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The Effects of Addition Calliandra Leaf (*Calliandracalothyrsus*) As A Source of Tannin and Myristic Acid in Complete Feed Based on Corn Straw to Volatile Fatty Acid Partial Concentration And Ammonia Concentration (Nh_3) *In Vitro*

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ABSTRACT
The research aimed to evaluate the effects of calliandra leaf and mono fat addition as myristic acid (MA) in complete feed based on corn straw to Volatile Fatty Acid (VFA) and ammonia concentration (NH_3) in vitro. The research materials were complete feed'sbased on corn straw consisted of corn straw, cascara, bran, tapioca residue, starch powder, soybean meal, palm kernel meal, copra meal, urea, molasses, calliandra leaf, and myristic acid. The research method was in vitro experiment in the laboratory and arranged in Randomized Block Design (RBD) with 5 treatments and 3 replications. The applied treatment was arranged based on DM with formulation P1 = Complete Feed (CF) (40% Corn Straw + 60% Concentrate), P2 = CF(40% Corn Straw + 60% Concentrate + 0% Calliandra)+ MA 30g/kg DM, P3 = CF (40% Corn Straw + 50% Concentrate + 10% Calliandra)+ MA 30g/kg DM, P4 = CF (40% Corn Straw + 45% Concentrate + 15% Calliandra)+ MA 30g/kg DM, P5 = CF (40% Corn Straw + 40% Concentrate + 20% Calliandra)+ MA 30g/kg DM. All treated feeds were arranged with iso protein with 14-15% protein content. The research result showed that the addition of calliandra leaf and MA did not have a significant difference (P>0.05) to partial VFA and NH_3 concentration. The conclusion was the addition of calliandra leaf and MA could decrease partial VFA and NH_3 on a complete feed based on corn straw. As the suggestion, it required further research using livestock to discover the clearer result in livestock productivity.
Keywords:*complete feed, VFA, and NH_3*

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I. INTRODUCTION

Indonesia is a tropical country that has 2 seasons, namely dry and rainy season. Forage in Indonesia is abundant during the rainy season but deficient in the dry season. Forage is a primary source of fiber feed for ruminant livestock from fresh forage, preserved, or agriculture waste. Agriculture waste is a side result of agriculture has low nutrients with crude fiber content and high lignin. Corn straw is one of the agricultural wastes utilized as feed for ruminant livestock in summer. Ruminants have two unique digestive systems because they have 4 stomachs that can digest high fiber as the source of energy, namely rumen.

The final product of carbohydrate fermentation inside rumen is *Volatile Fatty Acid* (VFA), with components consisting of acetate, propionate, and butyrate, a source of energy ruminant livestock (McDonald *et al.,* 1998). Besides carbohydrate, livestock feed should also fill protein needs, both pure protein or non-protein nitrogen (NPN) needed by livestock for reproduction. Tamminga (1979) stated protein inside rumen would be overhauled hydraulically by protease enzymes then became peptide, and amino acids mostly would be degraded and deaminated to organic acids, namely VFA, NH_3, CO_2, and CH_4.

Fermentation condition inside rumen was affected by types of feed provided to livestock. Feed with high fiber would be gradually revamped, caused to delayed enzyme work and retention inside rumen (Tomaszewska et al., 1993). The addition of tannin on feed could decrease total VFA on livestock (Patra, 2010)and also, the addition of fat could optimize VFA production on livestock (Ma'rufatunnisa, Tanuwiria, and Hernama, 2015). The change of VFA production could affect NH_3 concentrate on livestock.

The research aimed to evaluate tannin and fat which is a single fatty acidform *myristic acid* on a complete feed based on corn straw to VFA partial concentration and NH_3 concentration by *in Vitro*.

