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Research Article
Effect of Cassava Leaf Meal Supplementation on *In vitro* Digestibility of Ammoniated Palm Leaf Enriched with Sulphur and Phosphorus Minerals

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Abstract

Background: Palm leaf is a by-product of palm plantations that can be used as a feed alternative. Ammoniation is a potential approach to increase the digestibility of palm by-products. **Objective:** This study aimed to determine the cassava leaf meal supplementation level as a source of branched chain amino acids for ammoniated palm leaf enriched with sulphur (S) and phosphorus (P) minerals as feed for cattle as examined *in vitro*. **Materials and Methods:** Samples of S and P mineral-enriched ammoniated palm leaf supplemented with cassava leaf meal were analyzed for proximate composition. In this study, ammoniated palm leaf samples enriched with S (0.40%) and P (0.27%) were treated with several cassava leaf meal supplementation levels. This study used a randomized block design with 6 treatments and 4 replications. The treatments tested were 6 levels of cassava leaf meal (0, 5, 10, 15, 20 and 25% dry matter). Dry matter, organic matter, crude protein, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), cellulose, hemicellulose and rumen fluid characteristics (pH, VFA concentration and NH_3-N level) were evaluated. **Results:** The results showed that mineral (0.4% S and 0.27% P) and cassava leaf meal (5% of dry matter) supplementation exhibited higher digestibility compared to other treatments. This supplementation formula resulted in the following *in vitro* digestibility characteristics: dry matter (55.56%), organic matter (59.83%), crude protein (55.34%), NDF (56.99%), ADF (50.01%), cellulose (60.78%) and hemicellulose (71.35%). The rumen fluid characteristics of this formula were as follows: pH (6.70), VFA (129.89 mM) and NH_3-N (15.84 g/100 mL). **Conclusion:** Sulphur and P mineral-enriched ammoniated palm leaf supplemented with cassava leaf meal had significantly increased digestibility and rumen fluid qualities. Treatment with 5% cassava leaf meal produced the highest digestibility and rumen fluid characteristics.

Key words: Palm leaf, mineral supplementation, cassava leaf meal, ammoniation, digestibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Branched Chain Fatty Acid (BCFA) is required to synthesize cellulolytic bacteria such as *Ruminococcus* and *Bacteroides*. The BCFA in rumen is produced from decarboxylation and deamination of Branched Chain Amino Acids (BCAA), including valine, isoleucine and leucine. Such amino acids produce isobutyric acid, 2-methyl butyrate and isovalerat[1,2]. Cassava leaf is a BCAA source that contains a high percentage of crude protein with 6.7, 10.9 and 5.46 g/16 g N of isoleucine, leucine and valine, respectively[3].

Palm leaf midrib has great potential to be used as forage, but its utilization as feed is limited due to the low quality of palm leaf midrib. The nutrient content of palm leaf midrib is as follows: 54.12% dry matter, 89.86% organic matter, 8.51% crude protein, 28.48% crude fibre, 59.11% NDF, 42.87% ADF, 24.69% cellulose, 16.24% hemicellulose and 14.21% lignin[4]. The high content of lignin causes low digestibility and patability[5]. Efforts to optimize feed utilization of waste have focused on processing techniques, including physical, chemical, biological or a combination of techniques. Treatment alone results only in a small response to increase digestibility. Therefore, efforts to improve the digestibility of fibrous feed must also be combined with efforts to optimize the bioprocess in the rumen through increasing the rumen microbial population[6,7].

Sulphur (S) and phosphorus (P) are essential minerals for microbial protein synthesis. The content of both minerals are low and often deficient in waste feed, which negatively affects microbial protein synthesis and nutrient degradation. Both mineral supplementations are expected to support the growth and development of optimum rumen microbes, which in turn to improve feed digestibility in the rumen and increase the supply of microbial protein for ruminants. The S and P minerals are essential components for the synthesis of amino acids that contain S (methionine, cystine and cysteine). In addition, S plays an important role in the formation of vitamins and biotin. Sulphur, an inorganic form of ammonium sulphate may improve rumen microbial growth, cattle growth and digestibility[8,9].

In addition to S and P are also needed for microbial protein synthesis. Phosphorus is required by all microbe cells to maintain the integrity of the cell membrane, cell wall components, nucleic acids and high energy molecules (ATP, ADP and others). Specifically, P is needed as an essential element especially for cellulolytic cell walls that have a higher P requirement than hemicelluloses and amylolysis[10].

