Ruminal profile of completed feed as influenced by myristic and tannins addition

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Abstract. The study aimed to determine the effect of adding tannin and myristic acid in complete feed on nutrient content and the Profile of volatile fatty acids (VFA) in vitro. The materials used for this research were corn straw, condensed tannins, myristic acid, coffee waste, rice bran, tapioca by-product, soybean meal, copra meal, and palm kernel meal. The method was used in this experiment was laboratory experiment with four treatments and three replications. The treatment consisted of T_1 complete feed (40% corn straw + 60% concentrate (be based DM)), T_2 (complete feed + condensed tannins 3%/kg DM and myristic acid 2%/kg DM), T_3 (complete feed + condensed tannins 3%/kg DM and myristic acid 3%/kg DM), and T_4 (complete feed + condensed tannins 3% /kg DM and myristic acid 4%/kg DM). Every treatment was repeated three times and analysed statistically using Randomized Block Design (RBD). The adding of 4% myristic acid to the complete feed (T4) gives the best results, it increased nutrition and propionic acid, but decreased acetic acid, butyric acid, total VFA and C2/C3 ratio.

1 Introduction

One of the factors determining the success of a livestock business is feed. Animal feed is all feed ingredients that can be given and are beneficial for livestock and do not harm the body of animals. Each feed given must contain complete nutrition with a balanced composition so that the feeding is efficient according to the needs of the livestock [1]. However, ruminant feed is not available throughout the year, especially during the dry season. It is challenging to find forage which is the main feed for ruminants. Therefore, sufficient feed resources need to be sought to supplement the value of low forage quality and reduce dependence on grass. Sources of feed should be easy to obtain, available in large quantities at a relatively low cost, such as the utilization of corn straw waste. Corn straw consists of stalks (stems) and leaves. The percentages of each waste were 50% stalks, 20% leaves, 20% corn cobs, and 10% corn cobs [2]. In Indonesia, the amount of corn straw is 7,015,950 tons of dry matter and 25,056,965.21 tons of fresh material [3].

Corn straw is classified as a quality forage and low digestibility with a CP content of 6.37% and CF 27.61% [4]. [5] stated that corn and agro-industrial wastes have the potential

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as ruminant animal feed. [6] stated that corn straw as ruminant feed and a substitute for fibre sources must be balanced with concentrate to meet the animal's need. Concentrate as an additional feed in a complete feed is a feed ingredient that can improve the nutritional harmony of the whole feed and is mixed as a supplement (complementary). The concentrate has a CF content below 18% and is easy to digest. Concentrates are made from a mixture of several feed ingredients, namely sources of energy (grains), protein sources (types of cake and nuts), vitamins, and minerals [7].

Complete feed contains nutrients for livestock at a certain physiological level that is formed and given as the only feed that can meet the basic needs of production without additional substances, except water [8]. Complete feed technology is one of the efforts to increase the utilization of waste, either from agriculture or plantations, and the addition of non-conventional feed ingredients with physical treatment, supplementation, and considering the nutritional needs of livestock, both crude fiber, protein, energy, and others [9].

[10] stated that 15% of soybean meal protected with 1% tannin in complete feed contributed to the proportion of propionate and increased blood glucose in thin-tailed sheep but had not been able to reduce methane gas production. Adding more than 5% tannin in the diet will decrease both in vitro and in vivo digestibility and feed palatability. This will interfere with the use of nutrient digestibility and livestock productivity [11]. [11] stated that the addition of 2.5%, 5.0%, and 7.5% oil in animal feed was able to reduce the number of protozoa, reduce methane production without a negative effect on NH3 levels, VFA levels, activity CMC-ase, and microbial protein levels.

Volatile Fatty Acid (VFA) is one of the products of carbohydrate fermentation in the rumen, which is the primary energy source for ruminants and can contribute 55-60% of energy needs. The concentration of VFA in rumen fluid can be used to measure feed ferment ability and is closely related to rumen microbial activity [11]. Based on the description above, it is necessary to research the effect of adding condensed tannins and myristic acid to complete feed based on corn straw on nutrient content and concentration of VFA profile in rumen fluid in vitro.