II. MATERIALS AND METHODS

The research was held in the Laboratory of Animal Nutrition and Food, Faculty of Animal Husbandry, Brawijaya University of Malang. The applied method was a digestibility experiment by *in vitro* gas production in the laboratory using Randomized Block Design (RBD) with 5 treatments and 3 replications and feed protein arranged iso protein with 14-15% protein content. Grouping was based on three times of taking rumen fluid. The applied treatments were:

P1 = Complete Feed (40% Corn Straw + 60% Concentrate)
P2 = Complete Feed (40% Corn Straw + 60% Concentrate + 0% Calliandraleaf) + *Myristic Acid* 30 g/Kg DM
P3 = Complete Feed (40% Corn Straw + 50% Concentrate + 10% Calliandraleaf) + *Myristic Acid* 30 g/Kg DM
P4 = Complete Feed (40% Corn Straw + 45% Concentrate + 15% Calliandraleaf) + *Myristic Acid* 30 g/Kg DM
P5 = Complete Feed (40% Corn Straw + 40% Concentrate + 20% Calliandraleaf) + *Myristic Acid* 30 g/Kg DM

III. OBSERVATION VARIABLE

THE MEASUREMENT OF VFA PARTIAL CONCENTRATION

The analysis of partial VFA was conducted using chromatography gas (CG), supernatant partial VFA could be counted with the formula:

$$\text{Partial VFA} = \frac{Sample\ \ Area}{Standart\ \ Area} \times \text{concentration}$$

Note:
MW = Molecular Weight from acetate, propionate, and butyrate.

Analysis of NH_3 level from Residue of Incubation Gas Production 48 hours

The measurement of NH_3 was conducted using Conway Micro Diffusion method (1957), the calculation of NH_3 as follow:

$$\text{Level } NH_3 \text{ mM rumen fluid} = \frac{\text{ml } H_2SO_4 \times \text{n } H_2SO_4 \times 1000}{\text{ml Sample} \times \text{DM sample \%}} \times 100$$

Note:
ml H_2SO_4 = Titration H2SO4
N. H_2SO_4 = Normality H2SO4
ml Sample = total of used sample

DATA ANALYSIS

Data were analyzed statistically using Analysis of Variance (ANOVA) with Randomized Block Design. If there were significant or very significant distinctions in every treatment, it was continued with Duncan Multiple Range Test.

IV. RESULT AND DISCUSSIONS

The analysis result of feedstuff nutrient content can be seen in Table 1. The applied ingredient feeds in this research were cascara, bran, tapioca residue starch powder, soybean meal, palm kernel meal, copra meal, urea, molasses, corn straw, and calliandra leaf flour. The percentage of treatment feed can be seen in Table 2. The reduction of feedstuff percentage of protein source in P3-P5 was parallel with calliandra leaf addition. The reduction of feedstuff percentage was caused by calliandra, which had high protein and expected to change protein from the feed with the reduction of feed percentage of protein source. The applied *myristic acid* contained 99.7% *myristic acid*, 0.1% *lauric acid* and *palmitic acid*, and 0.1% other substances.

The tannin content in calliandra leaf can be seen in Table 1. The applied tannin content in the powder sample of calliandra Leaf was 8.86%, with tannin content in calliandra leaf flour was 0.46%. The result of calliandra tannin content was less than the result of Sestyawati, Putra, and Roni (2017), precisely 10%. It was caused by drying and the age level of calliandra leaf. According to Widarta and Wiadnyani (2019), drying and the leaf's age level could affect bioactive compounds in the particular material. According to Katno, Kusumadewi, and Sutjipto (2005) stated the duration of drying could affect the tannin level of material.

Table 1:The Raw Materialof Complete Feed Based on Corn Straw

Raw material	DM*	OM*	Ash*	CP*	CF*	EE*	Tannin (%)	C.Tannin (%)
Cascara	94.14	89.42	10.58	10.11	34.00	1.50	-	-
Bran	90.63	87.40	12.60	10.15	16.20	13.00	-	-
Tapioca residue	92.59	82.87	17.13	1.76	25.39	0.44	-	-
Soybean Meal	93.53	91.62	8.38	47.53	4.04	2.57	-	-
Palm Kernel meal	95.39	94.97	5.03	14.24	20.91	10.01	-	-
Copra Meal	95.69	92.23	7.77	22.12	21.78	2.45	-	-
Urea	99.88	99.93	0.07	244.60	-	-	-	-
Molasses	78.47	84.57	15.43	4.55	9.81	-	-	-
Corn Straw	94.46	89.83	10.17	5.13	36.43	0.63	-	-
Calliandra leaf flour	93.67	92.37	7.63	23.16	12.08	3.90	8.86	0.46

