Utilization of Fermented Cocoa Pod Husk (CPH) as Feed Ingredient for Sheep

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ABSTRACT
The experiment of sheep feeding with concentrate contained fermented cocoa pod husk (FCPH) has been conducted at the IRIAP research station. The experiment aimed to study and evaluate FCPH product for sheep. Fermentation of cocoa pod husk using Aspergillus oryzae was carried out for three days. The FCPH product was dried and ground. A feeding trial experiment was performed on weaned rams with 6 replications (6 heads) / treatment for 2 weeks of adaptation and 12 weeks of observation. The basal diet was chopped king grass, and the treatments applied as follows: Control (C: king grass+commercial concentrate), C20 (20% commercial concentrate was replaced with 20% FCPH), and C40 (40% commercial concentrate was replaced with 40% FCPH). The crude protein content of FCPH was higher than CPH (15.94 vs 6.71%). Replacement of commercial concentrate with 20 and 40% of FCPH increased protein digestibility from 45.32 (C) to 55.35 (C20) and 60.25 (C40), NDF digestibility was 50.14, 57.16, and 67.19%, for C, C20, and C40, respectively. The average dry matter consumption ranged from 600-900 g/head/day. The feed conversion ratio (FCR) at the adaptation week was very high (21-23.6). Moreover, the FCR was better during the observation period with a value of 14.02, 9.56, and 8.4 for C, C20, and C40, respectively. Bodyweight gain for C0, C20, and C40 was 65.80, 80.64, and 85 g/head/day. Rumen chemical characteristics were as follows: rumen ammonia in C40 was the lowest compared to C and C20. Meanwhile, the rumen pH ranged between 5.88 and 6.05. Acetic acid dominated the rumen environment, the level was 28.68% (C20), 31.37% (C), and 47.38% (C40). The content of VFA also indicated normal rumen digestive conditions. The biological processing of CPH increased the digestibility of protein and neutral detergent fibre. Cocoa pod husk fermented using Aspergillus oryzae can be used to replace commercial concentrates as much as 20 and 40% without causing a negative effect.

Keywords: Cocoa pod husk, Aspergillus oryzae, sheep, feeding

1. INTRODUCTION
Feed accounts for 65–75% of the total cost, and feed quality in the livestock business is essential, and its availability is not always met. For Indonesia, most of the feed ingredients (corn, soybeans) are still dependent on imports. As an agricultural country, Indonesia develops variety of agricultural/plantation commodities such as palm oil and cocoa. Indonesia is one of the top 5 cocoa-producing countries in the world and stay in the 3rd rank [1]. The overall annual production is 849,875 tons, with the majority coming from Sulawesi, and South Sulawesi alone produces 70% of Indonesia’s total cocoa production [2]. The proportion of waste by-products was 60% or 509,925 tons, equivalent to 152,977 tons of dry matter, which can accommodate around 115 thousand livestock units per year. Processing of cacao pod husk (CPH) into silage (North Sumatra) and fermentation using Aspergillus niger (South and Southeast Sulawesi, and Bali) and other biological agents (microbial) has been carried out. However, complete information on the characteristics of the products has not been available.

Several researchers have used various fungi for the CPH fermentation process, namely: Aspergillus niger, Rhizopus oligosporus and Trichoderma reesei [3], A. niger, Pestalotiopsis guepinii, Rhizopus oligosporus and Mucor circinelloides [4] [5], Phanerochaete chrysosporium [6], and Aspergillus sp [7]. CPH
fermentation using A. niger can reduce crude fibre content by 5.5% [8], and coconut cake fermentation using A. niger can reduce crude fibre content by 4.91% [5]. Fermentation of CPH showed an increase in protein content of 9% to 12% after fermentation with A. niger [3] and 13.38% [8]. Fermentation of CPH using A. oryzae can produce fermented CPH products with protein content reached 15.00% [8]. In this article, the product of A. oryzae fermented cocoa pod husk (FCPH) was used to replace 20 to 40% of feed concentrate in sheep feeding and evaluate the benefits of FCPH as a component of sheep feed concentrate.

2. MATERIALS AND METHODS

2.1. Materials

The fungus A. oryzae, was from the Balitnak culture collection. Cocoa pod husk (CPH) was collected from a plantation in West Bandung. Other chemicals purchased from the respected supplier were either pro-analysis grade or technical grade.

2.2. Methods

2.2.1. General methods

A. oryzae powder form was made according to previous study [9]. The mold was inoculated into rice media and incubated for 3 days. The spores formed were dried at 40°C and ground. The spore powder was added to the rice media and incubated for 3 days. The spores formed were ground into a powder. The mold was grown under laboratory conditions, where the mold was grown in rice media for 3 days. The mold was then ground into a powder.