2 Materials and methods

Making complete feed with each treatment in this study was carried out at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Brawijaya University, Malang. Analysis of the nutrient content of feed ingredients was carried out at the Nutrition and Animal Feed Laboratory, Faculty of Animal Science, Brawijaya University, Malang, and VFA concentrations were analysed at the Centre for Food and Nutrition Studies, Gadjah Mada University.

2.1 Materials

The main objective in analysing time series is to understand, interpret and evaluate changes in the phenomena in the hope of more correctly anticipating the course of future events. The materials used in this study were corn straw, concentrates consisting of coffee husks, rice bran, cassava, palm oil meal, copra meal, soybean meal, molasses, urea, salt, feed premix, condensed tannins, myristic acid, chemicals for proximate analysis and measuring in vitro gas and rumen fluid production.

2.2 Method

The research method used in this study was a laboratory experiment with four treatments and three replicate groups. Grouping based on the time of taking different cow rumen fluid. Complete feed was composed of forage and concentrate in a ratio of 40:60 (based on the percentage of DM), the concentration was composed of iso-protein with a CP content of 14% with the addition of condensed tannins (3%/kg DM) and myristic acid (2, 3, 4% /kg DM), as follows:

T_1: complete feed (60% concentrate + 40% corn straw).
T_2: complete feed (60% concentrate + 40% corn straw) + condensed tannins 3%/kg DM+ myristic acid 2%/kg DM.
T_3: complete feed (60% concentrate + 40% corn straw) + condensed tannins 3%/kg DM + myristic acid 3%/kg DM.
T_4: complete feed (60% concentrate + 40% corn straw) + condensed tannins 3%/kg DM + myristic acid 4%/kg DM.

Variables observed included nutrient content [12] and concentration of Profile Volatile Fatty Acids (VFA) [13].

2.3 Data analysis

The research data were analysed using a Randomized Block Design (RBD) and continued with Duncan's Multiple Range Test (DMRT).

3 Results and discussion

3.1 Proximate profile

Complete feed is a complete ration that has been formulated so that it contains all the nutrients according to the needs of livestock and is given as the only feed. [14] explained that complete feed for ruminants is a mixture of concentrate and forage feed ingredients. Feeding of complete feeds form to the ruminant animals must pay attention to rumen condition especially rumen pH. The results of the analysis of the nutrient content of the feed ingredients that make up the treatment feed, namely corn straw, concentrate, condensed tannins, and myristic acid, are presented in Table 1.

Corn straw has a CP content of 5.13%, CF is 36.43%, and EE is 0.63% (Table 1). The analysis results are higher than the analysis conducted by [15], namely the CP of 4.46%; EE is 0.85%, and CF is 33.12%. [16] stated that corn straw as forage is classified as low-quality forage with low digestibility because the CP content is 6.37% and CF is 27.61%. [16] revealed that forage feeding on fattening cattle would not significantly affect high body weight gain in a short time. The weight gain of cows is higher with a relatively short fattening time if the cows are given a ration consisting of concentrate and forage.

The concentrate used in this study had a CP content of 21.43%. This protein content is excellent to meet the nutritional needs of the diet. Concentrate function on complete feed corn straw-based with condensed tannins and myristic acid can improve the nutritional quality of diet. This is comparable to the statement by [17] that the provision of concentrate feed, which has a higher nutritional value than forage, is intended to provide opportunities for livestock to maximize growth or production. This is clarified by [16] statement that high CP content in concentrate can increase rumen microbial growth, resulting in increased activity in digesting feed ingredients. Giving concentrate can help rumen microbes to digest CF faster and increase levels of propionate, which is useful in the formation of meat.

