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Application of omega 3,6 with *Moringa oleifera* suplemented vitamin E on rumen fluid characteristics, microbial protein synthesis, and methane gas production in goats on suboptimal lands

Penerapan omega 3,6 bersama Moringa oleifera *suplementasi vitamin E terhadap karakteristik cairan rumen, sintesis protein mikroba dan produksi gas metan pada ternak kambing di lahan suboptimal*

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ABSTRAK

Untuk mengatasi masalah emisi gas metan dari ternak ruminansia dan masalah produktivitas yang rendah di tingkat peternak rakyat, diperlukan perubahan dalam pola pemberian makanan. Solusi ini melibatkan tindakan langsung terhadap gas yang dihasilkan di dalam lambung ternak ruminansia dengan memberikan pakan yang mengandung zat aktif untuk mengurangi mikroba yang menghasilkan gas dalam lambung. Salah satu alternatif pakan yang sering digunakan sebagai promotor perbaikan karakteristik cairan rumen adalah penggunaan sumber omega 3.6 bersama daun kelor yang diperkaya dengan vitamin E. Penelitian ini bertujuan untuk menginvestigasi pengaruh pemberian pakan terhadap produksi gas metan pada ternak kambing. Metode penelitian menggunakan Rancangan Acak Kelompok (RAK) dengan empat perlakuan dan lima ulangan secara *in vitro*. Dari hasil penelitian, dapat disimpulkan bahwa penggunaan sumber omega 3,6 bersama dengan daun kelor yang diperkaya dengan vitamin E mampu mengurangi produksi gas metan sebanyak 25,62% dengan nilai Volatil Fatty Acid (VFA) 144,53 mM, pH berkisar 6,99, NH_3 11,87 mg/100ml, biomassa mikroba 20,42 mg/ml, protein mikroba 217,58mg/ml, koloni bakteri $5{,}34 \times 10^9$ sel/ml dan populasi protozoa 29×10^6 sel/ml. Kesimpulan ini menunjukkan bahwa pemanfaatan sumber omega 3,6 bersama daun kelor dan vitamin E memiliki potensi untuk secara konsisten dapat memperbaiki karakteristik cairan rumen dan mengurangi emisi gas metan, meningkatkan sintesis protein mikroba dan penurunan populasi protozoa dengan menggunakan kombinasi sumber omega 3,6 dan daun kelor dilahan suboptimal, serta suplementasi vitamin E.

Kata kunci: daun kelor, kambing, gas methan, omega 3&6, vitamin E

ABSTRACT

Changes in feeding patterns were needed to overcome the problem of methane gas emissions from ruminant livestock and the problem of low productivity at the smallholder livestock level. This solution involves direct action against the gas produced in the stomach of ruminants by providing feed containing active substances to reduce the microbes that produce gas in the stomach. One alternative

feed that was often used as a promoter of improving rumen fluid characteristics was the use of omega 3.6 sources together with Moringa leaves enriched with vitamin E. This research aimed to investigate the effect of feeding on methane gas production in goats. The research method used a Randomized Block Design (RAK) with four treatments and five replications in vitro. From the research results, it could be concluded that the use of omega 3.6 together with Moringa leaves enriched with vitamin E was able to reduce methane gas production by 25.62% with a Volatile Fatty Acid (VFA) value of 144.53 mM, pH around 6.99, NH_3 11.87 mg/100ml, microbial biomass 20.42 mg/ml, microbial protein 217.58mg/ml, bacterial colony $5.34x10^9$ cells/ml and protozoa population $29x10^6$ cells/ml. This conclusion shows that the use of omega 3.6 with *Moringa oleifera* supplemented with vitamin E has the potential to consistently improve rumen fluid characteristics and reduce methane gas emissions by using a combination of omega 3.6 sources and Moringa leaves in suboptimal land, as well as vitamin E supplementation.