Note: - * Based 100%DM
- Analysis results of Animal Nutrition and Forage Laboratory, Faculty of Animal Science, Brawijaya University (2020).
- The analysis of tannin nutrient was conducted in Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta (2020)
- The analysis of tannin condensation content (c. tannin) was conducted at BPPT Ciawi, Bogor (2021)

The content of treatment feed nutrient can be seen in Table 3. The expected nutrient was feed protein 14-15%. The addition of calliandra leaf and *myristic acid* on the feed affected the nutrient content of the feed. The addition of calliandra leaf affected crude fiber (CF) content in the treatment feed showed an increase in the feed treatment's. The CF content in the treatment feed was almost no different if it was compared to the control feed without the addition of *myristic acid*. It was different from research conducted by Muchlas, Chuzaemi, and Mashudi (2020) using complete feed based on rice straw with the addition of myristic acid level 20, 30, and 40 g/Kg DM and tannin from mimosa powder. In this research, the addition of *myristic acid* level 20, 30, and 40 g/kg DM affected crude fiber on feed. The over high-fat level on feed (over 5%) had the negative effect of fiber digestibility inside rumen (Wina and Susana, 2013).

Table 2:Feed Treatment Percentages

	Treatments (%)				
Raw material	P1	P2	P3	P4	P5
Cascara	17	17	17	17	17
Bran	20	20	18	18	16
Tapioca residue Starch Powder	10	10	10	10	10
Soybean Meal	21.67	21.67	19	18	17
Palm Kernel meal	13.33	13.33	10	8	7
Copra Meal	12	12	10	8	7
Urea	1	1	1	1	1
Molasses	5	5	5	5	5
Calliandra leaf flour	0	0	10	15	20
Total	100	100	100	100	100
Myristic acid (%/kg DM)	0	3	3	3	3
Corn Straw	40	40	40	40	40

Table 3.The Content of Complete Feed Nutrient Based on Corn Straw

Treatments	DM (%)	OM (%)	Ash (%)	CP (%)	CF (%)	EE (%)
P1	92.54	93.38	6.58	15.41	17.12	3.71
P2	93.64	93.07	6.91	15.66	19.19	3.72
P3	93.12	93.15	6.85	14.36	19.99	3.65
P4	92.61	93.34	6.66	15.96	19.97	2.89
P5	92.72	93.28	6.72	15.04	19.64	2.79

Note:- Analysis results in Animal Nutrition and Forage Laboratory, Faculty of Animal Science,Brawijaya University (2020).

- P1 = Complete Feed (40% Corn Stover + 60% Concentrate), P2 = Complete Feed (40% Corn Stover + 60% Concentrate + 0% Calliandra) + MA 30g/kg DM, P3 = Complete Feed (40% Corn Stover + 50% Concentrate + 10% Calliandra) + MA 30g/kg DM, P4 = Complete Feed (40% Corn Stover + 45% Concentrate + 15% Calliandra) + MA 30g/kg DM, P5 = Complete Feed (40% Corn Stover + 40% Concentrate + 20% Calliandra) + MA 30g/kg DM.

Total VFA, Proportion of Acetic Acid, Propionic, Butyric Acid and the Ratio of Acetate and Propionate in Complete Feed by *In Vitro*

The analysis result of total VFA, acetic (C2), propionate (C3), butyrate (C4), and the ratio of acetate/propionate (C2/C3) in complete feed based on corn straw with the addition of calliandra leaf and *myristic acid* could be seen in Table 4.

Table 4: The Average Value of Total VFA Acetate (C2), Propionate (C3), Butyrate (C4) and the ratio of acetate/propionate (C2/C3).

