Nutrient Content and Total VFA Concentration Evaluation by Addition of Condensed Tannin and Myristic Acid in Complete Feed Through In Vitro Method

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ABSTRACT
The objective of this study was to determine the effect of condensed tannins and myristic acid addition in complete feed on nutrient content and VFA concentrations in vitro. The materials used for this research were maize straw, condensed tannins, myristic acid, coffee husk, rice bran, cassava waste, soybean meal, copra meal, and palm kernel meal. The method used in this research was an experiment in the laboratory using Randomized Block Design (RBD) with four treatments and three replications. The treatments in this study were T1 (complete feed (40% corn straw + 60% concentrate)), T2 (complete feed + condensed tannin 1.5% /kg DM and myristic acid 2% /kg DM), T3 (complete feed + condensed tannin 1.5% /kg DM and myristic acid 3% /kg DM), and T4 (complete feed + condensed tannin 1.5% /kg DM and myristic acid 4% /kg DM). The data obtained were analysed by variance analysis followed by Duncan's Multiple Range Test (DMRT). The results showed that the treatments gave a highly significant effect ($P<0.01$) on total VFA concentration. Percentage of Tannins and myristic acid in T2 can increase VFA 23% compared to control. It can be concluded that the addition of condensed tannins 1.5% + myristic acid 2% on complete feed (T2) was the best treatment in terms of nutrient content and the increase of total VFA.

Keywords: *Complete feed, Condensed tannin, Myristic acid, Nutrient content, VFA.*

1. INTRODUCTION

Feed is anything that can be eaten, digested, and absorbed by livestock without causing poisoning or harming the health of livestock that consume it [1]. Feed is the most crucial factor in the livestock business because its contribution reaches 60-70% of the total production cost [2].

The utilization of agricultural by-products as animal feedstuff is an alternative to meet the nutritional needs of livestock, either as supplements, concentrate components, or primary feed [3]. An agricultural by-product that is often used as animal feed is maize straw. The low nutrient content and high crude fibre of maize straw causing the low digestibility of maize straw [4]. Moreover, the availability of conventional feed in the dry season is relatively low. Decreased feed quality is indicated by the low digestibility and low nutrient content of the feed. To anticipate the low productivity of livestock, it is necessary to study the potential use of maize straw as feed, especially during the dry season. One way or method to increase the nutrient content of maize straw is by adding concentrate to become a complete feed.

The addition of condensed tannin and myristic acid and energy and protein source feedstuff can increase the nutrient content of maize straw and total VFA in complete feed. According to [5], the tannin content in animal feed has a beneficial effect if it is added to complete feed that is high in protein both in quantity and quality. This is because high-quality protein can be protected by tannin from rumen microbial degradation to be more available in the post-rumen digestive tract. This ultimately impacts energy use efficiency in the rumen due to the energy that was initially supposed to be used

to degrade protein in the rumen is diverted for other energy needs.

The myristic acid content in vegetable oil affects the decreasing of protozoa population and the increasing of bacteria that can be beneficial for livestock. According to Jordan et al., [6], the reduced protozoa population causes the activity of bacteria in rumen and propionate to increase, resulting in lower methane gas. The decrease of the protozoa population in the rumen can reduce methane production and increase the efficiency of energy utilization also utilization of microbial protein for ruminants, so it is expected to increase overall livestock production [7]. This makes tannin and myristic acid good compounds for manipulating the level of protein degradation in the rumen. According to this, it is necessary to study further about the best level of condensed tannin and myristic acid use in maize straw-based complete feed on nutrient content and total Volatile Fatty Acids (VFA) concentration.

2. MATERIALS AND METHOD

The research was conducted at the Animal Nutrition and Animal Feed Laboratory, Faculty of Animal Science, Brawijaya University, Malang.The analysis of total VFA levels was conducted at the Dairy Animal Nutrition Laboratory, Department of Nutrition Science and Feed Technology, Faculty of Animal Science, Bogor Agricultural University.