Cocoa pod husk (CPH) was chopped, sun-dried, and then ground. The dried substrate was added water up to 60% water content and then steamed for 30 minutes, stand to lukewarm, then a mineral mixture (consisting of 1% ZA, 0.5% urea and other macro minerals) was added. Fermentation lasted for 3 days with a substrate thickness of 1-2 cm on plastic trays. Laboratory analysis includes analysis of chemical composition (Proximate analysis: Dry Matter, Crude Protein, Crude Fibre, Fat, Ash, NDF, ADF, lignin), in vitro and in sacco digestibility.

Experiments on livestock using 3 ration treatments as follows: C0 (Control: King grass + feed concentrate); C20 (King grass + 80% Feed Concentrate + 20% FCPH) and C40 (King grass + 60% Feed Concentrate + 40% FCPH). In this study, weaning rams were used with 6 replications (6 heads) per treatment.

2.2.2. In sacco and in vitro pepsin Digestibility

The in sacco test was carried out to determine the level of FCPH degradability in the rumen as a source of protein for ruminant feed. Sheep with rumen fistula were given basic feed sufficient for basal living needs (50% grass and 50% concentrate). Feeding the sheep was done 2 times a day in the morning and evening. Five grams of the sample was put into a nylon bag that was surface-tied and weighted. The bags were incubated in the rumen for 0, 2, 4, 8, 12, 24, 48, 72 and 96 hours. After going through the specified incubation period, the nylon bag was washed with running water until it was clean and dried in an oven (50-60°C) so that a constant weight was obtained. Protein and fiber were analyzed and digestibility was calculated.

Furthermore, protein digestibility by pepsin was also tested as a measurement of post-rumen digestibility. Post-rumen crude protein digestibility (in vitro) was tested by measuring the sensitivity of the feed to the pepsin enzyme under acidic conditions [10, 11]. Weighed as much as 2 g of the remaining sample of the in sacco digestibility residue for 24 hours and put into a fermentation tube. Then 20 ml of 0.2% pepsin in 0.1 M HCl was added to the tube and incubated at 39°C for 24 hours. After 24 hours of incubation, it was filtered using a sintered glass filter (G2). The remaining feed was analyzed for crude protein content.

2.2.3. Feeding trial

All sheep were placed in individual cages of 20 stilts for easier observation. Placement of livestock in cage plots was carried out randomly according to the type of feed as treatment. Prior to the research, the sheep were given time to adapt to the cage environment and feed for 14 days with experimental rations. All sheep were dewormed using Kalbuzen during the adaptation period. Prior to the study, all animals were weighed live to obtain initial weight data.

During the study, the amount of concentrate feed was 2% of live weight based on dry matter (NRC, 1975). The concentrate was given before the grass. The remaining concentrate and grass were weighed the next day to determine consumption. Drinking water was available continuously in the cage. Live body weight during the study was carried out every week, in the morning before feeding and drinking water. The experiment was carried out for 3 months.

The experiment was carried out using a completely randomized design.

3. RESULTS AND DISCUSSION

3.1. Fermentation

The growth of A. oryzae on days 2 and 3 can be seen in Figure 1. The CPH substrate was filled with A. oryzae mycelia. At this stage, the mold has not yet started to form spores and was the highest protein content phase. Chemical composition analysis (Proximate analysis: dry matter, Crude protein and fibre, fat, ash, NDF, ADF, and lignin) of CPH and FCPH showed in Table 1. Crude protein content increased from 6.71% before
fermentation to 15.94% after fermentation. The nitrogen content was equivalent to 2.55%, while the addition of nitrogen from ZA (1%) and Urea (0.5%) contributes an additional 0.45% of nitrogen so that the net nitrogen content in FCPH was 2.1% or equivalent to 13.16% protein and an increase of 196.11%. Another result was the fat content decreased by 22.03%; from 2.36 to 1.84%.

3.2. In vitro and in sacco digestibility

The highest dry matter loss in sacco occurred in FCPH, which was 200-360 g/kg. Meanwhile, CPH without fermentation was 100-300 g/kg of material.