Treatments	C2 (mMol)	C3 (mMol)	C4 (mMol)	C2/C3 (mMol)	Total VFA (mMol)
P1	34.45	21.80	17.12	1.60	73.36
P2	33.81	18.65	16.69	1.82	69.15
P3	32.35	18.61	16.61	1.73	67.57
P4	30.43	17.77	15.07	1.66	63.27
P5	30.07	19.51	18.39	1.58	67.97

VFA (*Volatile Fatty Acid*) is a metabolic product produced rumen microbe from carbohydrate degradation. Carbohydrate, fiber, and non-nitrogen-freeextract (NNFE) (Ma'rifatunnisa, Tanuwiria, and Hernawan, 2015). The research result showed total VFA had no significant difference (P>0.05) to a given treatment. The highest concentration of VFA was P1, 73.36 mMol, and the lowest was P4, 63.27 mMol. Hidratiningrum, Bata, and Santosa (2011) the factors that affected VFA concentration were types of microbe inside rumen, absorption, and fermentability of carbohydrate source feed. Dhia, Kami and Tanuwiria (2019) stated that the product of total VFA for livestock survival was approximately from 70-150 mM. The total VFA of this research showed that P1 was still within the normal range, but P2-P5 showed insignificant degradation. It was due to P1 feed easily fermented by rumen microbe; therefore, it produced a high concentration of VFA while P2-P5 feed contained tannin and *myristic acid*; therefore, it was not degraded easily by microbe inside rumen.

Carbohydrate was the primary energy source from easily-fermented feed inside within rumen to produce VFA (Amri and Yurleni, 2014). Black and Kenny (1984) stated that carbohydrate within rumen was almost entirely fermented into VFA. Rumen microbe hydrolyzed cellulose into monosaccharides, and then it formed into VFA.VFA was an energy source for livestock and a source of carbon framework to form microbe protein.VFA concentration within rumen described the equilibrium between production speed and expanse for each VFA as an inter-conversion process. The speed of VFA absorption was affected by concentration, osmotic pressure, and pH rumen. The research conducted by Muslimah *et al.*, (2020) applied tengkawang meal added 9,75%, 19,64%, 30,09%, and 40,08% in beef cattle feed *in vitro*. The research stated that the more addition of tangkawang meal, the obtained VFA concentration would be higher. It showed that carbohydrates and protein in tengkawang meal were easily fermented by rumen microbe. Bhatta *et al.*, (2009) conducted research using

condensation and hydrolysis tannin provided to ruminant feed. The result stated that the addition of condensation and hydrolysis tannin decreased total VFA, which was better than only added condensation tannin. Patra (2010) stated that most tannin supplementation tannin decreased the digestibility of feed, for instance, CP, NDF, and ADF, and tended to decreased total VFA because tannin interacted with other macromolecules such as protein and carbohydrate.

Bauman *et al.*,(2003) stated that fat addition to feed had a negative effect on rumen microbe because it obstructed the activity of rumen microbe and decreased fiber digestibility. It was caused by fat that coated feed particles. Therefore, it could prevent bacteria attachment in feed even though fat was expected to increase energy in feed without depending on VFA production. Ma'rifatunnisa, Tanuwiria, and Hernaman (2015) stated that reducing the protozoa population obstructed bacteria activity, especially digestive bacteria. Therefore bacteria could not change carbohydrate optimally into VFA. It was also supported by Purwanto, Bata, and Rahayu (2019) that protozoa were the primary predator of rumen bacteria. Rumen bacteria were required for ruminants to digest crude fiber that would produce a product of rumen fermentation in VFA and $N\text{-}NH_3$ for livestock productivity. That statement was different from Suharti*et al.,* (2005) that conducted research using supplementation of canola and flaxseed oil protected in vitro. The result showed that supplementation of flaxseed oil tended to increase total VFA, and the proportion of propionate acid had the potency of energy source. The result showed that the addition of protected fat did not obstruct rumen microbe activity within feed fermentation.

ACETATE ACID

The research result of acetate acid proportion (C2) of total VFA on a complete feed based on corn straw added tannin from calliandra leaf, and *myristic acid* could be seen in Table 4. The result showed that the C2 proportion on completed feed was not a significant distinction (P>0.05). The highest content of C2 was P1, 34.45 mMol, and decreased continuously until the lowest was P5, 30.07 mMol. Those results showed that the addition of tannin and *myristic acid* could decrease C2 proportion in feed. The reduction of C2 concentration in feed was parallel to the addition of calliandra leaf as a tannin source that decreased the protozoa population within complete feed at the same time. Vogels, Hope, and Stumm (1980) stated that protozoa were agents changing acetate and butyric acid. The research conducted by Bhatta *et al.,* (2009) added tannin in the feed that produced proportion of acetate acid, which decreased with the addition of tannins, and was parallel to the reduction of protozoan population in that study.