Keywords: goat, methan gas, moringa leaves, omega 3&6, vitamin E

INTRODUCTION

Chemically, moringa leaves contain concentrated tannins. Condensed tannins are compounds that can protect proteins, increasing the amount of protein absorbed in the small intestine and reducing NH_3 levels. Complex compounds of tannins and proteins do not dissolve in the rumen, but the acidic atmosphere within the abomasum enzymatically digests these components, making the proteins soluble and available to livestock (Wood et al., 2018).

Microorganisms in the rumen are very important for ruminant production, especially when using fiber feeds. Ammonia is the main source of nitrogen and is essential for protein synthesis in microorganisms. Rumen N-NH_3 concentration is a major focus in ruminant livestock husbandry (Warly et al., 2015). The main factor influencing the use of NH_3 is the availability of carbohydrates in the feed, which serves as an energy source for the formation of microbial proteins. Therefore, nitrogen and energy availability within the rumen must be balanced to achieve maximum efficiency of microbial protein synthesis.

Balance is achieved through careful feeding, considering it as a source of protein and energy. Increasing the number of microorganisms, especially bacteria, in addition to increasing the digestibility of fibrous feeds, also provides a source of high-quality protein for ruminants. Microbial proteins can cover up to 90% of amino acid requirements. These amino acids are highly consistent and ideal for meeting the needs of suboptimal ruminants. Moringa leaves grown in suboptimal soils contain many different types of antioxidants, including ascorbic acid, flavonoids, phenols, and carotenoids, which together with vitamin E are a natural antioxidant.

The high concentration of ascorbic acid, protein, and essential amino acids, especially methionine, cysteine, tryptophan, and lysine in the leaves and pods make moringa leaves an ideal feed supplement. Therefore, combining low ammonia (NH_3) jackfruit leaves with moringa leaves may increase nutrient digestibility and reduce methane gas production (Zain et al., 2015). Together with moringa leaves from suboptimal locations, it provides a source of omega 3.

The addition of fish oil to ruminant feed should not exceed 6–7% of the dry matter of the feed as it affects the fermentation of ruminant microorganisms. The function of omega-3 fatty acids is to control the antimicrobial effect of fatty acids to minimize disruption of rumen fermentation and to ensure the highest possible fat content in the feed. Based on the above studies, the strategy is to increase the efficiency of microbial protein synthesis and reduce methane gas in suboptimal areas through research focused on providing jackfruit and moringa leaves as feed additives.

This was a very interesting limitation. The objective of this research was to reduce methane gas produced by goats on suboptimal land using feed made from Moringa leaves grown on suboptimal land and a source of omega 3 and 6 supplemented with vitamin E. The goal was to identify the goal of reducing generation.

MATERIALS AND METHODS

Materials

Moringa oleifera was often found in the Tapanuli area which has suboptimal land with andisol soil type, which has quite high nutrient elements resulting from volcanic ash. Omega 3,6 oil, vitamin E powder, and goat rumen fluid from slaughterhouses was used for *in vitro*.

Methods

This study used an experimental method using a randomized block design (RAK) consisting of four treatments and five replications *in vitro*. Treatment A consists of 40% concentrate+60% field grass, B consists of 40% concentrate+2.5% *M. oleifera*+3% Omega 3.6+0.2% vitamin E+55% field grass, C consists of 40% concentrate+2.5% *M. oleifera*+4% Omega 3.6+ 0.2% vitamin E+55% field grass, while Treatment D consists of 40% Concentrate+2.5% *M. oleifera*+5% Omega 3.6+0.2% vitamin E+55% field grass.

The parameters tested in this study were rumen fluid properties and methane gas production (Table 1). The composition of chemical treatment rations based on dry matter, organic matter, crude protein, crude fiber, crude fat, BETN, TDN was presented in Table 2.