Table 1. Proximate analysis of CPH and FCPH

| Component       | CPH | FCPH |
|-----------------|-----|------|
| Moisture (%)    | 9.88| 5.4  |
| Crude Protein (%)| 6.71| 15.94|
| Fat (%)         | 2.36| 1.84 |
| Gross Energy (kcal/kg) | 3814 | 3983 |
| Crude fibre (%) | 42.71| 48.39|
| Ash (%)         | 10.19| 11.25|
| Ca (%)          | 0.49 | 0.5  |
| P (%)           | 0.1  | 0.11 |
| NDF (%)         | 68.63| 79.09|
| ADF (%)         | 64.58| 71.36|
| Lignin (%)      | 30.7 | 35.36|

Meanwhile, it was reported that digestibility in sacco with an incubation period of 48 hours for rice straw, *Acacia auriculiformis*, sugarcane shoots, and *Acacia mangium* were 25, 30,30 and 32%, respectively [12]. Dry matter digestibility in sacco CPH, FCPH in 48 hours incubation were 25.4 and 35.11% (Figure 2), equivalent to the above ingredients except for FCPH (35.11%), which was higher than *Acacia* sp and sugarcane tops.

The highest loss of protein content or protein digestibility in sacco occurred in KBCFAn while KBCFAo was the lowest compared to KBC and KBCFAn. The low digestibility of protein in sacco allowed digestion in the post-rumen digestive system. Such conditions are desirable so that with the help of enzymes in the intestine, protein can be digested into amino acids and can be absorbed by livestock (Figure 3).

![Figure 1. FCPH fermentation day 2 (above) and day 3 (below)](image1.png)

![Figure 2. Dry matter loss in sacco of FCPH (upper line) and CPH (lower line)](image2.png)
The digestibility of protein and in vitro NDF (rumen-pepsin) of feed ingredients and rations can be seen in table 5. Replacement of concentrate with FCPH 20 and 40% increased protein digestibility from 55.32 to 60.25% and NDF from 57.16 to 67.19%. It was confirmed that FCPH contains fibre hydrolase enzymes, including mannanase (1700 U/g), and contribute to increase DM loss in FCPH.

### 3.3. Feeding trial

Feeding trial shown no difficulty in sheep accepting feed concentrate containing 20% and 40% FCPH.

Nutrient content of feed at the beginning and end of the study especially protein content, varied between 15 and 18%. Protein content at the beginning of experiment was 17.91 (C0); 16.27 (C20) and 16.70% (C40) and 15.58 (C0), 15.59 (C20) and 14.12% (C40) at the end of the experiment. Replacement of concentrate with 20 and 40% of fermented cocoa pod husk increased protein’s digestibility from 55.32 to 60.25% and NDF of 57.16 to 67.19%.

### Table 2. In vitro digestibility of protein and NDF of feed materials

| Materials | Digestibility  |
|-----------|----------------|
|           | Protein (%)    | NDF (%)      |
| CPH       | 48.96 ± 1.40   | 49.50 ± 0.76 |
| FCPH      | 64.35 ± 0.55   | 61.83 ± 0.76 |
| King Grass| 70.02 ± 0.60   | 66.99 ± 1.74 |
| K0        | 55.32 ± 0.72   | 57.16 ± 0.83 |
| C20       | 59.67 ± 1.02   | 66.09 ± 3.60 |
| C40       | 60.25 ± 1.09   | 67.19 ± 1.49 |

Daily live weight gain followed the pattern of the 3rd order polynomial equation. In comparison, the analysis of variance on live body weight was not significantly different compared with control. Daily live weight gain ranged between 65-85 g/head/day. Dry matter intake ranged between 600-900 g/head/day). Results of analysis of variance on dry matter intake within treatments was significantly different.

Feed conversion ratio (FCR) two-weekly intervals during the adaptation period was high (between 21 and 23.6) and for subsequent trial weeks, ranged between 8.48 and 14.02. The FCR between 9.53 to 12.3 was reported for yearling sheep when wheat bran was partially replaced by air-dried Moringa stenopetala [13] for 70 days trial.

Analysis of variance for FCR was significantly different between treatments.

Intake, excretion, and absorption of protein in sheep for C0, C20 and C40 ranged from 90-100 g/h/day, with up to 60% being absorbed and the rest eliminated in urine and feces. Protein intake was not significantly different between treatments. Fiber intake (measured as neutral detergent fiber, NDF) between 400-600 g/h/day and 50% metabolized in the body. The rest was wasted through feces. Meanwhile, analysis of variance showed that the NDF fibre uptake was not significantly different between treatments.

### 4. CONCLUSION

FCPH protein content is higher than CPH. Fermentation increases the digestibility of protein and fibre. The product of CPH fermented using Aspergillus oryzae can replace commercial concentrates as much as 20 and 40% without causing negative effects for sheep.

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