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Effect of Replacing Alfalfa Hay with Dried Moringa Oleifera Leaves on Rumen Fermentation, Rumen Microbial Protein Synthesis and Methane Production in Lactating Dairy Cows

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Abstract

Alteration of the microbial ecosystem and ruminal function can be used to improve feed utilization and production performance of ruminant animals. This study aimed to assess the effect of supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay on rumen fermentation, microbial protein production, and ruminal methane gas production. Nine lactating Holstein-Friesian cows (444±39.7 kg) were randomly assigned to three dietary treatments supplemented with 0, 5 and 10% of Moringa leaves in place of 10, 5 and 0% of Lucerne hay on a DM basis in a randomised complete block design for 8 weeks. There was no significant difference in the pH, VFAs, estimated methane, microbial protein, and microbial mass, even though there was a variation in the Moringa-supplemented treatment and the control. Compared with the control, Moringa leaves decreased (P=0.0017) the rumen protozoa and increased (P=0.0092) rumen Ammonia-N concentration. It is concluded that Moringa leaves enhanced rumen fermentation kinetics in lactating Holstein-Friesian cows and can be used as a protein supplement for dairy cows and a substitute for Lucerne hay.

***Keywords**: Ammonia-N, Feed efficiency, Methane, Moringa oleifera, Ruminal fermentation*

Introduction

Despite the economic potential of the dairy industry in developing countries, they are constrained by numerous factors, including a shortage of year-round supply of high-quality feeds, low-quality milk-producing breeds, poor management, and their impacts on climate change, and this has led to poor livestock productivity (Hao et al., 2017).

Smallholder dairy farmers constitute the higher percentage of dairy producer in the tropics, and they use crop residues for their livestock with little or no supplementation and their storage methods are insufficient to preserve the quality of the feeds, which results in seasonal variations in feed availability and a reduction in milk production (Njarui et al., 2016).

Animal nutritionists and microbiologists have altered the microbial ecosystem and rumen function to improve feed utilization and performance by including Ionophores and antibiotics. This intervention's metabolism and residual effect have led to anti-microbial resistance, which has caused great public concern and strict regulations on ruminant production (Matloup et al., 2017).

Hence, there is a need to explore alternative resources, such as fodder trees and shrubs, that can replace antibiotics in manipulating the rumen function to increase productivity on a sustainable basis. In this regard, the drought-tolerant Moringa (*Moringa oleifera*) tree can be considered one of the numerous top feed resources in the tropics. Moringa is a tree native to India but has spread to many areas worldwide. It's a perennial evergreen tree (Palada et al., 2007) whose forage contains 5.9% moisture, 38.6% carbohydrates, 27.2% protein, and 17.1% fat (Yameogo et al., 2011). Moringa's versatility allows multiple harvests throughout the growing season, and the dried leaves can be stored for extended periods without losing nutritional content (Mendieta-Araica et al., 2011).

Numerous studies have demonstrated that Moringa can enhance the health and performance of ruminants, such as oxidative status and milk production (Babiker et al., 2016), improve their nutrition via microbial protein synthesis (Soliva et al., 2005), and regulate rumen microflora as it contains large quantities of active substances, such as flavonoids, and other phenolic compounds (Kholif et al., 2016). The various nutrients in Moringa, including protein, fatty acids, minerals, and vitamins, make it a good feed resource for livestock (Moyo et al., 2012).

Several studies have reported that the inclusion of Moringa leaves in ruminant diets have improved animal performance quantitatively and qualitatively (Mendieta-Araica et al., 2011; Sultana et al., 2015; Babiker et al., 2016; Cohen-Zinder et al., 2016; Kholif et al., 2016; Kholif et al., 2017; Zeng et al., 2018). Therefore, this study aimed to assess the effect of supplementing Moringa leaves in dairy cows fed basal Rhodes grass hay on ruminal fermentation, microbial protein production, and ruminal methane gas production.

Materials and Methods

Study Site

This research was conducted at Tatton Agriculture Park (TAP), Egerton University, Njoro, Kenya. It is located between longitude 35º 57'E and latitude 0º 23'S at an elevation of 2,200 and 2,280 m above sea level, with a daily mean temperature of 21ºC. The precipitation pattern is bimodal, with mean annual precipitation between 900 and 1,020 mm. The long rain occurs between March and May, occasionally extending into June, and the short rain occurs between September and November.

