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Energy Metabolism of Sheep Supplemented with Complete Rumen Modifier (CRM)

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ABSTRACT

This study aimed to examine the effect of supplementing the Complete Rumen Modifier (CRM) which consisted of 30% *Morinda citrifolia* leaf flour, 30% *Camellia sinensis* leaf dregs flour, 30% *Ipomoea batatas* leaf flour, 3% *S. cerevisiae*, 3% metionin and 3% sulfur on the energy metabolism of 18 local male sheep aged 6-8 months. The experimental feed consisted of ammoniated rice straw and concentrate (40 : 60) weighing 4% DM body weight. This study was subjected to a randomized complete block design (RCBD), analyzed six groups of initial weight of sheep allotted into three treatment groups: 0%, 1%, and 2% CRM of DM feed. The measured variables were energy intake (EI), digestible energy (DE), metabolizable energy (ME), energy retention (RE), and efficiency of RE to EI, RE to DE, and RE to ME. The results showed significant effects ($P<0.01$) of supplementing CRM in the feed on EI, DE, ME, RE, and efficiency of RE to EI, RE to DE, and RE to ME. The orthogonal polynomial test revealed that a higher dose of CRM supplementation in the feed has quadraterally increased ($P<0.01$) EI, DE, ME, and RE with peak points on 0,62%; 0,80%; 1,05%; 1,09%, respectively, and linearly increased ($P<0.01$) the efficiency of RE to EI, RE to DE, and RE to ME. It was concluded that the addition of CRM at the level of 2% was able to produce optimal energy efficiency in sheep.

Key words: Complete rumen modifier (CRM), Energy efficiency, Energy metabolism

Introduction

Sheep is a ruminant cattle with great potentials for meat production (Novaiza *et al*., 2012; Rusdiana and Praharani, 2015). While meat production can be maximized through maintenance intensification using high concentrated feed, it allows lactic acid to accumulate which results in the decreased ruminal pH and a higher risk of acidosis (Pantaya *et al*., 2016; Putra *et al*., 2022; Syahrir *et al*., 2010). The increased rumen pH may result in the lower feed intake and fiber digestibility (Salcedo *et al*., 2012), which potentially impede ruminal microbes in reducing VFA production as the main source of energy for ruminants.

Efforts to address this issue include incorporating buffers into feed. The commonly used buffers are NaCO3, Na_2CO_3, and MgO, which prevents the declining ruminal pH and affects rumen fermentation products, body weight gain, and feed conversion (Joseph, 2001; Sarwar *et al*., 2007; Gastaldello *et al*., 2013; Putra *et al*., 2022), However, buffer is now limited in use because it potentially accumulates and leaves residue in livestock products. This issue can be addressed by incorporating feed additive, such as complete rumen modifier (CRM), to substitute buffers.

Previous studies have examined CRM for different feeds and types of livestock, including rice straw for sheep and elephant grass for cows. Another study investigated dairy goats supplemented with CRM made of soapberries, *Albizia faltacaria* leaves, *Sesbania grandiflora, Acetoanaerobium noterae* and microbial growth factors to reduce methane production which evidently increase average daily gain (ADG) in sheep and improve milk quality and production in dairy cows and goats (Thalib *et al*., 2010; Sukmawati *et al*., 2011; Thalib *et al*., 2011). However, the combination of high concentrate and CRM made of the same components can increase the risk of acidosis in livestock; therefore, it is crucial to modify the composition by incorporating flavonoid sources to prevent declining ruminal pH.

The Complete Rumen Modifier (CRM) in this study includes multiple sources of flavonoids, namely *Morinda citrifolia* leaf, *Ipomoea batatas* leaf, and *Camelia sinensis* leaf dregs (Irawan *et al*., 2020; Kurniawan, 2018; Sudarman *et al*., 2016; Tugiyanti *et al*., 2017) and growth promotor of rumen bacteria such as like *Saccharomyces* *cereviceae,* methionine amino acids, and sulfur minerals, (Puastuti, 2009; Wiyatna and Hernaman, 2016). Balcells *et al*. (2012) reported that supplementing 300 mg/kg DM plant extracts containing flavonoids into feed has successfully reduced the risk of acidosis by decreasing the population of lactate-producing bacteria *Streptococcus bovis,* and increasing the population of lactic-user bacteria *Megasphaera elsdenii*, pH value, and propionate production.

