Research Article

In-vitro Digestibility of Ammoniated Aromatic Supplemented Lemongrass Waste

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Abstract | Aromatic lemongrass waste is promising as an alternative roughage source for grass substitution for ruminants, but the lignin content is relatively high, so it has low digestibility. We evaluated the effectiveness of precursor rumen microbial growth supplementation to increase the digestibility of ammoniated aromatic lemongrass waste. The precursor rumen microbial growth supplement contains cassava leaves as a source of branched-chain amino acids, phosphorus, and zinc minerals, which was administered as follows: T0= ammoniated aromatic lemongrass waste, T1= T0 + 5% cassava leaf, T2= T1 +0.4% mineral phosphorus, T3= T1 +100 ppm mineral zinc, and T4= T1 + 0.4% mineral phosphorus and 100 ppm mineral zinc. The degradation of various components such as dry matter, organic matter, crude protein, neutral detergent fiber, acid detergent fiber, cellulose, and hemicelluloses in the ammoniated aromatic lemongrass waste samples was evaluated by incubating them on *in-vitro* media at 39°C/48 h. The analysis of variance following a 5 x 4 randomized block design was used. The results revealed a significant (P<0.05) increase in the digestibility of ammoniated aromatic lemongrass waste in the rumen. The degradation of ammoniated aromatic lemongrass waste was found to be significantly (P<0.05) higher when supplemented with cassava leaves and mineral zinc (T3) compared to others. However, it was observed to be significantly (P<0.05) lower than T4. Cassava leaf, mineral phosphor, and zinc (T4) were found to be the most effective in enhancing the digestibility of ammoniated aromatic lemongrass waste in the rumen.

Keywords | Ammoniated aromatic lemongrass waste, Cassava leaf, Digestibility, Phosphor, Zinc.

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INTRODUCTION

The use of agricultural and plantation waste is very potential to overcome the crisis of ruminant roughage. Among the potential agricultural wastes is aromatic lemongrass (*Cymbopogon nardus L.*) waste. Aromatic lemongrass is one of the most famous volatile plants in Indonesia which is obtained from the distillation process of aromatic lemongrass to produce essential oils. Elihasridas et al. (2023) reported that aromatic lemongrass waste nutritional content was adequate with a protein content of 7.72%, higher than rice straw (3.93%). Aromatic lemongrass waste is promising as an alternative roughage source for grass substitution for ruminants, but the lignin content is relatively high at 27.7% (Kamoga et al., 2013) so it has low digestibility.

Chemical processing technology, namely ammoniation, has succeeded to improve the digestibility of the waste, but its application did not provide maximum results (Suyitman et al., 2021). To increase the digestibility of aromatic lemongrass waste, the bioprocess in the rumen can be optimized through the supplementation of microbial growth precursor nutrients. Supplementation is useful in overcoming division and will increase the digestive capacity of an animal via improved metabolism and rumen microbial performance. Rumen microbial growth can be driven by the addition of branch chain amino acids that can be supplied from cassava leaf (Suyitman et al., 2017; Arief et al., 2023a, 2023b). Rumen microorganisms require trace element supplementation especially phosphorus and zinc (Jamarun et al., 2020).

Efforts to increase the digestibility of aromatic waste from lemongrass in the rumen must use a holistic approach. Microbes and feed fermentability strongly influence the rumen bioprocess. When all nutrient precursors are available at an optimal concentration, the maximum rate of microbial growth can be attained. Therefore, a combination of feed processing technology and supplementation of nutrients precursor rumen microbial growth can optimize the digestibility of aromatic lemongrass waste in the rumen. We aimed to evaluate the effectiveness of precursor rumen microbial growth supplementation to increase the digestibility and microbial protein synthesis of ammoniated aromatic lemongrass waste.

MATERIALS AND METHODS

Ethical Approval

Since this study did not involve live animals, ethical approval was not necessary.

