Effect of Condensed Tannin and Myristic Acid in Corn Straw-Based Complete Feeds on NH_3 Concentration and Microbial Protein Synthesis

Siti Chuzaemi[1*], Mashudi Mashudi[1], Poespitasari Hazanah Ndaru[1], Asri Nurul Huda[1], and Eko Agus Siswoyo[1]

[1] *Departmen of Animal Nutrition, Faculty of Animal Science, Brawijaya University, Jalan Veteran No. 1, Malang City, Indonesia 65145*
[*] *Corresponding author. Email: schuzaemi@ub.ac.id*

ABSTRACT
This study aimed to determine the effect of condensed tannin (CT) and myristic acid in corn straw-based complete feed on NH_3 concentration and *in vitro* synthesis of microbial protein. The materials used in this study were corn straw, CT, myristic acid, coffee husk rice bran, soybean meal, copra meal, and palm kernel meal. The method used for this research was an experiment in the laboratory using Randomized Block Design (RBD), which consisted of four treatments and three replications. The treatments in this study were T1 (complete feed (40% corn straw + 60% concentrate), T2 (complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM + myristic acid 20 g/kg DM), T3 (complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM + myristic acid 30 g/kg DM), and T4 (complete feed (40% corn straw + 60% concentrate) + CT 30 g/ kg DM + myristic acid 40 g/kg DM). The results showed that the addition of CT and myristic acid in the complete feed had no significant effect ($P>0.05$) on NH_3 concentration. It could be concluded that the addition of CT and myristic acid could increase the nutrient content of the feed. Also, the addition level of CT 30 g/kg DM and myristic acid 40 g/kg DM (T4) gave the best result on synthesis protein microbial efficiency and NH_3 concentration.

Keywords: *Complete feed, Corn straw, CT, myristic acid, NH_3, Protein microbial synthesis.*

1. INTRODUCTION

Feed is an essential factor in the livestock business. Therefore improving feed management is highly expected to increase the efficiency of the livestock business. Ruminants need fiber feed as the source of energy and maintain normal rumen function and rumen microbial activity. The feed given by farmers is often nutrient deficiency which affects the fulfillment the livestock nutrients needs for essential requirement for maintenance and production because the lower quality of feed offered to animal is related to the farmer capital lack and also natural factor. Moreover, forage production is minimal during the dry season, so alternative feeds are needed as its substitute, one of which is corn straw.

Agricultural waste such as corn straw has considerable potential as a source of ruminant feed. The limiting factor for agricultural by product as feed is the low content of essential nutrients such as protein, energy, minerals, and vitamins. Besides, straw digestibility is also low because it is difficult to be degraded by rumen microbes [1].

Corn straw has more complex structure and it is difficult to degrade due to its high lignin content, which is 12.8%, and its high crude fiber (CF) content, which is 33.58%. The CF content could influence the rate of degradation of dry matter and organic matter; the higher the CF content in feedstuff, the lower the degradation rate of dry matter and organic matter [2]. Ruminants lose between 8–14% of the total digested energy. The low nutrient content in agricultural by-products is caused by the nutrient content of plants in stems and leaves that have been diverted to the main product in the form of seed or fruit [3]. Therefore, it is necessary to find a corn straw processing technology to improve nutritional quality and digestibility. The use of corn straw in

complete feed as a fiber source and combined with other feedstuffs higher in protein and energy content to obtain feed with good nutritional quality.

The addition feedstuffs contain energy, protein, CT and myristic are expected to improve the quality of corn straw. The CT content in animal feed has a beneficial effect when added to high protein feeds in quantity and quality. This is because tannins can protect the high-quality protein from the degradation of rumen microorganisms to be more available in the post-rumen digestive tract by making hydrogen bonds. The tannin-protein bond complex then can be released at low pH in the abomasum, and the p, pepsin enzyme can degrade the protein, so the amino acids in it are available for livestock. This mechanism ultimately impacts the 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 to other energy needs [4].