The increased pH value is expected to prevent sheep exposure to acidosis. Also, higher yield of propionic acid due to the conversion of lactic acid bacteria can benefit the livestock by improving energy supply sources for basic living and production, and stimulating more energy efficiency by livestock. Accordingly, it is important to examine the effect of incorporating CRM made of multiple sources of flavonoids (*Morinda citrifolia* leaf, *Ipomoea batatas* leaf), *S. cerevisiae*, methionine amino acids, and sulfur minerals into feed to prevent acidosis and increase metabolic and energy efficiency of sheep. In hypothesis, supplementing up to 2% of Complete Rumen Modifier (CRM) into the feed of sheep can increase energy efficiency in sheep.

Materials and Methods

Livestock and feed formulations

This study used 18 sheep aged 6-8 months weighing 18-25 kg. The sheep were sheared and weighed to determine the initial weight, then dewormed and injected with vitamin B complex. The sheep were placed in an individual platform cage with the size of the cage, the length of the cage is 1.5 m, the width is 1 m, the height is 1m and elevated 0.5 m above the ground. Each sheep wore harnessed diaper made of modified used-car tyres to contain urine, while feces holder was placed at the bottom of the cages. Feed was provided in a bucket and drinking water was given ad libitum at the front part of the cage.

The amount of feed offered to the sheep was 4% DM body weight, consisted of ammoniated rice straw and concentrate (40 : 60). The concentrate contained 49.5% cassava waste, 33% rice bran, 16.5% soybean meal, and 1% minerals. The ingredients of *Complete rumen modifier* (CRM) as feed *additive* were consisted of 30% *Morinda citrifolia* leaf flour, 30% *Camellia sinensis* leaf dregs flour, 30% *Ipomoea batatas* leaf flour, 3% *S. cerevisiae*, 3% metionin and 3% sulfur. This study aimed to examinethe effect of supplementing the Complete Rumen Modifier (CRM). The experiment was subjected to randomized complete block design (RCBD) applied to 18 experimental units consisted of six groups of initial body weight of sheep and three doses of CRM (0%,1%, 2% DM feed) randomly allotted to the sheep. The composition of diet available in Table 1.

The variables measured were energy intake (EI), digestible energy (DE), metabolizable energy (ME), energy retention (RE), efficiency of energy retention (RE) to energy intake (EI), efficiency of energy retention (RE) to digestible energy (DE), and efficiency of energy retention (RE) to metabolizable energy (ME), all calculated based on Tillman *et al*. (1989). Methane energy and heat production (HP) were calculated using the estimation method (Ryle and Ørskov, 1990), based the stoichiometry : Methane Production ie. (((2pa + 2pb) – pp) / 4), pa is the proportion of asetate, pb is proportion of butyrate, and pp is proportion of propionate; Heat Production ie. (100% - % glucose conversion efficiency to VFA - % methane production); Glucose Conversion Efficiency to VFA ie. percentage of (0,622pa + 1,092pp + 1,560pb) / (pa + pp + 2pb), pa is the proportion of asetate, pb is proportion of butyrate, and pp is proportion of propionate.

Chemical and statistical analysis

The proximate analysis of feed samples, feed residues, and feces referred to the AOAC procedure, followed by gross energy analysis carried out using the bomb calorimeter (Dittmann *et al*., 2014). The data obtained were subjected to analysis of variance and continued with the orthogonal polynomial test (Steel and Torrie, 1993).