Material

This study uses aromatic lemongrass waste that is ammoniated with urea $CO(NH_2)_2$, cassava leaf as a source of branched-chain amino acids, TSP fertilizer as a source of phosphor (P), and zinc sulfate ($ZnSO_4$) as a source of zinc (Zn) minerals. Aromatic lemongrass waste was acquired from refining citronella oil in aromatic lemongrass plantations at Limau Manis, Padang, Indonesia. Rumen fluid was obtained from a slaughter house of Kacang goat with an average BW ± 20 kg fed a diet napier grass, legume, and concentrate.

Method

The *in-vitro* study was carried out following Tilley and Terry (1963). This aromatic lemon grass waste increases digestibility through processing technology with ammoniation. The increased rumen bioprocess was carried out through supplementation precursor rumen microbial growth that are branched-chain amino acids from cassava leaves, mineral phosphor from TSP fertilizer, and mineral zinc from $ZnSO_4$ compounds. The treatment consists of: T0 = ammoniated aromatic lemongrass, T1 = T0 + 5% cassava leaf, T2 = T1 + 0.4% mineral phosphor, T3 = T1 + 100 ppm mineral zinc, and T4 = T1 + 0.4% mineral phosphor + 100 ppm mineral zinc. The addition of P mineral dose was following Pazla et al. (2018) and Zn mineral doses were following Elihasridas (2012). A Randomized Block Design with five different treatments and four replicates was utilized. The impact of each treatment was assessed through analysis of variance (ANOVA), while variations between the treatments were compared utilizing Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 2002). The descriptive method with three replications was used to compare the chemical composition of aromatic lemongrass with ammoniated aromatic lemongrass. The variables observed were chemical composition, nutrient digestibility (crude protein, dry matter, organic matter), Fiber fraction digestibility (neutral detergent fiber, acid detergent fiber, cellulose, and hemicellulose), and rumen fluid characteristics (NH_3, total VFA, and microbial protein synthesis). Proximate analysis following AOAC Association of Official Agricultural Chemists (2016) and the analysis of fiber fraction after Van Soest et al. (1991). The concentration of NH_3 after the Conway micro-diffusion method. The steam distillation method measured the VFA concentration.

Making Ammoniated Aromatic Lemongrass Waste

To produce ammoniated aromatic lemongrass waste, the method proposed by Elihasridas et al. (2023) was followed. Aromatic lemongrass waste is cut into 3-5 cm pieces and stirred evenly with dried chicken manure 10% from the dry matter of aromatic lemongrass waste (a source of urease enzyme for accelerating the conversion of urea into NH_3 and shorten the curing process). Urea 4% was dissolved in water, where the ratio of water and dry matter aromatic lemongrass waste used is 1: 1. Aromatic lemongrass waste is put into a 50 lt plastic drum layer by layer then sprayed with urea solution and compacted so that the atmosphere is anaerobic. After the drum was filled, cover it with 2 layers of plastic and tie it with a rope. The drum was stored in a safe and shaded place for 10 days. The drum was opened, and aromatic lemongrass waste was removed and dried (2 days) to remove excess ammonia gas and ground.

Statistical Analysis

A Randomized Block Design with five different treatments and four replicates was utilized. The statistical analysis of the data was conducted by utilizing Analysis of Variances via IBM SPSS Statistics (USA) Version 21.0 software. Additionally, the Duncan Multiple Range Test (DMRT) was employed for further testing.

Table 1: Chemical composition of aromatic lemongrass waste and ammoniated aromatic lemongrass waste (n=3)

Chemical composition (%)	Aromatic Lemongrass Waste	Ammoniated Aromatic Lemongrass Waste
Dry matter	34.87±0.44	32.17±0.07
Ash	8.04±0.32	9.66±0.19
Organic matter	91.96±0.23	90.34±0.05
Crude protein	7.21±0.18	11.08±0.45
Ether extract	2.24±0.52	1.97±0.17
Crude fiber	26.52±0.20	21.31±0.38
Ca	0.40±0.12	0.37±0.20
P	0.16±0.08	0.15±0.19