The decreasing of protozoa population due to the defaunation process causes a decreasing in the symbiosis between protozoa and methanogens [5]. Thus CT and myristic acid promising compounds to manipulate the level of protein degradation in the rumen. Therefore, it is necessary to study further the best level of CT and myristic acid use in corn straw-based complete feed in terms of the concentration of NH_3 and the efficiency of microbial protein synthesis in vitro gas production.

2. MATERIALS AND METHOD

This research was conducted in the Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang.

The material used in this study was a complete feed prepared from 90 days old corn straw (*Zea Mays*), and concentrate with a ratio of 40%-60% in DM bases. The complete feed were design in isoprotein content of 14% CP. and. Concentrate consisted of rice bran, copra meal, palm kernel meal, coffee husk, cassava waste, molasses, premix, urea, salt, soybean meal. The CT (CT) was added at 30g/kg DM and myristic acid at 20,30, and 40/g/kg DM of complete feed. Randomized Block Design (RBD) was used in this research, which consisted of 4 treatments and each treatment had 3 groups as replicates:

T1 = Complete feed (40% corn straw + 60% concentrate)
T2 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg + myristic acid 20 g/kg DM
T3 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM + myristic acid 30 g/kg DM
T4 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM+ myristic acid 40 g/kg DM

The observed research variables included nutrient content [6], NH_3 concentration, and the efficiency of microbial protein synthesis [7]

3. RESULT AND DISCUSSION

3.1. Nutrient Content of Feedstuff

Table 1 shows that the CP content of corn straw obtained in the study was 5.13%. Analysis of the CP content of corn straw in the study showed a higher number when compared to the results of a study by [8] which the CP content of corn straw is 4.1% and according to the research by [9] which is 4.46%. The crude protein content of the corn straw based on the analysis result in this study was also higher than reported in [10], which stated that the CP content of corn straw is 5.56%. This difference is caused by the location of the experiment, where the levels of soil fertility, irrigation, planting age, and fertilization are different. Forage feeding in cattle fattening will not significantly affect high body weight gain in a short time. The body weight gain of cattle is higher with a relatively short fattening time when the cattle are given a ration consist of concentrate and forage [11].

The concentrate that used in this study contains CP with a value of 21.43%. Analysis of the CP content of concentrate in this study had lower results when compared to the results of a study by Aprilia *et al*. (2018) in which the CP content of concentrate is 20.50%, CF is 17.16%, and NFE is 47.46%. The combination with concentrate in the making of corn straw-based complete feed aims to improve the quality of corn straw as animal feed. Concentrate contains low CF, relatively higher carbohydrates, proteins, higher fats but vary in value, and are easily digested.

This study used myristic acid from palm fat (99% pure), as a feed supplement to inhibit the protozoan population that was expected to reduce the concentration of NH_3. This caused the need for tannin supplementation which aimed to protect the protein so it can be absorbed by the small intestine [12] Mimosa flour t with tannin content of 23% was used in this study. The tannin content is higher compared to the results of a study which stated that the extraction of tannin from stems and leaves of the shame plant has a value of 3.65% [13]. Other research also reported that the roots of the shameplant contain tannin with the value of 10% [14], lower than the results of this study.

Table 1. Feedstuff Nutrient Content

Feedstuff	Nutrient Content (%)						
	DM (%)*	OM*	Ash*	CP*	CF*	EE*	Tannin(%)
Concentrate	90.18	88.58	11.42	21.43	18.74	7.12	-
Corn straw	94.46	89.83	10.17	5.13	36.43	0.63	-
CT	90.22	94.22	3.57	0.59	0.47	0.85	23
Myristic Acid	90.41	99.97	0.03	-	-	1.05	-

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

Table 2. Nutrient content of corn straw-based complete feed

Nutrient Content (%)	Diet Treatments			
	T1	T2	T3	T4
DM (%)	93.44	93.82	93.53	93.78
OM*	90.09	90.38	90.57	90.55
CP*	14.23	15.59	13.79	14.09
CF*	24	24.53	24.6	24.9
EE*	2.94	4.07	5.44	7.12

