The effect of dietary protein and energy supplementation on in vitro - rumen characteristics and digestibility of Bali bulls

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Abstract. The aim of this study was to measure the effect of dietary protein and energy supplementation on rumen characteristics and digestibility of Bali bulls, by in vitro. Leucaena leucocephala (LL) and Corypha elata Robx (CeR) as protein and energy sources, respectively, and Native Grasses (NG) collected from Natural Pasture (NP) in Timor-Leste, were used as dietary treatments. A 3 x 3 factorial design was used in this study, namely, protein and energy sources (LL, CeR and LL-CeR) and 3 levels of supplementation (10, 15 and 20%). Rumen characteristics (pH, NH₃, microbial protein (MP), and volatile fatty acids (VFA) production and composition) and dry matter (DM) and organic matter (OM) digestibility were measured. We found that dietary supplementation had a significant (P<0.05) effect on rumen characteristics in terms NH₃ concentration, total VFA, acetic acid, propionic acid but did not affect the pH, butyric acid and microbial protein (P>0.05). The digestibility of DM and OM were also significantly increased (P<0.05). It is concluded that dietary supplementation improves rumen characteristics and digestibility of Bali bulls. Leucaena Leucocephala and CeR are recommended as protein and energy supplementation, respectively, on NG diet in NP in Timor-Leste to optimize productivity of Bali cattle.

1. Introduction

Farmers in Timor-Leste generally raise cattle as additional income. Cattle are managed extensively in Natural Pasture (NP) [1]. In an extensive production system without improvement on the pasture, Native Grasses (NG) is commonly characterized by low nutrient quality, resulting in low cattle productivity. This condition is compounded by several factors, i.e. over grazing, high annual temperature varying between 25 and 31°C, with humidity ranging between 71 and 83% and annual rainfall less than 1000 mm/year [2].

Nutrient quality of forages in NP can be improved by utilizing protein and or energy supplements. In Timor-Leste, Leucaena leucocephala (LL) and Corypha elata Robx (CeR) grow widely and they are typically used as protein and energy supplements in cattle, respectively. Corypha elata Robx (CeR), harvested from CeR trees, is commonly supplemented in the cattle diet, which is usually offered to cattle when they return from grazing. CeR is a potential energy source due to its valuable nutritional composition, including 2% crude protein (CP), 17.5% crude fiber (CF), 1.2% crude fat, 71.5% NFE, 0.4% ash, 0.1% P and 3340 kcal energy. Furthermore, CeR contains 87.64% DM, 5.21% ash, 82.02 OM, 2.53% CP, 12.04% CF and 4210 kcal energy [3]. Synchronization of nutrient supply
in rumen is important to stimulate the microbial growth and maximize the RDP binding into microbial cells [4] because microbial protein content provides more than 50% of protein needed by cattle [3]. The aim of the study was to measure the effect of dietary protein and energy supplementation on NG basal diet on the rumen characteristics and digestibility of Bali bulls. The results of this study were expected to be used to identify the optimum level of energy-protein supplementation in the diet.

2. Material and methods

Materials used in this study were NG, collected from NP in 3 villages (Batugade, Sanerin and Aidabatelet), in Timor-Leste. A total of five plots (each for 1x1 m²) were sampled for each village. LL leaves and CeR as a protein and energy sources, respectively, were collected from Timor-Leste.

2.2. Methods In this study, treatments were assigned into 3 x 3 factorial design with five replications: NG + LL, NG + CeR, and NG + CeR + LL + CeR; and three supplementation levels: 10, 15 and 20%. Using in vitro method [5], the rumen characteristic (pH, VFA, NH₃ and MP) and digestibility of DM, OM, CP and CF were measured. Analysis of variance (ANOVA) was used to evaluate the effect of energy-protein dietary supplementation on the characteristics of in vitro rumen fluid. Mean differences between treatments were further tested using Duncan multiple range test (DMRT) [6].