Table 2: Nutrient digestibility of the treatment

Nutrient Digestibility (%)	T0	T1	T2	T3	T4
Dry matter	53.21±0.13^{d}	57.17±0.45^{c}	62.24±0.20^{b}	59.53±^{c}	67.84±0.30^{a}
Organic matter	58.34±0.52^{d}	62.18±0.39^{c}	68.45±^{b}	66.76±0.59^{b}	72. 81±0.65^{a}
Crude protein	61.23±0.19^{d}	68.75±0.42^{b}	74.67±0.48^{a}	69.62±0.24^{b}	76.72±0.36^{a}

Note: Different superscripts (a, b, c, d) in the same line showed significant differences (P<0.05).
T0 = Ammoniated Aromatic Lemongrass, T1 = T0 + 5% Cassava Leaf, T2 = T1 + 0.4% Mineral Phosphor, T3 = T1 + 100 ppm Mineral Zinc and T4 = T1 + 0.4% Mineral Phosphor + 100 ppm Mineral Zinc.

Table 3: Fiber fraction digestibility of the treatment

Digestibility (%)	T0	T1	T2	T3	T4
NDF	48.13±0.09^{d}	52.61±0.16^{c}	58.36±0.07^{b}	56.47±0.15^{b}	63.73±0.17^{a}
ADF	46.60±0.02^{c}	52.82±0.07^{b}	56.73±0.05^{b}	53.82±0.09^{b}	60.64±0.12^{a}
Cellulose	45.57±0.72^{c}	51.61±0.65^{b}	54.83±0.82^{b}	52.75±0.61^{b}	57.33±0.76^{a}
Hemicellulose	50.77±0.55^{d}	56.72±0.43^{c}	61.65±0.67^{b}	60.73±0.26^{b}	69.22±0.18^{a}

Note: Different superscripts (a, b, c, d) in the same line showed significant differences (P<0.05).
T0 = Ammoniated Aromatic Lemongrass, T1 = T0 + 5% Cassava Leaf, T2 = T1 + 0.4% Mineral Phosphor, T3 = T1 + 100 ppm Mineral Zinc and T4 = T1 + 0.4% Mineral Phosphor + 100 ppm Mineral Zinc.

Table 4: Total VFA, N-NH_3 and microbial protein synthesis (MPS) of the treatment.

Parameters	T0	T1	T2	T3	T4
V F A (mM)	102.56±0.08^{d}	110.44±0.12^{c}	121.97±0.29^{b}	112.63±0.43^{c}	133.47±0.21^{a}
N-NH_3 (mg/100 ml)	7.73±0.39^{b}	10.34±0.45^{a}	11.95±0.64^{a}	11.32±0.39^{a}	12.97±0.51^{a}
MPS (mg/l/h)	70.09±0.45^{d}	81.22±0.16^{c}	87.64±0.18^{b}	83.43±^{c}	94.87±0.28^{a}

Note: Different superscripts (a, b , c, d) in the same line showed significant differences (P<0.05).
T0 = Ammoniated Aromatic Lemongrass, T1 = T0 + 5% Cassava Leaf, T2 = T1 + 0.4% Mineral Phosphor, T3 = T1 + 100 ppm Mineral Zinc and T4 = T1 + 0.4% Mineral Phosphor + 100 ppm Mineral Zinc.

RESULTS

Chemical Composition of Aromatic Lemongrass Pre- and Post-Ammoniation

The crude protein contents of ammoniated lemongrass waste were higher than that of non-ammoniated waste. The ammoniation of aromatic lemongrass waste (Table 1) increased the crude protein contents from 7.21 to 11.08% and decreased the crude fiber contents from 26.52 to 21.31%.

In-Vitro Digestibility

The treatment had a significant impact (P<0.05) on the digestibility of dry matter, organic matter, and crude protein. The digestibility of dry matter and organic matter in the T0, T1, and T2 increased, decreased in the T3, and increased in the T4. The T4 had the highest digestibility for dry matter and organic matter, while the lowest digestibility was found in the T0 (Table 2).