The material used in this study was complete feed which was prepared from forage and concentrate with the ratio of 40%:60% (based on DM). The complete feed used was composed of isoproteins with CP of 14%. Myristic acid used to make complete feed contains fatty acid of 99% and condensed tannin content of 75% in mimosa powder. The forage used is maize straw (Zea mays) with a cutting age of 90 days. The concentrate consisted of rice bran (Oryza sativa), copra meal (Cocos nucifera), palm kernel meal (Elaeisguineensis), coffee husk (Coffeasp), cassava waste (Manihotutilissima), molasses (Saccharumofficinarum), premix, urea, salt, and soybean meal (Glycine max). The treatments used in this study were the addition of condensed tannin (CT) 1.5% /kg DM and myristic acid (2%, 3%, and 4% /kg DM) in complete feed.

The research method used was experimental in the laboratory using a Randomized Block Design (RBD), which consisted of 4 treatments and each treatment had three groups as replications, with the grouping based on the taking time of rumen fluidfrom different cows as follows:

T1 = Complete feed (40% maize straw + 60% concentrate)
T2 = Complete feed (40% maize straw + 60% concentrate) + CT 1.5% /kg DM + myristic acid 2% /kg DM
T3 = Complete feed (40% maize straw + 60% concentrate) + CT 1.5% /kg DM + myristic acid 3% /kg DM
T4 = Complete feed (40% maize straw + 60% concentrate) + CT 1.5% /kg DM + myristic acid 4% /kg DM

Research variables:
Variables observed included a test of nutrient content [8] and analysis of total VFA production [9]. Data analysis used analysis of variance with Duncan's Multiple Distance Test to find out the differences between each treatment.

3. RESULTS AND DISCUSSIONS

Maize straw is part of the stems and leaves of maize that have been dried in the field and are harvested when the maize cobs are picked [10]. Table 1 shows that the crude protein content of maize straw obtained in the study was 5.13%. Analysis of the crude protein content of maize straw in the study showed a higher number when compared to the results of a study [11] which the crude protein content of maize straw is 4.1%.

The concentrate is a fortifying feed composed of grains and waste from the food industry process that serves to increase the low nutritional value to meet the normal needs of livestock to grow and develop healthily [12]. The crude protein concentrate obtained in this study has met the protein needs of cattle by 21.43%. Analysis of the CP content of concentrate in this study had higher results when compared to the results of a study by [13] that the content of crude protein concentrate is 20.50%.

This study used myristic acid from palm fat (99% pure) as a feed supplement used to inhibit the protozoan population, which was expected to reduce CH4 in the rumen. This caused the need for tannin supplementation

Table 1. Nutrient content of feedstuff

Feedstuff	Nutrient Content (%)					
	DM	OM*	Ash*	CP*	CF*	EE*
Concentrate	92.83	89.83	10.05	21.43	17.58	5.08
Maize straw	94.46	89.83	10.17	5.13	36.43	0.63
Condensed tannin (Mimosa powder)	90.22	94.22	3.57	8.37	3.30	1.43
Myristic Acid	90.41	99.97	0.03	-	-	-

Description: 1) The results of the analysis by the Nutrition and Animal Feed Laboratory, Faculty of Animal Science, Universitas Brawijaya (2019)
*) Based on 100% DM

Table 2. Nutrient content average of treatment complete feed

Treatment	Nutrient Content (%)					
	DM	OM*	Ash*	CP*	CF*	EE*
T1	93.44	90.09	9.91	14.23	24.00	2.94
T2	93.82	90.38	9.62	15.59	24.53	4.07
T3	93.53	90.57	9.43	13.79	24.60	5.44
T4	93.78	90.55	9.45	14.09	24.90	7.12

Description: 1) The results of the analysis by the Nutrition and Animal Feed Laboratory, Faculty of Animal Science, Universitas Brawijaya (2019)
*) Based on 100% DM

which aimed to protect protein so it can be absorbed by the small intestine [14]. This study used mimosa powder which contains atannin content of 75%. The tannin content is higher when compared to the results of a study by [15] which stated that the extraction of tannins from the stems and leaves of shame plant has a value of 3.65%.

The proximate analysis results in Table 2 show that the value of crude protein content on maize straw-based complete feed with the addition of CT (mimosa powder) and different myristic acid ranges from 13.79-15.59% and T2 contain the highest CP comparing to other treatments. The low CP content in T3 was caused by tannin compounds that are able to form complex bonds with proteins and made these proteins unable to be degraded by rumen microbes so these proteins become by-pass proteins 16]. The crude protein content in each treatment feed was in accordance with [17] which stated that the crude protein requirement for beef cattle was at least 12% crude protein content.

