Initial assessment on the use of cocoa pulp in complete feed formulation: *in vitro* dry matter and organic matter digestibility

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Abstract. Cocoa pulp is a by-product from cocoa industry which is produced in large quantity, but very limited study has been carried out in utilizing it as energy source in animal feed. The purpose of this study was to assess the *in vitro* dry matter (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of complete feed containing different levels of cocoa pulp. The experiment was carried out according to completely randomised design consisting of four treatments and three replications. The treatments were P0 = Complete feed containing 0% cocoa pulp, P1 = Complete feed containing 5% cocoa pulp, P2 = Complete feed containing 10% cocoa pulp, and P3 = Complete feed containing 15% cocoa pulp on dry matter basis. The results of the study indicated that the average IVDMD was 567, 538, 566, and 526 g kg⁻¹ DM, while the average IVOMD was 522, 491, 502, and 461 g/kg DM, respectively for treatment P0, P1, P2, and P3. Statistical analysis indicated that increasing levels of cocoa pulp in the feed significantly affected (P<0.05) the IVDMD and IVOMD of the feed. In conclusion, cocoa pulp is potential to be used up to 10% in complete feed with corn cobs as the fibre source.

1. Introduction

Indonesia is one of the top six countries of cocoa production in the world. The cocoa production in Indonesia reached 661,243 tons in 2015 [1]. Among the provinces in Indonesia, South Sulawesi is one of the largest cocoa production region with an area of 246,223 ha producing around 143,237 tons of cocoa beans [2]. In addition to the main products which are cocoa butter and cocoa powder, the processing of cocoa fruit also resulted in byproducts such as cocoa pods, cocoa pods husk, and pulp which are usually disposed off as waste. In the cocoa processing industry, only 10% cocoa butter or cocoa powder can be derived from of the fresh cocoa and roughly 68.5% of fresh cocoa mass will become waste. The amount of cocoa pulp, one of the byproducts form cocoa processing, could reach up to 40% the fresh weight of cocoa beans [3]. Another study reported that processing of 1 kg of cocoa beans could yield 100-190 ml of cocoa pulp [4]. Actually, cocoa pulp has been used for various purposes, such as for acetic acid production [5], organic herbicides for the plants [6, 7], jam for human
consumption, juice and fermented beverages [8, 9, 10]. However, a large proportion is not utilized and considered as a waste.

One alternative on the utilization of cocoa pulp is by utilizing it as animal feed especially for ruminants such as cattle and goat. Based on its physical form, cocoa pulp is potentially useful as feedstuff if combined or mixed with other ingredients, especially those that could absorb water, such as corn cobs meal and rice bran. Chemically, cocoa pulp contains high percentage of sugar [11], and can be used to substitute molasses in complete feed or in the urea molasses block (UMB). Use of complete feed has some advantages, for example it has been shown to increase feed consumption, daily body weight gain of Bali cattle [12] and improved the quality and yield of goat milk [13]. Moreover, the use of complete feed formulated from materials such as corn cobs resulted in a good performance of local goats in South Sulawesi [14].

In order to optimally use the cocoa pulp as a feed ingredient in a complete feed it is important to have initial information related to its biological value when used as a part of the complete feed. Many methods have been applied to evaluate the nutritive value of a feedstuff. One of those is in vitro technique [15]. This study was carried out to determine the effects the increased levels of cocoa pulp in a complete feed using corn cobs as the main fibre source, on in vitro dry matter digestibility and in vitro organic matter digestibility.

2. Materials and Methods

2.1. Formulation of Complete Feed

Formulation of the complete feeds for this study was carried out according to the following protocols: All feed ingredients having large or rough particle size, such as corn cobs, coconut cake meal was groundto smaller particles using a hammer mills. Each feed ingredient was weighed according to the formulations in each dietary treatment (four different treatments). All feedstuff in each treatment were mixed thoroughly using a hand mixer. The mixture then was made into cube form using simple press equipment. The feed was then dried in oven at 65°C for 48 hours. One hundred g samples of each treatment diet was then ground to pass 1-mm sieve for both chemical analysis (proximate and fibre analysis) and for the in vitro study. The composition of each dietary treatment is presented in table 1.