Supplementation of phosphorus (P) and cassava leaves on ammoniated aromatic lemongrass waste (T2) showed a significant (P<0.05) increasing the digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and hemicellulose. Supplementation of Zn in the T3 revealed a significant (P<0.05) increasing the digestibility of Ammoniated aromatic lemongrass waste but was not significantly different from T2. Table 3 showed that the T4 exhibited the greatest digestibility for ADF, NDF, cellulose, and hemicellulose, whereas the lowest values were observed in the T0 treatment.

VFA, NH_3, and Microbial Protein Synthesis

The overall production of volatile fatty acids (VFA), ammonia (NH_3) concentration and microbial protein synthesis are presented in Table 4. VFA and NH_3 concentrations were significantly (P<0.05) influenced by the treatment, while microbial protein synthesis was significantly (P<0.05) different for each treatment. The highest microbial protein synthesis was produced in the T4, T2, T3, T1, and T0 respectively (Fig. 3). Concentrations of VFA, NH_3, and rumen microbial protein synthesis were the same as the digestibility patterns of dry matter, organic matter, and fiber fraction of the rations. The VFA concentration of the T4 was significantly (P<0.05) higher than the other treatments (Fig. 2), but the T1 and T3 were not significantly different, while the NH_3 concentrations of the T1, T2, T3, and T4 were not significantly different and tended to be high in the T4 (Fig. 1). Increased synthesis of microbial protein in the rumen will have an impact on increasing feed digestibility and increasing the supply of microbial protein for ruminants. There is a strong correlation between the availability of NH_3 and VFA and microbial protein synthesis (Fig. 4 and Fig.5) with correlation values of 0.91 and 0.95, respectively.