Experimental Animals and Design

A total of nine lactating Holstein-Friesian cows weighing 444±39.7 kg were housed in sheltered cubicles within the vicinity of the University Biogas unit. They were randomly assigned to three experimental groups of three animals each, using a Randomised Complete Block Design (RCBD). During the first week of the feeding trial, the animals were drenched with an anthelminthic to control internal parasites and sprayed weekly to control external parasites.

Experimental Diets and Treatment

Rhodes grass (*Chloris gayana*) hay served as the basal diet, while Lucerne (*Medicago sativa*) hay and dried Moringa (*Moringa oleifera*) leaves were used as supplements. Rhodes grass hay was acquired from neighbouring stores, and Lucerne hay was acquired from Kenya Agriculture and Livestock Research Organisation (KALRO), Naivasha. The hay was shredded using a fodder chopper and stored in gunny bags.

Moringa leaves were sourced from Kiorimba/Machegene, Meru County, Kenya. The leaves were removed from the branches, air-dried on plastic sheets under partial shade, and then stored in gunny bags.

The cows were fed three different experimental diets, as stated in Table 1, and were randomly assigned to one of three treatments as follows:

- T1: The basal diet with 0% Moringa (10% Lucerne); control
- T2: The basal diet with 5% supplementation of Moringa (5% Moringa + 5% Lucerne);
- T3: The basal diet with 10% supplementation of Moringa (10% Moringa).

Table 1. Composition of dietary treatments

Ingredients	Dietary treatments		
	T1 (Control)	T2	T3
Dried Moringa leaves (%)	0	5	10
Lucerne hay (%)	10	5	0
Rhodes grass hay (%)	90	90	90
Total (%)	100	100	100

Dry matter intake (DMI) was estimated as 4% of the live weight of each cow, the supplements (Moringa leaves and Lucerne hay) were fed at 10% of the total estimated daily DMI for each cow, and the basal diet (Rhodes grass hay) was offered ad libitum. The basal diets were offered to each cow ad libitum at 08:00 hr, 13:00 hr, and 16:00 hr, and to ensure that the supplement was wholly consumed, it was provided prior to ad libitum feeding of the basal diet. The basal diet was also adjusted based on the previous day's intake to ensure that the cow receives sufficient basal feed without leaving too many leftovers and to avoid selective feeding. There was also unlimited access to water and mineral supplement. The feeding trial lasted for eight (8) weeks, with two prior weeks assigned to adaptation to the treatments.

Collection of Rumen Liquor

During the last week of the feeding trial, rumen liquor was collected manually from the animals using a stomach tube connected to a vacuum pump 3 hr after the morning feeding (Lodge-Ivey et al., 2009). Feed particles were removed from the rumen liquor by straining it through four layers of cheesecloth. This was used to determine the pH, NH_3-N, VFA, protozoa number, and microbial protein synthesis.

Determination of pH and NH_3-N

The pH of the rumen liquor was measured immediately using a pH metre calibrated at pH 4.0 and pH 9.0 using respective pH buffers. To determine the NH_3-N, a portion (8 ml) of the rumen liquor was preserved by adding 2 ml of freshly prepared 25% metaphosphoric acid to prevent further microbial activity and NH_3 loss through volatilization. The mixture was stored in tightly capped tubes at -20°C until further analysis. The rumen Ammonia-

N was determined using the procedure of Smith & Murphy (1993). It is based on the principle that ammonia reacts with alkaline hypochlorite and phenol in the presence of a catalyst (sodium nitroprusside) to form indophenol (blue), and the concentration of ammonia is directly proportional to the absorbance of indophenol, which is measured spectrophotometricaly.

Determination of VFA, Protozoa Number and Microbial Protein

An aliquot of the rumen liquor was frozen at -20°C for analysis of the VFAs. The concentration of the VFAs (acetate, propionate, and butyrate) was determined using the spectrophotometric method for determining high-range VFA concentration in mixed-acid fermentation samples developed by Aramrueang et al. (2022) using acetic, propionic, and butyric acid as standards.