| Feedstuff (%) (DM basis) | Treatments |
|--------------------------|------------|
|                          | P0         | P1         | P2         | P3         |
| Corn cobs                | 50.0       | 50.0       | 50.0       | 50.0       |
| Rice bran                | 18.0       | 18.0       | 18.0       | 18.0       |
| Coconut cake meal        | 7.5        | 7.5        | 7.5        | 7.5        |
| Shrimp waste meal        | 5.0        | 5.0        | 5.0        | 5.0        |
| Cocoa pulp               | 0.0        | 5.0        | 10.0       | 15.0       |
| Molasses                 | 15.0       | 10.0       | 5.0        | 0.0        |
| Urea                     | 1.5        | 1.5        | 1.5        | 1.5        |
| Salt                     | 1.0        | 1.0        | 1.0        | 1.0        |
| Mineral Mix              | 2.0        | 2.0        | 2.0        | 2.0        |
| Total                    | 100.0      | 100.0      | 100.0      | 100.0      |

P0 = complete feed containing 0% cocoa pulp, P1 = complete feed containing 5% cocoa pulp, P2 = complete feed containing 10% cocoa pulp, and P3 = complete feed containing 15% cocoa pulp.
2.2. In vitro Study
The in vitro study was carried out according to completely randomized design consisting of four treatments, P0, P1, P2, and P3 and three replications for each treatment giving a total of 12 experimental units [16]. The treatments were P0 = complete feed containing 0% cocoa pulp, P1 = complete feed containing 5% cocoa pulp, P2 = complete feed containing 10% cocoa pulp, and P3 = complete feed containing 15% cocoa pulp. In the formulations the molasses were replaced by cocoa pulp. The pepsin-cellulase in vitro digestibility technique [15, 17] was used in order to determine the in vitro dry matter disappearance (IVDMD) and in vitro organic matter disappearance (IVOMD) of the samples. This technique is a two-stage procedure requiring the addition of cellulase in an acid buffer and subsequent addition of pepsin in an acetate buffer into the samples incubated at 50°C for a total of 120 hours.

2.3. Laboratory Analysis and Calculation
Sample DM content was determined by drying at 105°C in the oven for 24 h (Memmert, Germany). The percentage of ash was determined by combustion of samples in the furnace (Nabertherm, Germany) for 6 h at 550°C. Organic matter (OM) was calculated as 100-%ash (DM basis). Total N content was determined by the Kjeldahl procedure [18] and percentage of crude protein (CP) was calculated as total N*6.25. Fibre composition (NDF and ADF) was analysed according to the procedure of Goering and Van Soest [19].

IVDMD and IVOMD were calculated as follows:
IVDMD = (DM incubated (g) - DM residue (g)) x 100% / DM incubated (g)
IVOMD = (OM incubated (g) - OM residue (g)) x 100% / OM incubated (g)

2.4. Data Analysis
All the experimental data were analysed using analysis of variances according to the completely randomised design with experimental model: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where $Y_{ij}$ = observation value, $\mu$ = general mean, $T_i$ = effects of treatment ($i = 1, 2, 3, 4$), and $\varepsilon_{ij}$ = experimental error ($i = 1, 2, 3, 4$ and $j = 1, 2, 3$).

3. Results and Discussion
The Proximate analysis indicated that the crude protein content, ether extract, and ash of the treatments increased as the cocoa pulp levels in the diets increased, while fibre content and nitrogen free extract components decreased as the levels of cocoa pulp increased (table 2). Based on Van Soest analysis, Neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the complete feed decreased as the levels of cocoa pulp in the diets increased (table 2). Statistical analysis was not carried out for chemical components of the diets. The change of the chemical components of the diets as the levels of cocoa pulp increased is understandable as the chemical component of molasses, in which the cocoa pulp intended to substitute was not exactly the same. For instance, both ingredients are different in their protein and fibre contents. Therefore, the fibre content of the complete feed for instance increased when cocoa pulp increased due to crude fibre content of cocoa pulp was somewhat higher than that of molasses The change of chemical components of the complete feed was merely a consequence of substituting molasses with cocoa pulp in the diets, in which both feedstuffs do not share exactly similar chemical components. In general, however, the chemical component of the complete feed (all treatments) was quite satisfactory for ruminants with the crude protein content of the diets varied from 12.7% (P0) to 16.8% (P3) (table 2). Ruminant animal requires at least 7.5% crude protein in their diet [20].
Table 2. Chemical components of the experimental diets

| Components (g/kg DM)             | Treatments |
|----------------------------------|------------|
|                                  | P0         | P1         | P2         | P3         |
| Proximate components             |            |            |            |            |
| Crude protein                    | 127        | 131        | 166        | 168        |
| Ether extract                     | 32         | 45         | 59         | 57         |
| Crude fibre                      | 178        | 177        | 174        | 161        |
| Nitrogen free extract            | 582        | 548        | 487        | 484        |
| Minerals                          | 82         | 99         | 114        | 129        |
| Fibre components                 |            |            |            |            |
| NDF                              | 528        | 469        | 395        | 448        |
| ADF                              | 251        | 238        | 233        | 225        |

*Animal Feed Chemical Laboratory, Hasanudin University, 2017. P0 = complete feed containing 0% cocoa pulp, P1 = complete feed containing 5% cocoa pulp, P2 = complete feed containing 10% cocoa pulp, and P3 = complete feed containing 15% cocoa pulp.

