility of dry matter and organic matter cocoa pod. This may be due to the low S content (0.007%) of the cocoa pod, which is far below the needs of S (0.14 to 0.26%) [16]. Therefore, S supplementation as much as 0.2% gives a positive response to microbial activity resulting in better digestibility. P supplementation had no effect on dry matter and organic matter of fermented cocoa pod. This may be due to the P content of fermented cocoa pod at 0.67% has exceeded the standard requirements, namely 0.18% - 0.36% [16]. The higher digestibility of feed means greater benefit in supporting the productivity of livestock. Nutrient digestibility in this study is only around 28.62% - 30.52% which indicated that the fermented cocoa pod is a low quality feed. Nutrients contained in the material is not entirely available for ruminants. Feed quality is determined by the digestibility of nutrients of these materials, which describes what percentage of substances that are digested and what percent is excreted through feces [11].

3.1 Bacterial Population and Cellulolitic Bacteria

The total population of bacteria ranged from (log) 4.66 to 6.15, while the population of cellulolytic bacteria ranged from (log) 4.81 to 5.79. S, P, and Zn supplementation had no significant effect (P>0.05) on the total population of bacteria and cellulolytic rumen bacteria (Table 2).

Bacteria play an important role in the process of fermentative digestion of feed in the rumen. Bacteria population in the rumen ranged from 10⁹ to 10¹² per ml of rumen fluid. To obtain a higher digestibility of low quality wastes, an increase in bacterial population should be pursued through the provision of nutrient precursor for rumen microbial growth. S is essential for fiber digestion in the rumen, and adequate sulfur supply will optimize the digestibility of cellulose through the stimulation of specific cellulolytic bacteria and the activity of ciliate protozoa and fungi in rumen [9]. P needed by rumen microorganisms for cellulose digestion, but it is not easy to prove that P can stimulate the production of VFA [17]. P is specifically required for cell wall digestibility as main elements, especially for selulolisis requiring higher than for hemiselulolisis and amilolisis. In most in-vivo study, deficiency of P showed a negative effect on the digestibility of the fiber fraction and the ability to digest organic matter [9].
### Table 2. Total Bacterial Population and Cellulolitic bacteria

| Treatments | Total Bacteria (log) | Cellulolitic Bacteria (log) |
|------------|----------------------|-----------------------------|
| T<sub>0</sub> | 6.15                 | 5.38                        |
| T<sub>1</sub> | 5.84                 | 5.58                        |
| T<sub>2</sub> | 4.66                 | 4.81                        |
| T<sub>3</sub> | 5.97                 | 5.48                        |
| T<sub>4</sub> | 5.92                 | 5.79                        |
| SE         | 0.038                | 0.035                       |

3.2. Rumen Fluid Characteristics

Characteristics of rumen fluid is generally indicated optimum rumen conditions for microbial growth and activity. This condition is a necessary condition that must be met for better support high livestock production. Three main factors can be used as criteria in assessing the condition of rumen i.e., pH, total VFA and NH3 concentration rumen fluid. The results of measurements of pH, total VFA and NH3 concentrations in this study can be seen in Table 3.

### Table 3. Rumen Fluid Characteristics.

| Treatments | pH     | VFA (mM) | NH<sub>3</sub>-N (mg/100ml) |
|------------|--------|----------|----------------------------|
| T<sub>0</sub> | 7.18<sup>b</sup> | 85.01<sup>*</sup> | 5.16<sup>a</sup> |
| T<sub>1</sub> | 7.00<sup>ab</sup> | 106.28<sup>ab</sup> | 6.30<sup>b</sup> |
| T<sub>2</sub> | 7.08<sup>ab</sup> | 96.90<sup>ab</sup> | 6.79<sup>b</sup> |
| T<sub>3</sub> | 7.00<sup>a</sup> | 99.23<sup>ab</sup> | 6.15<sup>b</sup> |
| T<sub>4</sub> | 6.78<sup>a</sup> | 115.52<sup>b</sup> | 6.50<sup>b</sup> |

Notes: Different superscripts in the same column shows significantly different (P<0.05)

Results of analysis of variance in Table 3 showed that the mineral supplementation significantly (P<0.05) on the parameters of rumen fluid characteristics. Rumen fluid pH values obtained ranged from 6.78 to 7.18. Near-neutral pH value is obtained for the use of artificial saliva as a buffer capable of maintaining stability conditions of the rumen fermentation activity influence. This is in accordance with D C Church [17], who reported that saliva acts as a buffer to maintain the stability of rumen fluid. High or low pH of rumen fluid is determined by the type of food, the time available for the rumen microbes to ferment food ingredients, buffers and system capacity levels and total VFA NH3 rumen fluid, so that the rumen pH can vary in the time before and after meals. The pH value obtained in this study are in optimal conditions to support microbial growth and activities. Our findings in accordance with R A Erdman [18] and E R Orskov and M Ryle [19], they reported that rumen pH range of 6.3 to 7.0 can assure the growth and activity of rumen microbes. If the rumen pH below 6.2 then the cellulolitic microbial life will be disrupted and the digestibility of the fiber will decrease. Production of total VFA in this study ranged from 85.01 to 115.52 mM. Statistical analysis showed that the effect of different treatments were highly significant (P <0.01) of total VFA. DMRT highest VFA concentration 115.52 mM obtained at SP + Zn supplementation treatment. The high production VFA at SP + Zn supplementation treatment is in line with the high dry matter digestibility. It is easy to understand, because the VFA is the end product of carbohydrate fermentation by rumen microbes so that the increase in the digestibility will lead to an increase in the final product, i.e., VFA.

H L Davies [20] suggested that elevated levels of VFA reflects the increased solubility soluble carbohydrate feed. VFA ruminants have a dual role as a source of energy for livestock and a source of carbon skeletons for the growth of microbial protein [21]. VFA is a dynamic element that amount depends on the material fermentability, absorption in the rumen wall and utilization by rumen microbes [22]. VFA production is highly dependent on a variety of carbohydrates contained in a
material. Increased production obtained in this study could support microbial growth and activity. This is in accordance with P J Van Soest [24], who reported that total VFA needed for the growth and activity of microbial range of 80-160 mM. NH3 is the result of the digestibility of protein or NPN compounds by rumen microbes. The concentration of NH3 obtained in this study ranged from 5.16 to 6.79mg/100 ml rumen fluid. Consentration of NH3 rumen fluid is strongly influenced by the type of food, solubility of nitrogen and the level of digestibility of protein, concentration of nitrogen ration, time after feeding, rate of use of nitrogen for microbial biomass rumen, absorption of NH3-N or recycling of urea and nitrogen rumen bacteria [3]. The concentration level of NH3-N in this study was at S supplementation, supplementation of P, SP, and SP + Zn was not significantly different. Overall concentrations of NH3-N in this study have been indicated to sufficient for NH3-N for growth of rumen microbes. This findings is supported by L D Satter and L L Slyter [25], they reported that the concentration of NH3-N the rumen varied between 0-130mg/100 ml rumen fluid, whereas the minimum levels for rumen microbial protein synthesis that is optimal is 5 mg / 100 ml rumen fluid.

4. Conclusions
Supplementation of S, P and Zn simultaneously on fermented cocoa pod improved the digestibility of nutrients in vitro, but it did not affect the population of bacteria and cellulolytic bacteria in rumen. The mineral supplementation maintained rumen fluid characteristics under optimal conditions for growth and microbial activity.

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