3. Results and discussion

3.1. Digestibility

The digestibility of NG supplemented with LL, CeR and LL-CeR at different levels of 10, 15 and 20% is presented in Table 1. The results showed that LL and CeR supplementation had a significant effect on the digestibility of DM and OM (P<0.05), possibly due to CP addition from LL leaves. Because protein in the rumen is degraded to NH₃[7]. NH₃ is used for microbes protein synthesis [8], and thus, rumen microbes population increases. The increased microbes’ population and activity will improve digestibility of NG, since microbes produce cellulose enzyme, which is responsible to digest the cellulose of the NG. Supplementation at a level of 20% increased DM digestibility because energy and protein sources from CeR and LL leaves are able to supply the rumen microbes need. DM digestibility in diets with LL-CeR supplementation in this study is in agreement [1], who found 39.2% for DM digestibility.

In this study, OM digestibility of NG was 33.75%. OM digestibility increased along with the levels of LL leaves, which were 40.4% at 10% level, 41.0% at 15% level and 40.3% at 20% level. LL supplementation increased OM digestibility (P<0.05). This increase is possibly due to CP availability in LL leaves. CeR supplementation significantly increased OM digestibility (P<0.05), but OM digestibility was not significantly affected by different supplementation levels, 46.8% at 10% level, 48.8% at 15% level and 49.5% at 20% level. OM digestibility in diets supplemented found an increase of OM digestibility from 49.4% to 59.7% after concentrate supplementation. The increase in OM digestibility is possibly due to a higher OM content in CeR [9]. NG and CeR contain 77.88% and 89.96% of OM, respectively. The use of CeR as supplement increases rumen microbes population and fermentation process [10].

The LL-CeR supplementation increased OM digestibility (P<0.05), but OM digestibility in LL-CeR group was lower than that in LL and CeR groups. A lower OM in LL-CeR group combination was possibly due to imbalance nutrient composition. Supplementation levels had no significant effect on OM digestibility, partly because the nutrient composition in diet did not meet the ideal balance between energy and protein sources used for microbial activity. LL leaves supplementation increased CP digestibility from 17.24 to 23.6% at a level of 20%, because LL leaves contain adequate amount of protein and N used for ruminal activity. Also, CP and CF digestibility increased in LL-CeR supplementation group (Table 1). A high CP digestibility increases microbial activity, resulting a faster nutrient degradation in the rumen. LL leaves supplementation in combination with NG increased CF digestibility in all supplementation levels. The highest mean of CF digestibility in diets supplemented with LL-NG was found at a level 20%, with a mean value of 31.3%. The highest CF
digestibility was found in the LL-CeR supplementation at 10% level, with a value of 34.0%. This high CF digestibility is attributed to the high fiber digestibility in CeR and a combination of LL-CeR increases nutrient degradation by rumen microbes. CeR is collected from the stem of CeR tree, containing high starch and CF, but its protein content is low, with an energy content of 3.480 kcal/kg [11].

**Table 1.** Native grasses digestibility supplemented with LL, CeR and LL-CeR at different levels

| Parameter | LL (%) | CeR (%) | LL-CeR (%) |
|-----------|--------|---------|------------|
| DM (%)    | 37.3±1.44a | 38.1±1.41a | 37.6±1.96c |
| OM (%)    | 40.4±1.13a | 41.0±1.38a | 40.3±2.19a |
| CP (%)    | 22.1±1.26a | 22.7±2.31a | 23.6±2.97a |
| CF (%)    | 29.6±2.10a | 32.8±1.46a | 31.3±2.17a |