T1 = Complete feed (40% corn straw + 60% concentrate), T2 = Complete feed (40% corn straw + 60% concentrate) +CT 30 g/kg + myristic acid 20 g/kg DM, T3 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM + myristic acid 30 g/kg DM, T4 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM+ myristic acid 40 g/kg DM

Crude protein P(CP) content in the treatment of corn straw-based complete feed with the addition of CT and myristic acid was different, ranging from 13.79-15.59%. According to the analysis results of table 1 it can be concluded that the protein in corn straw-based complete feed was slightly lower than the control treatment. CP in complete feed was adjusted [16], in which the ration for fattening beef cattle feed is 13%. Corn straw has low CP i.e. 5.13%, so in this study,corn straw was processed as a complete feed. In terms of nutrition and fodder aspects, the average of the CP content of corn straw is not sufficient to fullfil the beef cattle requirements of CP, which is 5.9% [17] and for the general minimum requirement of ruminants, which is 8% [17. 18]

Mystric acid supplementation in dairy cattle feed increased the production [19]. Ether Extract (EE) in corn straw-based complete feed has increased as shown in Table 2. T1, T2, T3, and T4 were consecutively 2.94, 4.07, 5.44, and 7.12%. Differences in EE content in the corn straw-based complete feed was related to the differences level of the myristic acid addition. According to SNI 3148-2:2017, the maximum amount of EE in complete feedstuffs for beef cattle is 7%.

The analysis of variance result of corn straw-based complete feed was not significantly different ($P > 0.05$) with in vitro NH_3 concentration. Ammonia is one of the products of fermentation activity in the rumen, which comes from feed protein degradation and become a quite important nitrogen sources for the synthesis of rumen microbes. Ammonia concentration in the rumen is determined by the protein level in consumed feed, the degradability degree,the length of feed stay in rumen, and the rumen pH [20]. Ammonia level in the rumen fluidwith fermented corn strover-based complete feed, was in the normal range (9.89 mM) whereas the optimum ammonia level in rumen was between 4-12 mM. The range of NH_3 produced in this study did not meet the needs for optimum microbes growth, which was around 2.60 - 3.70 mM. The rumen fluid level of ammonia was not statistically significant ($P>0.05$) in all treatments. The low number of protein that degraded in feed causes low concentration of NH_3. The concentration of NH_3 in rumen fluid is influenced by the consumed protein and the process of protein degradation. Synthesis protein microbial will be optimal if there is a synchronization of the release time between nitrogen source and carbon skeleton in rumen. The concentration of ammonia decreased along with the increased level of tannins protected protein [21]. Complex bonds between tannins and proteins that formed as insoluble tough compounds that allow the occurrence of protein deposition. This occurrence was illustrated by the decreasing of ammonia.

Table 3. NH_3 concentration and microbial protein synthesis of corn straw-based complete feed

Treatment	NH_3 (mM)	Microbial Protein Synthesis (g/kg DOMR)
T1	3.01 ± 0.44	31.57±4.18
T2	3.07 ± 0.58	36.97±5.42
T3	2.92 ± 0.53	36.34±3.01
T4	2.60 ± 0.57	38.93±5.58

T1 = Complete feed (40% corn straw + 60% concentrate), T2 = Complete feed (40% corn straw + 60% concentrate) +CT 30 g/kg + myristic acid 20 g/kg DM, T3 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM + myristic acid 30 g/kg DM, T4 = Complete feed (40% corn straw + 60% concentrate) + CT 30 g/kg DM+ myristic acid 40 g/kg DM

The bonding between tannin-protein is bond between the phenol group of tannins keto tannins, a hydrophobic interaction between the aromatic ring structure of proteins and tannins. The interaction with protein can be seen from the formed ring. The wider the formed ring, the greater the ability of tannins to interact with proteins [22]. The decrease in ammonia concentration is evidence of the decreased ability to break down proteins in rumen.