To determine the protozoa number, a subsample of the rumen liquor was fixed with 18.5% formalin immediately (Dehority, 1984), and a portion of each sample was stained and stored in methyl green formalin saline (MFS) solution (Gürelli, 2014). A well-mixed sample was used for the determination of the population of protozoa in the rumen fluid (Gürelli and Göçmen, 2012) with the aid of a microscope.

Microbial protein was determined using Lowry's procedure, where centrifugation was used to separate bacterial cells from the rumen liquor. Proteins from cells were released by suspending them in 0.25 N NaOH and heating them in a boiling water bath for 10 minutes. Microbial crude protein was then determined by the Folin phenol method (Lowry et al., 1951).

Estimation of Microbial Mass and Ruminal Methane Gas Production

The concentration of the VFAs was used to estimate the values of ATP using the equation described by Widiawati and Thalib (2009):

ATP_{pr} =[2.5x(A)] +[2.75x(P)] +[3.5x(B)]

where: ATP_{pr} – is the amount of ATP produced (mole)

(A)– is the concentration of acetate (mole)
(P) – is the concentration of propionate (mole)
(B) – is the concentration of butyrate (mole)

As Widiawati and Thalib (2009) suggested, the values of ATP produced and the values of ATP required for synthesizing a unit of the microbial cell were employed to predict the microbial protein synthesized in the rumen during fermentation. It is based on the assumption that each mole of ATP produces 10 g of the microbial cell. As a result, the microbial mass was computed using the equation described by Widiawati and Thalib (2009):

Microbial mass = 10 x ATP_{pr}

where: Microbial mass – is the amount of microbial cells produced (g)

ATP_{pr} – is the amount of ATP produced (mole)

Ruminal methane gas produced during the fermentation of the diets was estimated from the VFA proportions using the equation described by Widiawati and Thalib (2009):

CH_4 =[0.5x(A)] +[0.5x(B)] –[0.25x(P)]

where: CH_4 – is the amount of methane produced (mole)

(A) – is the concentration of acetate (mole)
(B) – is the concentration of butyrate (mole)
(P) – is the concentration of propionate (mole)

Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) procedure following a Randomised Complete Block Design (RCBD) using the Generalized Linear Model (GLM) procedure of SAS (SAS, 2009). The different means were separated using Tukey's Studentized Range (HSD) Test at $P<0.05$.

The statistical model of Randomised Complete Block Design (RCBD) was used:

$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

where: Y_{ij} - the j^{th} observation of the i^{th} treatment
μ - the population mean
α_i - the effect of the i^{th} treatment, i = {1, 2, 3}
β_j - the effect of the j^{th} block
ε_{ij} - the random error of the i^{th} treatment on the j^{th} block

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Results

Rumen Fermentation Kinetics

The effect of supplementing lactating Holstein-Friesian cows with different levels of Moringa leaves, and Lucerne hay on their rumen fermentation kinetics are presented in Table 2. The highest pH was observed in T2 (6.5), and no significant difference was across the treatments. The Ammonia-N content significantly differed across the treatments, and the highest was recorded in T2 (41.03 mg/L). Supplementing with Moringa leaves did not affect the concentration of acetate, butyrate, or propionate (P=0.0092), and there was no difference (P<.0001) in the acetate: propionate concentration across the treatments.

There was a decrease in protozoa number and an increase in microbial protein synthesis in the Moringa-supplemented treatment compared to the control. There was no significant difference (P=0.0092) across the treatments regarding the estimated methane gas and microbial biomass.