DM = Dry matter; OM = organic matter; CP = crude protein; CF = crude fiber

**Table 2.** The characteristics of *in vitro* rumen fluid

| Parameter | LL leaves (%) | CeR (%) | LL-CeR (%) |
|-----------|---------------|---------|------------|
| pH        | 7.2±0.05b     | 7.1±0.08b | 7.1±0.08b |
| VFA       | 39.8±2.12c    | 38.9±4.12c | 37.3±1.51c |
| NH₃       | 11.6±1.20c    | 9.1±1.31c | 9.4±1.79b |
| MP        | 4.2±0.91c     | 5.8±0.48c | 5.2±0.60c |

abcMean within a row with different superscripts differ significantly (P<0.05)

**3.2. Rumen fluid characteristics**

Rumen fluid characteristic are presented in **Table 2**. pH. LL and CeR supplementation did not significantly change pH, which ranged between 6.9 and 7.1. The results indicated that the rumen fluid in all groups had a normal pH. Normal ruminal pH is between 6 and 7 [9]. Microbial activity in rumen reaches optimum when ruminal pH is in normal range. Contrarily, feed digestion is poor when ruminal pH falls to less than 6. The highest pH value was found in groups with LL leaves supplementation at a level of 10%, with an average pH of 7.2, possibly due to high protein content in LL leaves. LL leaves contain high protein[12]. Also, a high fiber composition in LL leaves increases feed digestion in rumen that leads to increased ruminal pH to 7.00. A normal ruminal pH plays an important role in rumen microbial growth [13].

**3.3. VFA production**

LL and CeR supplementation increased VFA production. The highest VFA production was found in diets supplemented with CeR at levels of 15 and 20%, as well as with LL-CeR at a level of 20%. CeR contains a high EFN content (87.03%), which is rapidly degraded in rumen, with VFA as a final product. LL supplementation at a level of 10% increased VFA production. Carbohydrate composition in CeR is sufficient to meet the rumen microbial activity and it increases the hydrolysis of energy into organic acids. Carbohydrates in rumen will be hydrolysed by the activity of microbial enzymes, which produces pyruvic acid, followed by glycolysis which produces VFA as a final product [14].

**3.4. NH₃ production**

NH₃ concentrations in diets with LL and / or CeR supplementation at various levels did not clearly indicate which treatment is more dominant. However, a range value between 7.85 and 11.62mg/mL was obtained (**Table 2**), indicating that protein content in each treatment is sufficient to produce a suitable rumen environment for microbial activity. Ammonia concentration for microbial activity in the rumen normally ranged between 5 and 7 mg/100 [15]. Tahuk et al. The ratio of feed
supplementation of *Gliricidia sepium*, corn flour and rice bran significantly affected the amino acid (NH₃) production in rumen fluid of Bali bulls [15]. The high ammonia production is due to the availability of protein content in LL leaves, which increases protein degradation by microbes to NH₃ in rumen. In rumen, protein and other nitrogen compounds will be broken down into ammonia and peptides. Ammonia is further used for protein synthesis in rumen.

In this study, an average value of 11.6 mg / 100 mL for ruminal NH₃ production in Bali bulls supplemented with LL leaves at a level of 100% was higher than that in Bali bulls supplemented with CeR or LL-CeR combination. The results indicated that protein content in LL leaves increased NH₃ production in rumen.

3.5. Microbial protein production
The results showed that protein-energy dietary supplementation increased microbial production. CeR supplementation at a level of 10% had the highest microbial production (6.36 mg/mL). The increase of microbial production in other supplementation groups did not significantly different, ranging between (4.19 and 5.82 mg/mL).

4. Conclusion
Our finding concludes that *Leucaena Leucocephala* supplementation has a significant effect on DM and OM content, total VFA, acetic, propionic and acid production and NH₃ concentration. However, it has no significant effect on pH, butyric acid production and microbial protein in rumen fluid. The production of pH, acid: Propionate (A: P) and NH₃ in groups supplemented with LL leaves is higher than that in groups supplemented with CeR and LL-CeR. CeR supplementation increased DM, OM, total VFA and butyric acid production. In general, LL and CeR are recommended as protein and energy supplementation on native grass diet in natural pasture in Timor-Leste to optimize productivity of Bali cattle.

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