Mimosa leaves (*Mimosa pudica* L.) used as tannins in this study had a CP content of 0.59%; CF is 0.47%, and EE is 0.85%. This is following [18] statement that shy daughter contains 8.37% CP; EE 1.43%; CF 3.3% and contains flavonoid compounds, alkaloids, phenols, glycosides, tannins, saponins, terpenoids, and coumarins. [19] added that providing tannins in feed ingredients can have a defaunation effect. The presence of protein-tannin complexes can pressure the rumen protozoa population, thus causing an indirect impact on the decline of protozoa.

Myristic acid used in this study has an EE content of 1.05%. [20] explained that myristic acid is one of the three most abundant saturated fatty acids in coconut oil, in addition to lauric acid around 44-52% and palmitic acid around 7.5-10, 5%.

Table 1. Nutrient content of diet treatments' ingredients

Feedstuff	Nutrient value (%)						
	DM	**Ash***	**OM***	**CP***	**CF***	**EE***	**Tannin**
Corn straw	94.46	10.17	89.83	5.13	36.43	0.63	-
Concentrate	90.18	11.42	88.58	21.43	18.74	7.12	-
Condensed tannin	90.22	6.41	93.59	0.59	0.47	0.85	23
Myristic acid	90.41	0.03	99.97	-	-	1.05	-

Feed Analysis result from Laboratory of Animal Feed and Nutrient, Faculty of Animal Husbandry, University of Brawijaya (2019).
*) Based on 100% Dry Matter

The primary saturated fatty acids in meat are myristic, palmitic, and stearic. The use of coconut oil in feed can reduce the population of protozoa in the rumen and reduce methane gas emissions per kg live weight of sheep. A decrease of total volatile fatty acids in the rumen and the concentration of acetic and butyric acids were also seen due to the administration of coconut oil. The results of the analysis of the nutrient content of the treatment feed consisting of corn straw, concentrate, condensed tannin, and myristic acid in each treatment are shown in Table 2. The results showed that the CP content in the complete feed was following the initial target of the formulation of 14%. The composition of the CP content of complete feeds is based on the CP needs of ruminants ranging from 9-15%, and especially the CP needs of beef cattle ranging from 13-16% [18].

The CP content in each treatment ranged from 13.05 to 16.18% (Table 2). The CP content in the T1 treatment had the highest value from each treatment of 16.18% because there was no addition of condensed tannins and myristic acid in this treatment. The CP content in treatments T2, T3, and T4 increased in each treatment. This is presumably due to the addition of protein sources derived from concentrates and condensed tannins, and myristic acid. The CP content in each treatment was close to the formulation target of 14%. [19] stated that the high levels of CP in complete feeds are thought to come from additional protein sources from legumes with high protein content. [21] stated that feed with high protein content could be used as a nitrogen source for microbial growth in the rumen of ruminants. The benefits of tannins in feed include preventing bloating in cattle and sheep. Condensed tannins also protect the protein from rumen microbial degradation (increase bypass protein), so the small intestine can directly absorb that protein.

The content of CF in each treatment ranged from 22.87-24.00% (Table 2). The CF content in each treatment decreased due to condensed tannin and myristic acid with a CF content of

3.30%. [22] explained that CF is the primary energy source for ruminants. This is supported by [23] that ruminants can digest CF in DIET well, and an average of 70-80% of energy needs come from fiber. High CF tends to reduce protein digestibility. If an increase follows the increase in protein in the diet in CF, there will be a slight change in protein digestibility. Still, if the CF is reduced and protein is increased, protein digestibility will increase. The EE content in each treatment ranged from 2.94-6.19% (Table 2). The EE content in each treatment decreased due to condensed tannins and myristic acid in the complete feed. The EE content in the T3 treatment had the highest value of 6.19%; the high EE content in ruminant feed ingredients could interfere with the fermentation process of feed ingredients in the livestock rumen. The EE content in this study was higher than the research by [24] that the EE content of the feed consumed was relatively high, around 3.78%, but was still within the safe limit of the EE content for consumption ruminants (below 5%). [25] stated that fat content in the feed that is too high (above 5% of the total diet) would negatively affect the digestibility of CF in the rumen. It can affect the ability of livestock to utilize the nutrients of the feed consumed.