The results of the analysis showed that the myristic acid used in complete feed had a fatty acid content of 99%. The addition of myristic acid (2%, 3%, 4% / kg of DM) was able to increase the amount of EE in the treatment feed. This is proven by the higher the proportion of myristic acid used, the higher the ether extract amount obtained in the treatment feed. The increase of ether extract (EE) content in feed was suspected due to the presence of myristic acid which is a saturated fatty acid and can be obtained from natural ingredients.

In this study, the complete feed was composed of more than one feedstuff. It is intended that the feed used is able to meet the nutritional needs of livestock and minimize feed costs. Complete feed is a method of feeding ruminants where all forage/agricultural waste feedstuff and concentrates are mixed into a mixture that has a balanced nutrient content and meets the needs of

Table 3. Total VFA concentration in vitro gas production by incubation for 48 hours

Treatment	Group (ml Mol/L)			Average±SD
	1	2	3	
T1	151.89	150.01	152.90	151.60 ± 1.47^{b}
T2	116.45	116.50	116.81	116.59 ± 0.20^{c}
T3	116.21	116.81	115.81	116.28 ± 0.50^{b}
T4	92.04	98.58	93.02	94.55 ± 3.53^{a}

Description: Different superscripts in the same line show a highly significant difference (P<0.01) in the average of total VFA concentration/

livestock [18]. The use of various kinds of agricultural and agro-industry by-products can be used as an alternative as concentrates are increasingly expensive in the market [19].

The statistical test of the variance analysis calculation of the effect of treatment on VFA production showed that the addition of tannin and different levels of myristic acid in complete feed in vitro gave a highly significant effect (P<0.01) on the total VFA production. The VFA value in the treatment feed had lower yields when compared to the control feed (T1), which the control feed had the VFA concentration of 151.60 mM, while for the results shown by treatment feed with condensed tannin and myristic acid (T2, T3, and T4) consecutively had the VFA concentration of 116.59 mM; 116.28mM; and 94.55mM. This is because the level of tannin addition is still quite low so the bacteria are still able to adapt.

T2 has the highest total VFA comparing to the other treatments, however T4 has the lowest total VFA. The total concentration of VFA decreased due to defaunation [20]. However, there was an increase in molar propionate and a decrease in methane production. The increase in propionate is beneficial for livestock because it affects nutritional efficiency, growth, also body and carcass composition [21]. A total 3% of fatty acid supplementation will best affect livestock because fat serves as an energy source [22]. The addition of myristic acid as much as 2% can increase propionic acid the total VFA which is expected to reduce methane gas production so the efficiency of energy used is higher [23]. The use of fat content below 5% of the total ration will not cause a negative effect on fat on the fiber feed digestibility in rumen [24].

The decrease in total VFA concentration maybe caused by the inhibition of fiber digestion by tannin contained in mimosa powder. The decreased total VFA concentration is a reflection of changes in the rumen microbial population. Tannin compounds can reduce the

population of protozoa, the concentration of methane gas, and increasing in feed efficiency [25]. The provision of 1% tannin and 0.6% saponin in feed was able to give the best effect on defaunation and fermentability of feed but did not significantly suppress the production of methane gas [26]. The addition of 1.5% condensed tannin to the total ration had a positive impact on feed. According to Kusmartono [27], the use of tannin as feed has a positive impact if its content does not exceed 4% in the ration. Tannins are compounds that can be used to protect proteins from microbial degradation in rumen because tannins are able to bind proteins by forming complex compounds that are resistant to proteases so protein degradation in rumen decreases [28].

Table 3 shows that the total VFA concentrations obtained ranged from 94.55mM-151.60mM. The VFA produced from each treatment was still in the normal range. The VFA concentration that supports rumen microbial growth ranged from 80 mM to 160 mM [29]. The fermentability level of feedstuff influenced the high and low of VFA production, the number of soluble carbohydrates, the rumen pH, the feedstuff digestibility, and the number also the types of bacteria present in rumen [30].

4. CONCLUSIONS

4.1. Conclusions

The addition of condensed tannin 1.5% /kg DM and myristic acid 2% /kg DM in complete feed (T2) was the best treatment in terms of nutrient content and the increase of total VFA.

4.2. Suggestion

It is necessary to conduct further research on the use of condensed tannin with optimal levels in complete feeds and it is necessary to conduct an experiment using the in vivo method.

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