Table 1. Nutrient content of experimental feed

Composition	Treatment		
	P1	P2	P3
Ammoniated rice straw (%)	40	40	40
Concentrate (%)	60	60	60
CRM (%)	0	1	2
Nutrient content			
Dry matter (%)	74.31	75,20	76,09
Ash (%)	12.31	12.40	12.50
Organic matter (%)	62.00	62.79	63.59
Crude protein (%)	12.79	12.95	13.10
Crude Fiber (%)	21.33	21.56	21.78
Ether extract (%)	2.15	2.19	2.23
NFE (%)	25.73	26.11	26.49
Gross energy (Kcal/g)	3.31	3.35	3.39
Tannins from CRM (%) based DM concentrate*)	0	0.87	1.74

P1 = basal feed (40% amoniated rice straw + 60% concentrate); P2 = P1 + 1% complete rumen modifier; P3 = P1 + 2% complete rumen modifier.

Source: results of proximate analysis of the laboratory of nutrition and animal feed Sciences, Faculty of Animal Science, Jenderal Sudirman University in 2021.

*) results of Laboratory Center for agricultural postharvest research and development, Bogor.

Results and Discussion

Table 2 illustrates the results of the average energy intake (EI), digestible energy (DE), metabolizible energy (ME), energy retention (RE), and efficiency of RE to EI, RE to DE, RE to ME, as well as daily fecal energy, daily urine energy, and methane energy. The effect of CRM (P<0.01) on energy intake was calculated using the equation $y = -197x2^2 + 245.89x + 3034.7$ with the coefficient of determination (r^2) = 0.7373 (Figure 1), meaning that energy intake was 73% influenced by CRM and 27% by other factors. Figure 1 illustrates that, compared to control group, incorporating 1% of CRM into feed resulted in a higher energy intake (3,034.70 kcal vs. 3,083.25 kcal), but 2% of CRM produced a lower energy intake (2,737.11 kcal). In general, energy intake is determined by the quantity of feed intake and the proportion of energy feed.

Based on the line graph in Figure 1, the highest energy intake was 0.62%. The decrease of energy intake was probably due to lower feed intake because CRM supplementation reduced feed palatability. It was suspected that tannins in CRM emmited odor which sheep repelled. While Rapisarda *et al*. (2012) stated that ruminant feed intake is strongly influenced by the aroma of concentrate feed, Septiadi *et al*. (2015) mentioned that the smell, texture, and shape of feed largely determined feed palatability that affected feed intake. Tannin (Table 1) is suspected to make feed bitter, and thus less favored by the sheep. Furthermore, Wina (2001) reported that tannin compounds can affect animal feed intake and bind protein feed, and therefore, decrease feed efficiency.

Similar results were observed in digestible energy (DE), metabolizable energy (ME), and energy retention (RE). A quadratic growth (P<0.01) occurred in the equation of each line of $Y\text{-}DE = -143.4x^2 + 229.78x + 1874.3$ coefficient of determination (r^2) = 0.49 which peaked at 0.80% (Figure 2). Similarly, $Y\text{-}ME = -135.34x^2 + 282.89x + 1208{,}6$ coefficient of determination (r^2) = 0.57, peaked at 1.05% (Figure 3). Lastly, $Y\text{-}RE = -122.53x^2 + 266.81x + 1{,}012.6$ coefficient of determination (r^2) = 0.56, peaked at 1.09% (Figure 4). The increased digestible energy was probably due to the improved rumen conditions, resulting in increased feed digestibility. It was supported by the decreased energy value of feces produced as the CRM levels increased.

According to Amtiran *et al*. (2016), feed intake increases with feed digestibility, and thus higher feed energy absorbed by the livestock. Based on the results in Table 3, supplementing CRM has increased metabolizable energy increased and it peaked at 1.05% CRM. In addition, high level of metabolizable energy is attributed to wasted energy through methane which is probably due to defaunation. Tannins and saponins in CRM are defaunators which diminish the population of methanogenic bacteria. This finding was in accordance with Makkar (2003) that tannins in tea dregs are able to suppress methane gas emissions in livestock by diminishing the population of methanogenic bacteria. Wina *et al*. (2005) also reported that the addition of saponins from tea dregs was able to reduce methane production and increase the proportion of propionate. Wahyuni *et al*. (2014) stated that the addition of saponin tannins at the right dose could increase feed fermentability.