The purpose of this study was to determine the level of cassava leaf as a source of branched chain amino acids on *in vitro* digestibility of refined palm leaf enriched with S and P minerals. The benefits of this study were to increase the diversity of feedstuffs by plantations, which has great potential as ruminant feed and an alternative solution to address the problem of providing green feed while improving the environment. In addition, the results of this study may also improve the welfare of society and workforce expansion. This study may also be beneficial to the development of science in general and at particular farms.

MATERIALS AND METHODS

Materials: Palm leaves were collected from a palm plantation in Sitiung II, West Sumatra, Indonesia. Sulphur sourced from brimstone and phosphorus sourced from SP-36 fertilizer were purchased from a chemical store in Padang, West Sumatra. McDougall's solution was prepared as a buffer. The milling machine was used to grind the material before analysis. This study was performed during January through May 2016 at the Ruminant Nutrition Laboratory, Faculty of Animal Husbandry, Andalas University, Padang, West Sumatra, Indonesia.

Methods: Palm leaves enriched with S (0.40%) and P (0.27%) minerals were supplemented with various percentages of cassava leaf meal as a source of branched chain amino acids required by cellulolytic bacteria.

The experimental method used was a randomized block design with 6 treatments and 4 replications. The treatments tested were six levels of cassava leaf meal supplementation (0, 5, 10, 15, 20 and 25% of dry matter). The design model used was as follows[11]:

$$Y_{ij} = \mu+P_i+K_j+€_{ij}$$

Parameters observed: The following parameters were measured: dry matter, organic matter, crude protein, NDF, ADF, cellulose and hemicellulose. The following rumen fluid characteristics were measured: pH, VFA concentration and NH_3-N levels.

Statistical analysis: Duncan Multiple Range Test (DMRT) was used to determine significant level at 0.01.

RESULTS AND DISCUSSION

Nutrient digestibility: The nutrient digestibility results for the cassava leaf meal supplementation to ammoniated palm leaf midrib enriched with 0.4% sulphur and 0.27% phosphorus (which yielded the best results in previous studies) are shown in Table 1.

Table 1: Effect of cassava leaf meal supplementation level on the nutrient digestibility of ammoniated palm leaf midrib enriched with sulphur and phosphorus minerals

	Cassava leaf meal (%)						
Digestibility	0	5	10	15	20	25	SE
Dry matter	44.51^{c}	55.56^{a}	50.83^{b}	50.16^{b}	47.59^{bc}	45.38^{c}	1.04
Organic matter	51.09^{c}	59.83^{a}	55.87^{ab}	54.19^{b}	52.04^{bc}	49.96^{c}	0.97
Crude protein	55.21^{ab}	55.34^{a}	64.04^{a}	60.70^{a}	56.43^{b}	50.56^{b}	2.01
NDF	45.42^{c}	56.99^{a}	51.91^{ab}	50.71^{b}	48.78^{bc}	50.19^{b}	1.32
ADF	36.35^{c}	50.01^{a}	44.86^{a}	43.87^{a}	43.71^{ab}	40.78^{bc}	1.69
Cellulose	50.39^{c}	60.78^{a}	56.35^{b}	53.80^{b}	52.29^{bc}	50.27^{c}	0.94
Hemicellulose	65.59	71.35	61.56	65.67	58.41	61.57	3.46

NDF: Neutral detergent fibre, ADF: Acid detergent fibre, values with different superscripts in the same row indicate significant difference ($p<0.05$)

Table 2: Effect of cassava leaf meal supplementation level on rumen fluid characteristics of ammoniated palm leaf midrib enriched with S and P

	Cassava leaf meal (%)						
Rumen fluid characteristics	0	5	10	15	20	25	SE
pH	6.56	6.70	6.47	6.52	6.44	6.41	0.09
Prod. VFA (mM)	96.90^{c}	129.89^{a}	117.41^{ab}	108.02^{b}	104.11^{b}	103.01^{bc}	4.16
NH_3-N (g/100 mL)	11.15^{c}	15.84^{a}	14.39^{ab}	12.67^{b}	12.79^{b}	12.36^{bc}	0.45

Values with different superscripts in the same row indicate a significant difference ($p<0.05$)

The cassava leaf meal supplementation increased the digestibility of nutrients. The highest digestibility was obtained with 5% cassava leaf meal supplementation. Increased levels of cassava leaf meal led to decreased digestibility with 20 and 25% digestibility of the control digestibility. This phenomenon was due to the increase in the number of branched chain amino acids, which are a source of carbon skeletons for rumen microbe synthesis, without sufficient energy for microbial protein synthesis, as indicated by VFA production (Table 2) suggesting that the carbon skeleton provided could not be utilized. Even though there was enough branched carbon skeleton, there was not sufficient quantities of other precursor nutrients, such as energy, protein and minerals for balanced microbial protein synthesis to occur at its optimum.