Table 1. Composition of concentrate feed ingredients based on dry matter (%)

Concentrates	(DM %)
Fine Bran	40
Tofu residue	23
Coconut Flour	34
Fish Meal	2
Premix	1
Total	100

Table 2. Chemical composition of research rations

Nutritions	Ransum (%)			
	A	B	C	D
DM	42.69	45.67	43.39	45.84
OM	92.96	92.94	92.74	92.72
CP	12.62	12.60	12.56	12.53
CFat	25.20	24.59	24.90	24.29
CFiber	4.08	4.24	4.14	4.30
BETN	51.06	51.51	51.14	51.60
TDN	63.03	59.46	61.71	58.90

pH

pH meter method was used to measure the pH value of rumen fluid. The first step was to turn on the pH meter and allow it to stabilize for 15 to 30 minutes. Standardization was then performed using a standard buffer at pH 7. The pH meter was then cleaned with distilled water and dried with a cloth. Insert the pH meter electrode into the fermentation tube and measure the pH value based on the number displayed on the monitor screen.

Volatil Fatty Acid (VFA)

Steam distillation method was used to determine the production of Volatil Fatty Acid (VFA) 5 ml of supernatant contained in the tube was removed and placed in a distillation tube. Next, 1 mL of 15% H_2SO_4 was added to the distillation tube and the tube was immediately covered with rubber, which could be connected to a Leibig refrigerator.

Next, insert the distillation tube into the distillation flask containing distilled water. During the distillation process, the still was heated and water vapor pushes out the VFA, which was condensed in the Leibig condenser. The water condensed during the distillation was collected in an Erlenmeyer flask along with 5 ml of 0.5N NaOH solution and added until a volume of 250–300 ml was reached. After the distillation process was complete, add 2–3 drops of phenolphthalein indicator and titrate with NHCl solution until the color changes from pink to colorless (Suyitman et al., 2021).

NH_3

The Conway cup method was used to determine NH_3 production. In this method, 1 mL of supernatant was added to the right side of the Conway beaker, and 1 mL of 40% NaOH was added to the left side of the Conway beaker. Next, 1 mL of H_2BO_3 was dropped into the center of the Conway cup. The Conway cup was then covered with a lid and the rim of the cup coated with petrolatum and stored for 24 hours. After 24 hours, titration was performed with 0.005N H_2SO_4 solution until the color changed to red-green. Used 0.2 grams of feed sample was placed in a serum bottle and filled with 30 ml of a mixture of rumen fluid inoculum and McDougall buffer using an automatic pipette dispenser (Uddin et al., 2015). Serum vials were sealed with rubber stoppers, clamped with aluminum, and incubated in a 39°C incubator for

24 hours. A 5 mL gas sample was collected using a syringe, and the gas was filled into a 5 mL serum bottle capped with a rubber stopper and closed with an aluminum clamp. A gas chromatograph with a thermal conductivity detector was used to measure methane gas. Helium gas was used as carrier gas at a flow rate of 10 ml per minute. The detector and column temperatures were 250 °C and 60 °C, respectively. Methane gas production was calculated after cultivation was completed, taking into account the gas amount and gas composition. Methane gas was calculated using the following formula (Tavendale et al., 2005):

CH4 = (GV + HS) x concentration.

Where:

GV = gas volume (ml)

HS = Serum bottle headspace volume (ml)

Concentration = % of methane gas in sample

Microbial Protein Synthesis

Microbial protein synthesis was calculated based on the calculation method of Chen and Gomes (1995). Before calculating microbial protein synthesis, first calculate microbial nitrogen production. Microbial nitrogen (MN) production is calculated using the following formula: MN = 32 g/kg DOMR DOMR (Digestible organic matter fermented in the rumen) is calculated using the following formula: DOMR= feed consumption x BK feed x BO feed x digestibility BO x 0.65 Rumen microbial protein synthesis (SPM) is calculated using the following formula: SPM (g/day) = MN x 6.25.

Protozoa Population

The protozoan population was calculated based on the method used by Hristov (1998) using a hemocytometer chamber, with a dilution of 1:5 (1 ml sample with 5 ml methyl green formaldehyde solution). Protozoa were counted under 0.2 mm deep counting chambers and 6 classes were identified based on the method of Dehority (1993).