In condition which feed protein content is low, rumen ammonia concentration will be lowered and rumen microbial growth will decline, resulting in inhibited carbohydrate degradation [23]. The NH_3 concentration required by rumen microbes to digest feed optimally was 5-20 mg/dL, equivalent to 3.57-14.28 mM [24]. A concentration of 5 mg/dL NH_3 is equivalent to 3.57 mM [25]. Ammonia concentration decreased along with the increased level of protein protection using tannins.

Based on the NH_3 concentration obtained in each treatment, it can be concluded that the addition of myristic acid and CT in corn straw-based complete feed gave an effect on the decreasing of NH_3 concentration and the increasing of N production in rumen. Rumen microbes have utilized the given additive as a source of N for microbial protein synthesis [26].

High NH_3 is influenced by the level of feed protein solubility, the higher the protein solubility of an ingredient, the more easily the protein is degraded in rumen. The quality of protein in feedstuffs varies widely. Those diversity consider in nutrient solubility, the ability to produce NH_3 for rumen microbes, potency to provide protein which is affected by nitrogen, tannin, and sulfur that escapes degradation in the rumen, sensitivity toward post-rumen proteases; and the biological value regarding the concentration of NH_3 [27].

The decrease of NH_3 concentration means that there is a decrease in protein degradability by rumen microbes. This occurs because protein is protected by the addition of CTs, which forms complex compounds with corn the protein of straw-based complete feed, thereby reduce protein solubility and decrease the degradability in rumen, and ultimately reduce NH_3 concentrations. The concentration of NH_3 in the rumen is influenced by the protein level of the feed, the existence of protein source, and solubility [27]. The results of the analysis of variance showed that the treatments gave no significant effect ($P>0.05$) on the efficiency of microbial protein synthesis in corn straw-based complete feed supplemented with CT and different levels of myristic acid. Corn straw contained in T4 has high protein content. The factors that affect the efficiency of microbial protein synthesis include dry matter consumption, the ratio of forage and concentrate in ration, synchronization of nitrogen and energy, rumen environment, feed rate, and vitamins and minerals [28]. Ammonia is the main nitrogen source for the formation of rumen microbial protein synthesis. Approximately 70%-80% of protein is degraded to peptides and amino acids then further converted into ammonia.

The protein concentration increased in T3 and T4 treatments (Table 2), because of the effect of CT as a protein protection agent. Protein concentration in T3 was higher than in T1. This can be assumed that CT complex in rumen is occurred, so the protein is not degraded and cannot be hydrolyzed by rumen microbes. The efficiency of microbial protein in T1 was lower than T3 so the protein produced decreased. [27]. The concentration of ammonia that supports rumen microbial growth is 4-12 mM [23]. Table 4 shows that the highest microbial protein synthesis was found in treatment T4 (38.93±5.58 g/kg DOMR), followed by T2 (36.97±3.01 g/kg DOMR), T3 (36.34 g/kg DOMR), and the lowest microbial protein efficiency was found in treatment T1 (31.57±5.42 g/kg DOMR). This case is due to the different provisions of myristic acid and CTs. Optimal microbial protein efficiency requires a supply of nitrogen and organic acids. Nitrogen supply comes from ammonia production. The low concentration of ammonia indicates that microbes utilize more nitrogen sources to synthesize their body cells [29].

The results in Table 2. showed that the addition of CT and myristic acid increase the nutrient content of feed with the addition level 30g/kg DM of CT and 40g/kg DM

(T4) mystic acid gave the best results on microbial protein efficiency. So, the microbial protein degradation decreases and the protein well protected in rumen with pH ±7. The use of tannins in feedstuffs has the advantage of reducing protein degradation in rumen and being able to increase protein flow into duodenum [30]. Protein will be released from the tannin-protein complex bond in the abomasum (pH 2.5 to 3.5).

4. CONCLUSION

Based on the results of the study, it can be concluded that the addition of CT and myristic acid increase the nutrient content of feed, and the level of addition of CT 30 g/kg DM and mystic acid 40 g/kg DM (T4) gave the best results on the efficiency of microbial protein synthesis and ammonia concentration in rumen.

Recommendation for further research on the addition level of CT and myristic acid in complete feeds on fermentation product in rumen and *in vitro* digestibility.

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