The *in vitro* technique is commonly used to evaluate the nutritive value of feeds for ruminant animals. The differences or variation of the chemical components of the diets affected the parameters observed. Statistical analysis indicated that increasing levels of cocoa pulp in the complete feed affected (P<0.05) the IVDMD and IVOMD of the whole feed. The average IVDMD value ranged from 526 g kg⁻¹ DM (P0) to 567 g kg⁻¹ DM (P3), and from 461 g kg⁻¹ DM (P3) to 522 g/kg DM (P0). Further statistical analysis indicated that the average percentage of IVDMD was the lowest for the treatment P3, while the IVDMD for the treatment P2 was similar (P>0.05) to P0 and much higher (P<0.05) than that for P1. Based on the IVOMD, the average value for treatment P0 was much higher (P<0.05) than that for P1, P2, and P3. Moreover, IVDMD for P2 was much higher (P<0.05) than that of P1 and P3, while IVOMD for P1 was higher than P3.

Table 3. The means of IVDMD and IVOMD of the experimental diets

| Parameters          | Treatments |
|---------------------|------------|
|                     | P0         | P1         | P2         | P3         |
| IVDMD (g/kg DM)     | 567⁠ᵃ       | 538⁠ᵇ       | 566⁠ᵃ       | 526⁠ᵇ       | P<0.05     |
| IVOMD (g/kg DM)     | 522⁠ᵃ       | 491⁠ᵇ       | 502⁠ᶜ       | 461⁠ᵈ       | P<0.05     |

⁠ᵃ,b,c,d⁠ means at the same row sharing different superscript were different at 5% level of significant. IVDMD = *In vitro* dry matter disappearance, IVOMD = *In vitro* organic matter disappearance, P0 = Complete feed containing 0% cocoa pulp, P1 = Complete feed containing 5% cocoa pulp, P2 = Complete feed containing 10% cocoa pulp, P3 = Complete feed containing 15% cocoa pulp.

The data (table 3) indicates that the change of IVDMD and IVOMD values of the diet did not follow the pattern of increasing levels of cocoa pulp in the complete feed, in which the change in the value of IVDMD and IVOMD of the four treatments was not consistent. From this study it seems that the optimal level for inclusion of cocoa pulp in the complete feed was up to 10%. There is no exact explanation why the value of IVDMD and IVOMD was fluctuated and did not follow the pattern of increased levels of cocoa pulp. The most possible reason for this phenomenon, however, might be related to associative effects. This effect is known when the apparent
digestibility of a mixture of feeds is not equal to that of the sum of the individual component feeds. Associative effects are broadly categorised into negative and positive associative effects [21, 23]. Even though both ingredients, molasses and cocoa pulp, have similar characteristics in terms of provision of degradable carbohydrate, differences in their fat content might be also contribute to this differences. The fat content of the feed increases as the level of pulp cocoa in the diet increase. It seems that fat content of the diet containing 15% cocoa pulp has passed the tolerable level of fat for this particular diet. As a result, the extent of IVDMD and IVOMD for the feed containing 15% pulp was the lowest compared to other treatments [22]. Other factor that might have an effect on digestibility of the diet is the fibre source of the diet [24, 25]. Akhirany et al [26] reported that different forages has its own rumen degradation characteristics and may have an impact on affecting the digestibility when combining with other feedstuff as a complete feed. Therefore it is interesting to further explore the digestibility of the diet when cocoa pulp is combined with other fibre sources such as rice straw, corn stalk, or peanut straw, or soy bean straw.

4. Conclusion
Cocoa pulp has the potential to be used as an alternative feedstuff to substitute the use of molasses in complete feed using corn cobs as the main fibre source. This was based on the fact that the inclusion 10% (dry matter basis) in the complete feed has similar IVDMD and IVOMD with the complete feed containing 15% molasses. However, further study is required in order to determine whether the complete feed containing cocoa pulp is acceptable by the ruminant animal and could result in a comparable effects on the animal performance fed on conventional diet.

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