Decreased production of methane (CH_4) (22.74%) is expectedly due to the enhanced formation of propionic fatty acid which requires hydrogen (H_2) that is also used for methane production, and therefore, lower production of methane (Utami *et al*., 2021). Another contributing factor to the enhanced propionate activities was the flavonoids contained in CRM which accounted for 97.85 mg/100 g. Flavonoids play a role in stimulating the growth of lactic acid bacteria (LAB) to be converted into propionic acid. Olagaray and Bradford (2019) also mentioned that flavonoids stimulated the growth of LAB in the rumen (*Megasphaera elsdenii*) so that the LAB produced from concentrated fermentation can be utilized as propionic fatty acid. The conversion of LAB into propionic acid leads to enhanced energy supply for livestock to improve energy retention and energy metabolism and further contribute to the increased energy efficiency.

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Table 2. Average effect of CRM addition on energy metabolism of sheep

Variables	Treatment			Sig.
	P1	P2	P3	
Energy intake (Kcal)	3,034.70 ± 92.01	3,083.25 ± 96.44	2,737.11 ± 111.44	0.001
Fecal energy (Kcal)	1,160.35 ± 65.51	1,122.52 ± 57.35	976.80 ± 87.16	0.009
Digestible energy (Kcal)	1,874.35 ± 84.64	1,960.73 ± 93.69	1,760.31 ± 95.95	0.005
Urine energy (Kcal)	17.10 ± 2.74	23.81 ± 2.39	24.86 ± 4.05	0.002
Methane energy (Kcal)	650.35 ± 58.95	580.79 ± 47.05	502.45 ± 32.67	0.001
Metabolizable energy (Kcal)	1,208.57 ± 54.35	1,356.12 ± 71.80	1,233.00 ± 58.35	0.006
Energy retention (Kcal)	1,012.57 ± 69.20	1,156.83 ± 51.83	1,056.06 ± 55.21	0.005
Efficiency RE to EI (%)	33.31 ± 1.37	37.55 ± 2.41	38.56 ± 2.18	0.005
Efficiency RE to DE (%)	53.96 ± 1.44	58.96 ± 2.20	59.90 ± 1.84	0.001
Efficiency RE to ME (%)	83.74 ± 0.57	85.28 ± 0.57	85.59 ± 0.65	0.001

P1 = basal feed (40% amoniated rice straw + 60% concentrate); P2 = P1 + 1% complete rumen modifier; P3 = P1 + 2% complete rumen modifier.

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Energy retention (net energy) can be defined as food energy used by livestock for maintenance (basic life) and production. Net energy will be converted into mechanical energy used by livestock for basic living activities, and the rest will be stored as new tissues in the body. The increase of energy retention indicates that livestock only lose a little energy and have more energy in store for maintenance and production. Therefore, a linear increase (P<0.01) was evident in the efficiency of RE to EI, RE to DE, and RE to ME (Figures 5, 6, and 7).

The efficiency of RE to EI can also be interpreted as the percentage of energy consumed and remained in the livestock body (Utami *et al*., 2021). Figures 5, 6, and 7 which illustrate an increased energy efficiency show that incorporating CRM into feed has positive effects on

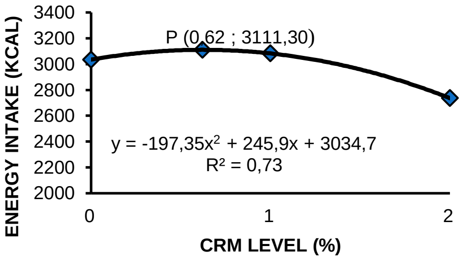

Figure 1. Relationship of CRM additions to energy intake.