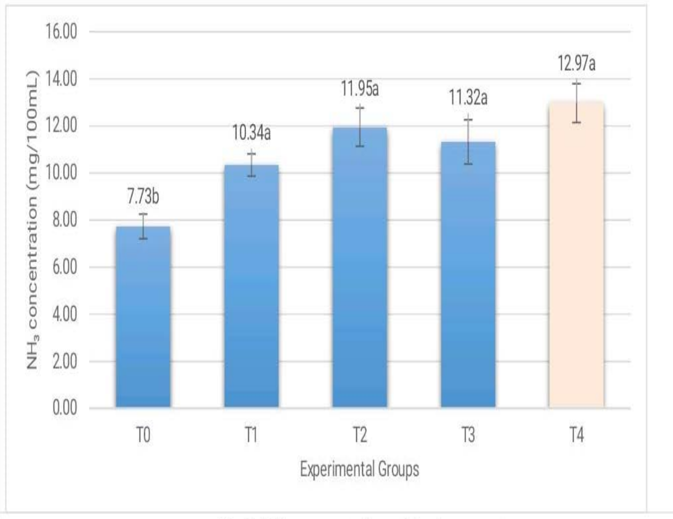

Figure 1: NH_3 concentration of the treatment

Figure 2: Total VFA production of the treatments

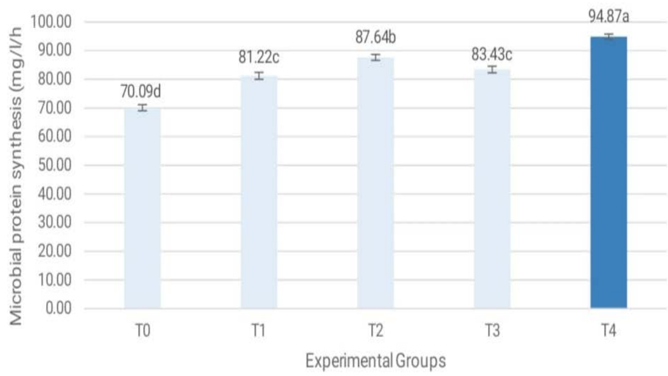

Figure 3: Microbial protein synthesis of the treatments

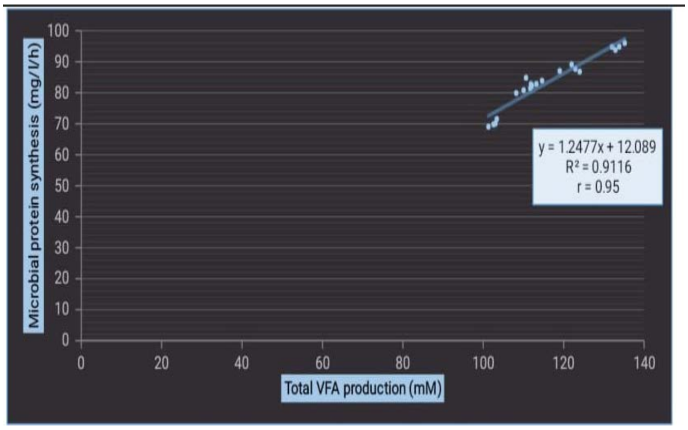

Figure 4: Correlation Microbial protein sysnthesis with Total VFA production

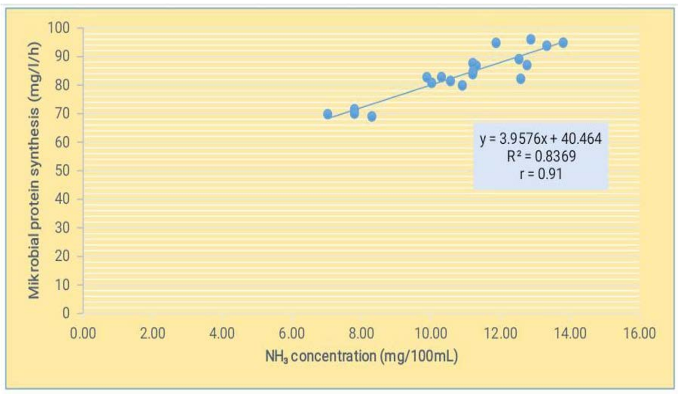

Figure 5: Correlation Microbal protein sysnthesis with NH_3 Concentration

DISCUSSION

Chemical Composition of Aromatic Lemongrass Waste Before and After Ammoniation

The ammoniation on aromatic lemongrass reduced dry matter by 7.74%. This decrease is due to the incubation treatment with urea's addition, which can remodel the aromatic lemongrass's ridged texture to become softer by NH_3 from urea (Pazla et al., 2018a). The softening of the material's texture is due to the dissolution of silicate minerals and the hydrolysis of lignocellulose and lignohemicellulose bonds (Rusli et al., 2021). These results followed Muthalib et al.'s (2018) opinion that urea's addition to low-quality materials can hydrolyze the bonds that exist between lignin and both cellulose and hemicellulose, break the bonds between hemicellulose and acetyl groups and reduce or eliminate cellulose crystals. Research by Suryani et al. (2016) showed that ammoniation on palm leaves and fronds with added urea could reduce the dry matter contents.

The addition of urea will cause ammonia to be absorbed and bound to bind to the acetyl group of the feed ingredients to form ammonium acetate salt, which is eventually counted as crude protein (Maluyu, 2014). We recorded an increase in the crude protein contents of the aromatic lemongrass by 53.68% which was higher than the 32.2% by Fariani and Akhadiarto (2009) in ammoniated corn cobs. The decrease in crude fiber contents by 19.61% was caused by optimal urea in hydrolyzing the course fiber bonds of the aromatic lemongrass so that the ammoniated aromatic lemongrass had a softer texture. Besides, the optimal density of feed ingredients causes anaerobic conditions in the feed ingredients. If the feed ingredients are in an anaerobic state, the glucose in these materials can be converted into pyruvate, combining with hydrogen to form lactic acid and ethanol. Anaerobic conditions can generate heat from ammonia gas, speeding up the ammonia process time because it makes it easier to break the lignocellulose bonds (Kucharska et al., 2018).

In-vitro Digestibility

Feed digestibility in the rumen is not only determined by the fermentability of the feed but also determined by the proliferation of microorganisms within the rumen. Our results showed that supplementation of cassava leaf (T1) caused a significant increase in the digestibility of ammoniated aromatic lemongrass waste. This proves that cassava leaf as a supplier of branched-chain amino acids could increase rumen bioprocess or increase rumen microbial growth, especially cellulolytic bacteria. Degradation of the branched-chain amino acid (BCAA) in the rumen produces branched-chain fatty acids (BCFA).