Table 2. Nutrient content of diet treatments

Treatments	DM (%)	OM*(%)	Ash*(%)	CP*(%)	CF*(%)	EE*(%)
T_1	93.44	90.09	9.91	16.18	24.00	2.94
T_2	93.66	90.56	9.44	13.73	23.31	5.33
T_3	93.51	90.69	9.31	13.05	23.26	6.19
T_4	93.46	90.56	9.44	14.12	22.87	3.35

Feed Analysis result from Laboratory of Animal Feed and Nutrient, Faculty of Animal Science, University of Brawijaya (2019).
*) Based on 100% Dry Matter

3.2 VFA Profile

Volatile Fatty Acid is one of the end products of carbohydrate fermentation from feed given in the rumen of livestock. Volatile Fatty Acid also affects the digestibility of feed in the rumen because VFA results from the breakdown of carbohydrates in the rumen. [26] explained that VFA is the main product of carbohydrate digestion in the rumen and the final product of the fermentation of organic matter, which is used as the primary energy source for ruminants from the rumen. [21] added that the production of VFA in rumen fluid can be used to measure feed fermentability and microbial activity and characterize the amount of VFA produced. In otherwise, the provision of feed for animals is a major contributor to land and water use greenhouse gas emission [19, 21]. The most common compositions in rumen fluid are acetic, propionic, and butyric acids, while small amounts of VFA are formic, isobutyric, valeric, isovaleric and caproic acids [27]. The results of the VFA concentration can be seen in Table 3.

The addition of condensed tannins and myristic acid in complete feed on the concentration of acetic acid (C2) had a very significant effect and decreased ($P<0.01$), and propionic acid (C3) had a very significant effect and increased ($P<0.05$), while the addition of condensed tannins and myristic acid to complete feed had no effect on butyric acid (C4) ($P>0.05$).

The lowest concentration of acetic acid occurred in treatment T_4 of 14.76 mMol with the addition of 3 grams of condensed tannin and 4% myristic acid/kg DM. [28] explained that myristic acid supplemented in a complete diet of 5% could reduce 36% of CH_4 gas production without affecting feed consumption and milk production. Condensed tannins can reduce

nutrient availability and enzyme activity and affect fiber degradation). The concentration of acetic acid in this study was 14.76±3.30-28.21±1.36 mMol. If the acetate produced is low, then the production of CH_4 gas has been successfully suppressed. This indicates that the T_4 treatment suppressed the CH_4 gas, which produced lower acetic acid than the other treatments.

The highest propionic acid concentration occurred in treatment T4 of 13.31 mMol with the addition of 3 grams of condensed tannin and 4% myristic acid/kg DM. The concentration of propionic acid in this study ranged from 7.30±0.64-13.31±1.88 mMol. [29] revealed that the total concentration of VFA decreased due to defaunation, but there was an increase in propionate concentration. [11] showed that the average propionic acid content of coconut oil was higher than that of sunflower seed oil and palm oil, each of which had a propionic acid content.

Table 3. The average of VFA concentration from rumen of in vitro gas production with 48 hours incubated

Treatments	VFA (m Mol)				
	Acetic Acid (C2)	**Propionic Acid (C3)**	**Butyric Acid (C4)**	**VFA Total**	**C2/C3 Ratio**
T_1	28.21±1.36^{c}	7.30±0.64^{a}	6.44±1.80	41.95±1.94^{b}	3.88±0.29^{b}
T_2	21.17±0.99^{b}	9.78±1.92^{ab}	4.44±0.93	35.39±3.69^{a}	2.21±0.32^{a}
T_3	19.89±0.47^{b}	10.74±1.08^{ab}	4.22±1.89	34.86±2.49^{a}	1.87±0.22^{a}
T_4	14.76±3.30^{a}	13.31±1.88^{a}	3.99±1.84	30.98±5.08^{a}	1.14±0.38^{a}

Notes: Different superscripts in the column mean value of acetic acid, total VFA, and C2/C3 ratio of 48 hours incubation showed a very significant difference ($P<0.01$). Different superscripts in the column mean value of propionic acid incubation 48 hours showed significant differences ($P<0.05$).