Improved digestibility of nutrients was obtained by supplementing cassava leaf meal containing branched chain amino acids. Decarboxylation and deamination of branched chain amino acids produces branched chain fatty acids, including isobutyrate, 2-methyl butyrate and isovalerate, which composes the branched carbon framework required for the synthesis of cellulolytic bacteria, such as *Ruminococcus* and *Bacteroides.* A previous study has reported that the addition of isovalerate, isobutyrate and 2-methyl butyrate increases cell wall digestibility and nitrogen utilization[9].

In this study, cassava leaf meal supplementation at the level of 5% of dry matter increased the dry matter, NDF and ADF levels by 24.83, 25.47 and 37.58%, respectively, compared to the control. Another study has shown that the optimal cassava leaf meal supplementation level is 15% of the dry matter for the ammoniation of palm fibre[8]. Increased cell wall digestibility (ADF and NDF) in this study favoured cellulolytic bacteria. The highest increase in cell wall digestibility was obtained at 5% cassava leaf meal supplementation. In addition, a previous report has also shown that the use of cassava leaf meal as a source of branched chain amino acids (valine, leucine and isoleucine) in ammoniated palm fibre improves the digestibility of feed and growth in ruminants[8].

Rumen fluid characteristics: The results of the rumen fluid characteristics in this study are shown in Table 2. The pH values obtained in this study ranged from 6.41-6.70. Statistical analysis showed that cassava leaf meal supplementation provided no significant effect ($p>0.05$) on rumen fluid pH. Optimal rumen fluid pH values that ensure growth and microbial activity range from 6.3-7.0[1]. If the rumen pH is below 6.2, then the cellulolytic microbial life will be disrupted and the digestibility of the fibre will decrease. The ideal pH value is achieved due to the use of artificial saliva, which serves as a buffer, allowing the rumen fluid pH to remain stable[1] and allowing maintenance of the balance of fermentation products, mainly VFA and NH_3-N.

Production of total VFA in the study ranged from 96.90-129.89% mM. There was a significantly higher VFA resulting from the cassava leaf meal treatments compared to the control ($p<0.01$). The highest VFA concentration (129.89 mM) was obtained with the treatment of 5% cassava leaf meal supplementation. Increased levels of cassava leaf meal supplementation resulted in an increased concentration of VFA, which was lower with the 5% supplementation level. Even at the level of 20 and 25% supplementation, the VFA concentration was equivalent to the control (0% cassava leaf

meal). This result was in line with the increase in the digestibility of nutrients. High digestibility was obtained at the level of 5% cassava leaf meal and higher levels of cassava leaf meal resulted in lower digestibility. The VFA is the end product of carbohydrate fermentation by rumen microbes. Thus, an increase in digestibility leads to an increase in the final product, namely, VFA.

The VFA concentration obtained in this study should support the growth of rumen microbes according to other studies reporting that the VFA amount required for rumen microbial growth is 80-160 mM[7]. Elevated levels of VFA reflect the increased solubility of the soluble carbohydrate feed. The VFA has a dual role as a source of energy for animals and a source of carbon for microbial growth[1].

The concentration of NH_3 obtained in this study ranged from 11.15-15.84 mg/100 mL rumen fluid. In this study, the highest concentration of NH_3-N was obtained at the 5% cassava leaf meal supplementation level. The high concentrations of NH_3-N and VFA resulting from this treatment will result in high microbial protein synthesis. Thus, higher enzyme concentrations produced reflect higher nutrient digestibility rates. Overall, the NH_3-N concentrations obtained in this study had sufficient NH_3-N for rumen microbial growth and activity in accordance with a previously published range[1].

These results indicated that 5% cassava leaf meal was sufficient to produce expected digestibility and rumen fluid characteristics. This study identified an alternative feed source that will be beneficial for ruminant farmers. This study also uncovered a critical resource for an agricultural by-product that has not been previously explored. Thus, a new use for cassava leaf meal/ammoniated palm leaf has been discovered.

CONCLUSION

The use of cassava leaf supplementation successfully increased nutritional composition and digestibility of ammoniated palm leaf. Supplementation with 5% cassava leaf meal provided the best result for its efficiency to produce higher digestibility and better rumen fluid characteristics.

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