If the feed has low protein contents (the use of fiber feed), fatty acid usually becomes a limiting factor for rumen microbial growth, especially cellulolytic bacteria. Zain et al. (2002) showed that the addition of amino acids or peptides tends to increase the number of cellulolytic bacteria and rumen microbial growth. Zain and Elihasridas (2003) and Suyitman et al. (2020) reported that supplementation of cassava leaf as a source of branched amino acids increased the digestibility of nutrition ammoniated palm fiber and palm leaf. The same results were reported by Nurhaita and Ningrat (2011) in ammoniated palm leaf rations. Arief and Pazla (2023) reported that cassava leaves can increase the digestibility and milk production of the Etawa crossbreed. Supplementation of P and cassava leaf to ammoniated aromatic lemongrass waste (T2), significantly increased the dry matter, organic matter, crude protein, and fiber fraction (NDF, ADF, cellulose, and hemicellulose) digestibility. Phosphorus is needed by all microbial cells primarily to maintain the integrity of cell membranes and cell walls, components of nucleic acids, and parts of high-energy molecules (ATP, ADP, etc.) (Pazla et al., 2020). *In-vitro* batch system research or continuous culture showed that P is needed for the cell wall fractions degradation, and the need for cellulolytic bacteria against P is more crucial compared to hemicellulolytic or amylolytic bacteria (Jamarun et al., 2017a, 2017b, 2017c). Kennedy et al. (2000) showed that supplementation of P in phosphate form *in-vitro* was able to increase NDF digestibility from bagasse. Supplementation of P, S, and Mg in palm fronds fermented with *Phanerochayte chrysosporium* increases the characteristics of rumen fluid and rumen microbial protein synthesis (Febrina et al., 2016; Febrina et al., 2017). Pazla et al., (2018b) reported that palm fronds fermented with *Phanerochayte chrysosporium* plus 2000 ppm P minerals resulted in the highest cellulose digestibility. High available P in fermented tithonia forage can increase nutrient digestibility (Pazla et al., 2021a, 2021b).

Our results revealed that Zn supplementation (T3) had a significant effect on increasing the digestibility of ammoniated aromatic lemongrass waste, but it was not significantly different from T2. The reason for this is that P and Zn are vital for the growth and activity of rumen microbes. Although the difference was not significant, the digestibility value was higher in the T2 when P was added than in the T3. P is a macro, whereas Zn is a micromineral. Both minerals play a critical role in enhancing the growth and activity of rumen microbes. Zn is an activator of many enzymes that are involved in carbohydrate metabolism, energy, degradation, protein synthesis, and nucleic acids (Jamarun and Pazla, 2022). Zinc (Zn) is often deficient for rumen microbial growth and can maximize feed degradation (Petrič et al., 2020). Insufficient Zn tends to limit microbial protein synthesis (Pathak, 2008). Additionally, Zn activates a variety of enzymes (Ibrahim et al., 2016). Elihasridas (2012) reported that Zn increased the digestibility of fiber fraction (NDF, ADF, and cellulose) of ammoniated corn cobs.

The results showed that treatment T4 had a significant effect on the digestibility of dry matter, organic matter, crude protein, NDF, ADF, cellulose, and hemicellulose compared to the other treatments. The adequacy of nutrients needed by rumen microbes increased their growth and activity, thus increasing the rumen's food degradation. Zain et al. (2019) and Zain et al. (2020) stated that the supplementation of a nutrient must be harmonized with the availability of other nutrients for optimal results. The growth and development of rumen microbes not only require nitrogen, but also the availability of other nutrients such as energy, minerals, and amino acids. (Pazla et al., 2021c; Pazla et al., 2023a, 2023b). Nurhaita and Ningrat (2011) reported that cassava leaves, S, and P supplementation increased the digestibility nutrients of ammoniated palm leaf.