In this study, the concentration of butyric acid had no significant effect on the addition of condensed tannins and myristic acid in complete feed ($P>0.05$). The proportion of butyric acid at T_2 was 4.44 mMol; T_3 was 4.22 mMol, and T_4 was 3.99 mMol. The concentration of butyric acid in this study ranged from 3.99±1.84-6.44±1.80 mMol. [27] explained that the formation of C_4 has faster absorption properties than C2 and C3, so the proportion of C4 in VFA is also low. [30] added that if starch increases or propionate and butyrate increase, the pH will decrease to 4.5-5. At low pH conditions, it will inhibit the growth of cellulolytic bacteria, inhibiting the digestion of forage. [31] stated that propionate and acetate have slower absorption properties than butyrate. The formation of butyric acid is closely related to the formation of acetic acid so that when the concentration of acetic acid increases, the concentration of butyric acid increases. Some of the butyric acid formed is used as a precursor for milk fatty acids into the blood and is used as an energy source for body tissues.

The total VFA concentration in this study had a very significant effect and decreased ($P<0.01$) in each treatment. The total VFA concentration ranged from 30.98±5.08 to 41.95±1.94 mMol. Volatile Fatty Acid produced in each treatment was lower than the control, so all treatments could suppress CH_4 gas. The T_4 treatment had the lowest total VFA value compared to the other treatments. [32] explained that the total VFA concentration (mmol) in the rumen fluid resulted from complete feed degradation with UDP supplementation of 0, 2.5, 5.0, 7.5, and 10.0%, respectively was 100.82; 94.12; 87.02; 106.32, and 88.33 mmol. The statistical analysis results showed that the difference in the level of UDP supplementation in complete feeds did not affect the concentration of total volatile fatty acids. The concentration of VFA in this study was not affected by the level of

UDP supplementation given in 1% formaldehyde-protected soybean meal. One of the causes of the low concentration of total VFA can be influenced by the amount of non-structural carbohydrates composed of complete feeds. This is supported by [33] stating that the decrease in total VFA concentration was due to VFA absorption and VFA as a carbon framework for rumen microbial protein synthesis.

The ratio of acetic acid and propionic acid (ratio C2/C3) aims to measure the efficiency of ruminant energy use and the quality of the product produced. The C2/C3 ratio results in the study had a very significant effect and decreased between treatments ($P<0.01$). The results of this study obtained the results of C2/C3, namely T_2 (2.21 mMol), T_3 (1.87 mMol), and T_4 (1.21 mMol). The low ratio of C2/C3 in this study is thought to be due to the high efficiency of livestock energy use, so the quality of the resulting product is more optimal. If the proportion of propionic acid increases, the balance of acetic acid will decrease. This causes the C2/C3 ratio to decline, and the low C2/C3 ratio is good for beef cattle.

4 Conclusion

The results of the study can be concluded, namely treatment T_4 [complete feed with the addition of the proportion of condensed tannins (3%/kg DM) and myristic acid (4% /kg DM)] is the best treatment by increasing the nutrient content and acid concentration. Propionic acid can reduce acetic acid, butyric acid concentration, total VFA concentration, and the ratio of C2/C3. Complete feed with the addition of condensed tannins (3%/kg DM) and myristic acid (4%/kg DM) resulted in the best nutrient content and concentration of VFA profile suitable for application to livestock. Further research needs to be done by applying complete feed based on corn straw with condensed tannins and myristic acid directly on livestock in vivo.

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