PROPIONATE ACID

The research result of propionate acid proportion (C3) of total VFA on a complete feed based on corn straw added tannin from calliandra leaf, and *myristic acid* could be seen in Table 4. The result showed that the C3 proportion on completed feed was not a significant distinction (P>0.05). The highest content of C3 was P1, 17.12 mMol, and the lowest was P4, 17.77 mMol. Those results showed that the addition of tannin and *myristic acid* on complete feed decreased the C3 proportion of total VFA. Those results were different from Wina, Muetzel, and Becker (2005) stated that the primary effect of tannin addition to rumen fermentation was pole conversion of short-chain fatty acid that increased propionate proportion and decreased ratio of acetate: propionate (C2/C3).

BUTYRIC ACID

The research result of butyric acid proportion (C4) of total VFA on a complete feed based on corn straw that was added tannin from calliandra leaf and *myristic acid* could be seen in Table 4.Those results showed that C4 proportion on completed feed was no significant difference (P>0.05). The highest content of C4 was P1, 21.80 mMol, and the lowest was P4, 15.07 mMol. Those results showed that the addition of tannin and *myristic acid* on complete feed decreased the C4 proportion of total VFA. According to Suhartanto (2014) stated that 90% of *butyric acid* was changed into a ketone object which was applied to arrange fat or applied as an energy source in the citrate cycle. The addition of tannin and *myristic acid* on the feed decreased C4 proportion because it was protected by tannin; therefore, it was not degraded easily by rumen microbe.

THE COMPARISON OF ACETATE AND PROPIONATE ACID

The research result of ratio proportion of acetate and propionate acid (C2/C3) of total VFA on a complete feed based on corn straw that was added tannin from calliandra leaf and *myristic acid* could be seen in Table 4.Those results showed that C2/C3 proportion on completed feed was not significantly different (P>0.05). The highest C2/C3 was P2, 1.82 mMol, and the lowest was P1, 1.60 mMol. Those results showed that the addition of tannin and *myristic acid* on complete feed increased the C2/C3 proportion of total VFA. It indicated that feed was not degraded easily by rumen microbe; therefore, it was expected absorbed in the post-rumen channel then utilized directly by livestock. According to Yost *et al.*, (1997), the maximal ratio of acetate and

propionate to add ruminant weight was 3:1. The current research had filled the ratio of ruminants weight addition.

Ammonia Concentration (NH_3)

The research analysis of ammonia and Microbe Protein Synthesis (MPS) concentration on complete feed based on corn straw by adding calliandra leaf flour and *myristic acid* could be seen in Table 5.

Table 5.Average Value of Ammonia Concentration

Treatments	Amonia (NH_3) (Mmol)
P1	6.77
P2	6.51
P3	6.64
P4	6.35
P5	6.54

NH_3 concentration was the result of degradation within rumen by proteolytic bacteria required for rumen microbe growth because NH_3 was a primary source for microbe protein synthesis and applied to fulfill the needs of rumen microbe protein (Dhia, Kamil and Tanuwiria, 2018). The result of variance analysis can be seen in Table 5. In Table 5, it was discovered that the addition of tannin derived from calliandra leaf and *myristic acid* was no significant difference in NH_3 concentration. The highest concentration of NH_3 was P1, 6.77 mMol and the lowest was P4 6.35 mMol. Although it was not significantly different from variance analysis, the addition of tannin and *myristic acid* in feed could decrease NH_3 concentration than P1. The research result was comparable to the research conducted by Ningrat*et al.,* (2016) using tannin derived from the gambir leaf. Those results showed that the addition of tannin from the gambir leaf on feed did not significantly differ in NH_3 concentration invariance analysis, but it could decrease NH_3 concentration.