VFA, NH_3, and Microbial Protein Synthesis

VFA and NH_3 concentrations reflect the fermentability of a ration, the higher the concentration of VFA and NH_3 produced, the higher the level of fermentability of the ration (Pazla et al., 2021d, 2021e; Ikhlas et al., 2023). Supplementation of cassava leaves, P, and Zn succeeded in increasing the ammoniated lemongrass waste digestibility in the rumen. This proves that the addition of supplements can increase the growth and performance of rumen microbes. Concentrations of VFA, NH_3, and rumen microbial protein synthesis produced were the same as the digestibility patterns of dry matter, organic matter, and fiber fraction of the rations. The highest concentrations of NH_3 and VFA were obtained in the T4 treatment.

The VFA and NH_3 production in the rumen is the fermentation result of food substances by rumen microbes, especially carbohydrates, and proteins (Zain et al., 2023). The total VFA production can be used as an indicator of energy availability for livestock. VFA components such as acetic, propionic, and butyric acids will be absorbed through the rumen wall and used as an energy source in various livestock organs through oxidation in the tricarboxylic acid cycle (Jamarun et al., 2021).

The addition of P, Zn, and cassava leaves in the T4 significantly improved the performance of rumen microbes in degrading feed to produce nutrient digestibility, the highest VFA, and NH_3 concentrations (Jamarun et al., 2017a, 2017b, 2017c). Zinc is important for rumen microbial growth and can enhance feed degradation, VFA, and NH_3 concentrations (Petrič et al., 2020). Meanwhile, cassava leaves provide branched-chain amino acids that can stimulate the growth of rumen microbes, particularly cellulolytic bacteria (Suyitman et al., 2020).

The results showed that microbial protein synthesis was significantly different for each treatment. Rumen microbes are the spearhead of food digestion in the rumen, the higher the microbial population in the rumen, the higher the rate of degradation of nutrients in the rumen. The maximum microbial growth rate can be achieved if the supply of all the nutrients needed for microbial growth and development is available in optimum concentrations, especially the supply of energy and protein (Putri et al., 2019, 2021). The high microbial activity requires the availability of sufficient nutrients, especially energy, and protein. An adequate and balanced supply of energy in the form of VFA and NH_3 will optimize fermentation conditions in the rumen and increase the growth and performance of rumen microbes so that feed digestibility increases (Ningrat et al., 2019; Ningrat et al., 2020). There is a strong correlation between the availability of NH_3 and VFA and microbial protein synthesis. According to Sugiyono (2007), a correlation value in the range of 0.8-1 is considered strong.

Increased microbial protein synthesis in the rumen will have an impact on increasing feed digestibility and increasing the supply of microbial protein for ruminants. Rumen microbes contribute quite a lot of protein to the needs of ruminants. According to Hackmann and Firkins (2015), rumen microbes supply 50 -80% protein to meet the needs of amino acids for ruminants. Therefore efforts to optimize microbial protein synthesis need to be a concern in meeting the needs of amino acids for ruminants.

CONCLUSION

Supplementation of nutrients precursor that promote the growth of rumen microorganisms increased the digestibility of ammoniated aromatic lemongrass waste. Supplementation of cassava leaf, mineral phosphor, and zink produced the best response to the digestibility of dry matter, organic matter, crude protein, NDF, ADF, cellulose, and hemicellulose of ammoniated aromatic lemongrass waste.

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CONFLICT OF INTEREST

The authors declare that they do not have any competing interests.

NOVELTY STATEMENT

Aromatic lemongrass waste, which is a low-quality fiber feed, has not been able to provide high livestock productivity. Supplementation of rumen microbial growth factors (cassava leaves as a source of branched-chain amino acids, Sulfur, and Zn) can improve the fermentability of aromatic lemongrass waste in the rumen and will later be able to provide sufficient nutrition for ruminants.

AUTHORS' CONTRIBUTION

Conceptualization: Mardiati Zain and Elihasridas, Data Curation: Roni Pazla and Mardiati Zain. Funding acquisition: Mardiati Zain. Methodology: Elihasridas. Project administration: Simel Sowmen, and Qurrata Aini. Supervision: Mardiati Zain. Validation: Roni Pazla and Elihasridas. Writing-original draft: Elihasridas. Writing-review & editing: Roni Pazla.

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