RESULTS

The results of applying omega 3.6 together with *Moringa oleifera* vitamin E supplementation on rumen fluid characteristics and methane gas production were presented in the Table 3. Methane gas production could see Figure 1 and microbial biomass in vitro could see in the Figure 2.

Table 3. Results of feed application on the characteristics of rumen fluid and methane gas production

Treatment	Variable			
	pH	VFA (mM)	NH_3 (mg/100 ml)	Total CH_4 (%)
A	6.45[a]	160[a]	14.32[a]	28.06[a]
B	7.21[b]	155.23[c]	18.85[d]	27.79[d]
C	7.05[b]	135[d]	12.04[a]	25.62[c]
D	7.10[b]	139.64[b]	13.34[b]	25.76[b]
SE	3.23	3.65	1.56	0.26

Note: Values with different superscripts in same row were significantly (P<0.05) different. Source: Nutrition laboratory, Faculty of Animal Husbandry, Andalas University, Padang (2023).

SE: Standar Error

Figure 1. Methane Gas Production *in vitro*

Figure 2. Microbial Biomass

The results of applying omega 3.6 together with *Moringa oleifera* vitamin E supplementation on microbial protein synthesis, bacterial colony, and protozoa population were presented in the Table 4. Protozoa population could be seen in Figure 3 and 4.

Table 4. Results of feed application on microbial protein synthesis, bacterial colony, and protozoa population

Treatment	Variable			
	Microbial Biomass (mg/ml)	Protein Microbial Biomass (mg/ml)	Bacterial colony total (sel/ml)	Protozoa Population (sel/ml)
A	6.45	75.77	$4.31x10^{9a}$	$24.45x10^{6a}$
B	17.43	173.22	$4.64x10^{9b}$	$36.86x10^{6c}$
C	11.67	104.64	$6.31x10^{9d}$	$33.21x10^{6c}$
D	20.42	217.58	$5.34x10^{9c}$	$29.00x10^{6b}$
SE	3.78	12.53	0.23	2.85

Note: Values with different superscripts in same row were significantly (P<0.05) different. Source: Nutrition laboratory, Faculty of Animal Husbandry, Andalas University, Padang (2023).

Figure 3. Protozoa Population

Figure 4. calculation of total protozoa

DISCUSSION

Table 3 shows that the treatment of applying omega 3.6 with moringa leaves supplemented vitamin E had a significant effect on the pH of rumen fluid. The pH of rumen fluid is neutral and ranges from 6.45 to 7.21. This means that the administration of omega 3.6 with moringa leaves supplemented with vitamin E affects the effectiveness of feed conversion in ruminants depending on the digestive process within the rumen. Low levels of digestion in the rumen result in poor feed efficiency. Normal rumen pH is 6.0–7 to maintain normal ruminal metabolism (Angelia & Silaban, 2019). A decrease in rumen pH to 6.0 can effect fiber digestibility. The results of the treatment showed that it had a significant effect (P<0.05) on the concentration of rumen fluid characteristics.

Moringa leaves were a high fiber feed which not only reduces the efficiency of feed use but also increases the production of methane gas (CH_4). The release of methane not only causes an increase in CH_4 concentrations in the air, but also causes a loss of 6–13% of energy from feed (Danang & Yulianto, 2016). Methane gas production shows that a lot of feed energy was wasted so that reducing methane gas production can reduce the loss of wasted feed energy (Jayanegara et al., 2015).

The higher the rate of addition of Moringa leaf flour, the more efficient the use of feed will be. Tannin has two mechanisms for reducing methane gas production, namely directly by inhibiting the activity and growth of methanogenic bacteria, and indirectly by inhibiting fiber digestion thereby reducing H_2 production (Suyadi & Wahyuningsih, 2017). This can also be seen from the increasing amount of NH_3 production produced. Judging from NH3 production, the results of the analysis of variance showed that the treatments had a significantly different effect (P<0.05) on the provision of jackfruit leaves and moringa leaves. Rumen fluid NH_3-N levels ranged from 13.34 mg/100ml to 18.85 mg/100 ml, this was caused by an increase in NH_3 levels from each treatment indicating that the breakdown of protein in the rumen into microbial protein came from each treatment providing field grass with a source of omega 3.6 with Moringa leaves supplemented vitamin E.