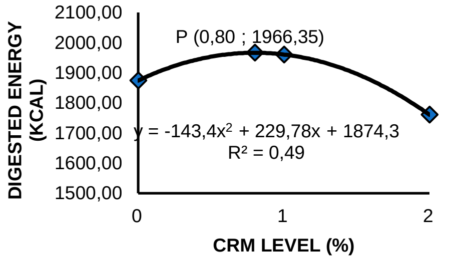

Figure 2. Relationship of CRM additions to digested energy.

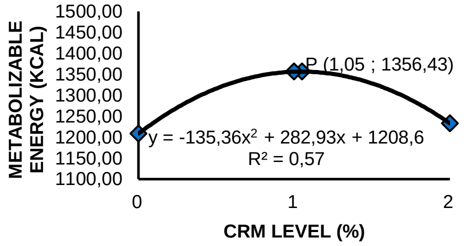

Figure 3. Relationship of CRM addition to metabolizable energy.

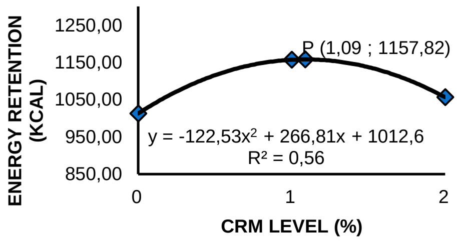

Figure 4. Relationship of CRM addition to energy retention.

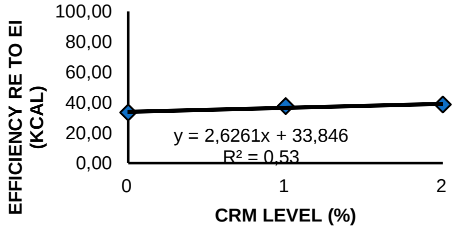

Figure 5. The relationship of CRM additions to the efficiency of RE to EI.

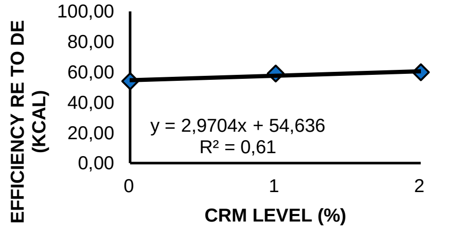

Figure 6. The relationship of CRM additions to the efficiency of RE to DE.

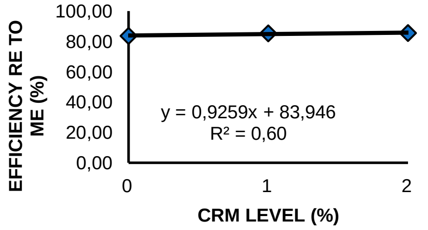

Figure 7. The relationship of CRM additions to the efficiency of RE to ME.

energy efficiency and metabolism, that are also attributed to the improved rumen conditions where ruminal bacteria allows more LAB converted into propionate.

The enhanced efficiency can reflect the ability of livestock to utilize feed which is parallel to the role of rumen bacteria. The addition of CRM has increased the population of rumen bacteria and improved fermentation activity, thus enhancing efficiency. In this study, rumen conditions improved because *S. Cerevisiae* in CRM utilized oxygen in the rumen, creating anaerobic conditions and stimulating the growth of rumen bacteria population (Hidayat and Rahwanandi, 2013).

The growth of rumen bacteria population is also attributed to the growth factors, namely amino acid, methionine, and sulfur minerals. Similarly, El-Tahawy *et al*. (2015) reported that incorporating amino acid methionine into the concentrate feed of Rahmani sheep could improve digestibility and growth. Sulfur minerals played an important role in the synthesis of microbial protein and amino acids containing sulfur groups such as cystine, cysteine and methionine (Dewi *et al*., 2015), and stimulated the growth of rumen microbes in sheep (Uhi *et al*., 2005). Table 2 indicates that the addition of 2% CRM in feed produces the most optimal efficiency for livestock in utilizing feed.

Conclusions

It was concluded that the addition of CRM at the level of 2% was able to produce optimal energy efficiency in sheep.

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