The degradability level of feed protein was one of the factors that affected NH_3 concentration (Prayitno, Wahyono, and Pangestu, 2018). Suparwi, Santoso dan Samsi (2017) stated that types of feed, chemistry composition of feedstuffs, and non-structural carbohydrate fraction in feedstuffs highly affected NH_3 level. Dhia, Kamil, and Tanuwiria (2018) stated that the high NH_3 concentration indirectly showed the more prominent feed protein degraded by rumen microbe; therefore, the more prominent protein was also wasted. Suparwi, Santoso, and Samsi (2017) added that the high NH_3 concentration was due to soluble crude protein and carbohydrate.

The reduction of NH_3 concentration was due to tannin addition derived from calliandra leaf and *myristic acid* on complete feed. Min *et al.,* (2003) stated that tannin could increase the feed benefit value of protein source by reducing the rumen's protein degradation. It caused more available amino acids (mainly essential amino acids) absorbed in the small intestine. Cahyani, Nurwantara, and Subrata (2012) said that tannin could decrease fermentability due to protein tannin complex bonds' formation. The NH_3 production was affected by a total of CP degradation in the rumen. The higher CP degradation in rumen would increase ammonia production. Otherwise, the lower CP degradation in rumen would decrease ammonia production. Abrar and Fariani (2018) conducted research using tannin from sorghum seed powder with 0.15% tannin concentration applied to elephant grass with *in vitro*. Those results illustrated that the tannin addition in feed could decrease NH_3 concentration. Other research conducted by Jenny, Surono, and Christiyanto (2012) applied tannin from tea dregs given to kapok seed meal with 0,25, 0,5, 0,75% tannin concentration. Those results showed that the tannin addition of tea dregs could decrease NH_3 concentration. The reduction of NH_3 showed protein degradability reduction of kapok seed meal by rumen microbe. It was due to the extract of tea dregs contained compensated tannin that could form a complex compound with kapok seed protein, therefore decreased the protein solubility of kapok seed meal and decreased its degradability in the rumen, which in turn will reduce the concentration of NH3.

The fat addition in feed could also decrease NH_3 concentration. Tiven*et al.,* (2012) conducted the research using crude palm oil, which was added to feed sheep feed. Those results showed that the addition of crude palm oil could decrease NH_3 concentration in livestock. The fat addition in livestock could obstruct microbe metabolism because the fat in feed could cover the microbe body; therefore, it interfered with enzyme production to degrade feed. This statement was also supported by the significant reduction of the protozoa population due to this research. Other research conducted by Onetti*et al.,* (2001) used types of fat with different levels given to dairy cattle with feedstuff in the form of corn silage. Those results showed that the fat addition in feed could decrease NH_3 concentration, but it was insignificant even though 4% fat addition was identical with NH_3 concentration of feed control without fat addition. Ainunsia*et al.,* (2020) conducted research using CPO with 0%, 4%, 8%, 12% concentration added to livestock feed such as grass and tofu dregs as a single protein

source in feed. Those results showed that the CPO addition in feed could decrease NH_3 concentration in the feed. The reduction of NH_3 concentration by CPO was due to feed protein protected by CPO from the fermentation process conducted by proteolytic bacteria.CPO is a type of oil with associated characteristics with feed particles; therefore, feed is covered by those compounds. As consequently of feed particles isolation by these compounds, the proteolytic enzymes produced by these bacteria were challenging to penetrate the feed protein. Besides, oil can be toxic to bacteria; therefore, it was suspected that rumen bacteria, especially proteolytic bacteria, would have their growth inhibited.

Protein synthesis would be maximized if supported by NH_3 production in the rumen for 4-12 mM (Cahyani, Nurwantara, and Subrata, 2012). The tannin addition of calliandra leaf and *myristic acid* to complete feed based on corn straw with a concentration range of NH_3 6.77 – 6.35 mMol. In the current research, it was found that the NH_3 concentration could meet the needs of livestock to carry out the protein synthesis process; therefore, the addition of tannins and myristic acid in feed decreased the NH3 concentration, but it did not interfere with protein synthesis in the rumen.

V. CONCLUSION

The use of calliandra leaf as a tannin source and *myristic acid* in complete feed based on corn straw did not affect partial VFA and NH_3 concentration. This research's best treatment was P4 because it had low comparison value C2:C3 and NH_3 value-filled livestock needs.

SUGGESTION

Based on the research result,further research was suggested by *in vivo* using livestock.

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