N-NH_3 production is the main product of the amino acid deamination process and its availability in the rumen for microbial growth is a top priority in optimizing forage fermentation. This is in accordance with the opinion of (Suwignyo et al., 2016) which states that the main factor influencing the use of NH_3 in rumen fluid is the availability of crude fiber for rumen microorganisms. The crude fiber available from field grass will function as an energy source for fermentation needs and rumen microbial growth, although the C NH_3 treatment results of 12.04

mg/100 ml were not as high as the NH_3 requirement for rumen fermentation activity, the maximum for coarse basal forage is 23 mg/100 ml. ml rumen fluid (Agung et al., 2016).

The presence of high VFA means that microbes can use N-NH_3 to form cell proteins. This is in accordance with the opinion of Tandi et al. (2018) who stated that the use of NH_3 needs to be accompanied by an energy source that is easily fermented. The average concentration of N-NH_3 obtained from the results of this research shows that it is sufficient and can be used for microbial growth and protein synthesis. NH_3 production in the C treatment is 12.04 mg/100ml, due to the ability of microbes to degrade feed ingredients in the rumen for body protein which is also found in the digestibility of protein in the rumen and NH_3 produced only to fulfill microbial protein in the rumen.

Bhatta et al. (2015) stated that the NH_3 concentration is influenced by several factors, including the type of food given, the source of nitrogen solubility, the level of protein degradation, the concentration of nitrogen in the ration, and others. This is in accordance with the opinion expressed by Harahap et al. (2021) that the NH_3 concentration of rumen fluid varies between 0–130 mg/100 ml, while the minimum limit for ammonia that can support rumen microbial growth is 5 mg/100 ml.

Furthermore, the highest VFA production was in treatment A, namely 160 mM and the lowest VFA production was produced in treatment C, namely 135 mM. The VFA production obtained in this study ranged from 135 – 160 mM, this was sufficient for VFA requirements for optimal microbial growth with the results showing significantly different ($P<0.05$).

According to Rahmawati et al. (2021), the VFA range required for optimal rumen microbial growth is 80 – 160 mM. High VFA production is energy sufficient for livestock (Laka & Kleden, 2019). VFA is the end product of carbohydrate fermentation and is the main energy source for ruminants from the rumen. VFA production in the rumen changes with differences in physical form, feed composition, level and frequency of feeding and processing obtained from the hydrolysis process of fat by lipolytic bacteria into fatty acids and glycerol. Fly fatty acids are the main energy source for ruminants produced from feed fermentation in the rumen. Therefore, VFA concentration reflects feed fermentability (Wang et al., 2018).

The research results showed that the application of omega 3.6 sources together with Moringa leaves supplemented with vitamin E had a significant impact ($P<0.05$) on methane gas production in vitro. The average total methane gas production ranges from 25.62% to 28.06%. Moringa leaves originating from suboptimal land have a high protein content, not only reducing the efficiency of feed use, but also increasing the production of methane gas (CH_4) (Bunglavan & Dutta, 2013).

The release of methane gas not only results in an increase in CH_4 concentrations in the air, but also causes an energy loss of around 6–13% of the feed. In addition, Moringa leaves have many benefits, including increasing feed efficiency, nitrogen retention, and reducing protozoa populations in the rumen. A decrease in this protozoa population can have an impact on reducing methane gas production. D treatment, which involves 5% omega 3 and 6 with vitamin E supplementation, has the potential to reduce methane production better together with Moringa leaves.

This indicates that when omega 3 and 6 along with Moringa leaves are supplemented with vitamin E, it can support a reduction in methane production during rumen fermentation activity. This may also be caused by the ability of tannins to reduce methane production (Abuelfatah et al., 2016).

Table 4 the results of the analysis show that the treatment of omega 3.6 sources together with Moringa oleifera vitamin E supplementation had no effect ($P>0.05$) on microbial biomass. The results of microbial biomass analysis in Treatment D were 20.42 mg/ml, higher than other treatments. This is because the digestion process in the rumen in ruminant livestock is very dependent on the population and types of microbes that develop in it, because the feed breakdown process is basically the work of enzymes produced by microbes in the rumen (Mosoni et al., 2011). Furthermore, the microbial biomass contained in the rumen is a combination of bacteria, ciliated protozoa, flagellated

protozoa, fungi, amoeba and bacteriophages (Morgavi et al., 2010). The diversity of microorganisms that are found in the rumen environment each has a specific function in the degradation of carbohydrates, proteins and fats originating from feed (Wang et al, 2018).

Furthermore, table 4 shows that the highest protein synthesis was in treatment D at 217.58 mg/ml, followed by treatment C at 104.64 mg/ml, treatment B at 173.22 mg/ml, and the lowest protein synthesis was in treatment A. amounted to 75.77 mg/ml. The results of microbial protein from each treatment were higher compared to the research results of Verma and Sukhla (2015), microbial protein synthesis which received the addition of 0% and 3% soybean oil in the ration respectively, namely 44.31 mg/ml and 56.95 mg/ml. ml. This could be caused by higher ammonia concentrations, thereby increasing the availability of nitrogen from protein degradation for microbial protein synthesis. Some of these carbohydrate sources include fermentable carbohydrates which are easily digested by rumen microbes (Hindratiningrum et al., 2011). Optimal microbial protein synthesis requires a supply of nitrogen and organic acids. The nitrogen supply comes from ammonia production, while organic acids will be met from VFA production which is the result of carbohydrate fermentation. The low concentration of ammonia indicates that more nitrogen sources are utilized by microbes to synthesize their body cells.

Utilization of omega 3.6 sources together with Moringa oleifera vitamin E supplementation showed a very significant effect ($P<0.05$) on total bacterial colonies and protozoan populations.

The total average of bacterial colonies ranged from $4.31x10^9$–$5.34x10^9$ cells/ml. Table 4 shows the best total bacterial colonies in treatment D of $5.34x10^9$. This is caused by the source of omega 3.6 together with Moringa oleifera supplementation with vitamin E, each of which *in vitro* has a significant effect on reducing the total population of rumen protozoa bacterial colonies. This is caused by the tannin content in the Moringa oleifera extract. The tannin content of Moringa oleifera at a level of 2.5% contributes 0.0951 mg/L and flavonoids are polyphenolic compounds which have antimicrobial activity which can suppress a number of microbial virulence factors, such as inhibiting biofilm formation, reducing host ligand adhesion, and neutralization of bacterial toxins (Harahap et al. 2023).

Furthermore, the results of using omega 3.6 sources together with Moringa oleifera vitamin E supplementation on protozoan populations ranged from $24.45x10^6$–$29x10^6$. This result is higher than that obtained by Wahyono et al. (2017) where the protozoa population obtained by tannin supplementation from jackfruit leaf flour ranged from $1.7x10^6$–$2.2x10^6$. This difference is caused by the tannin content of Moringa oleifera and vitamin E. The addition of Moringa oleifera with omega sources can reduce the total population of rumen protozoa (Harahap et al., 2021).

Table 4 shows that treatment D can very significantly reduce the protozoa population compared to treatments B and C, where the protozoa population decreased by $29x10^6$ except compared to treatment A as a control. In this study, there was a decrease in the total protozoan population because the saponin contained in the Moringa oleifera extract was relatively undetectable so it did not have the potential to become a protozoan defaunation agent. Protozoa can stabilize the fermentation process when given high concentrates because they are able to consume lactic acid more quickly than bacteria so that rumen pH increases and prevents acidosis (Newbold & Ramos Morales, 2020).

CONCLUSSION

Treatment of 5% omega 3 and 6 together with Moringa leaves supplemented with vitamin E in the ration can show the stability of rumen fluid characteristics, increased microbial protein synthesis of 217.58 mg/ml, and reduction in methane gas of 25.62%.

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