Table 2. Effect of supplementing lactating Holstein-Friesian cows with different levels of Moringa leaves and Lucerne hay on rumen fermentation, microbial protein and methane gas production

Parameters	Dietary treatments			SEM	P
	T1	T2	T3		
pH	6.17[a]	6.50[a]	6.30[a]	0.07	0.2563
Ammonia-N (mg/L)	10.31[c]	41.03[a]	24.25[b]	4.67	0.0092
Acetate (mmol/L)	27.51[a]	28.93[a]	27.67[a]	1.47	0.9912
Propionate (mmol/L)	41.27[a]	43.40[a]	41.50[a]	2.21	0.9912
Butyrate (mmol/L)	55.02[a]	57.86[a]	55.33[a]	2.95	0.9912
Acetate: Propionate	0.67[a]	0.67[a]	0.67[a]	0.00	<.0001
Protozoa number (x10^3 cells/ml)	70.57[a]	14.21[b]	4.26[b]	10.56	0.0017
Microbial protein (mg/L)	92.45[a]	92.38[a]	100.10[a]	4.28	0.3697
Methane (mmol/L)	30.95[a]	32.55[a]	31.12[a]	1.66	0.9912
Microbial mass (mmol/L)	3748.30[a]	3941.70[a]	3769.30[a]	200.88	0.9912

[abc]Means in the same row with the same letter are not significantly different at P<0.05; T1= Rhodes grass hay supplemented with Lucerne hay; T2= Rhodes grass hay supplemented with Lucerne hay and Moringa leaves; T3= Rhodes grass hay supplemented with Moringa leaves; SEM= Standard error of the mean.

Discussion

Rumen Fermentation Kinetics

The rumen microbiome is critical to the host animal's effective feed breakdown and utilisation, ultimately affecting its nutritional status. It is believed that the rumen microbiota of cattle produces around 70% of the total energy substrates ingested and utilised by the animal through their fermentation characteristics (Hungate, 1966).

The rumen is an ideal habitat for the growth of anaerobic microbes, and the normal pH of grass-fed ruminants is between the range of 6-7 (Russell et al., 2009). The pH observed in this experiment is within the range of 6.1-6.8, which shows that the animals had a healthy rumen, and the diets did not have a significant effect (P=0.2563) on the rumen pH. The result is consistent with that of Kholif et al. (2017), who studied the effect of Moringa leaf extract on rumen fermentation, and Soliva et al. (2005), who compared Moringa leaves with soybean and rapeseed meal.

The rumen Ammonia-N concentration ranged from 10.31-41.03 mg/L, and it falls within the range reported by Satter and Slyter (1974) to maximize microbial growth and activity in the rumen. The result showed an increase in rumen Ammonia-N concentration with supplementation with Moringa than the control diet.

This shows that the level administered can enhance rumen fermentation by making N available for microbial growth and enhancing microbial protein synthesis (Soliva et al., 2005). It explains the high microbial protein content observed in the Moringa-supplemented diet. However, Li et al. (2019) reported a decrease in ruminal Ammonia-N concentration in cows fed the Moringa diet compared to the control diet. Kholif et al. (2016) also observed a decrease in the concentration when they fed Moringa as fresh leaves, hay, or silage to lactating goats.

Ruminants can derive energy from complex carbohydrates through microbial fermentation to yield volatile fatty acids (VFAs), estimated to provide up to 75% of the total metabolizable energy (Bergman, 1990). The VFA production in the rumen depends on nutrient digestibility, the rate of absorption, the rate of digesta passage from the rumen, and the activity of the microbial population in the rumen (Li et al., 2019).

This study showed no significant difference in the acetate, propionate, butyrate, and acetate: propionate concentration of the Moringa-supplemented treatments compared to the control. It can also be observed that there was an increase in the concentration of the VFAs with the addition of Moringa leaves, although the combination of Moringa and Lucerne showed the highest increase. This increase indicates an improvement in fermentation due

to the increase in the microbial mass, mostly bacteria (Li et al., 2019). This result agrees with Kholif et al. (2015) and Li et al. (2019), who reported that feeding Moringa improves the VFA concentration in lactating goats and cows.

The rumen protozoa have been demonstrated to enhance methanogenesis and impact intraruminal recycling of microbial protein even though they contribute to fibre degradation and stabilize ruminal pH changes (Puniya et al., 2015). Some of the methanogenic bacteria have a symbiotic relationship with rumen ciliate protozoa and remain either inside the body of the protozoa or are attached to their surface, and this is the reason why defaunation is usually associated with reduced production of methane in the rumen as the methanogens lose their symbiotic association resulting in their reduced biological activity (Santra and Karim, 2003).

In this study, there was a drastic decrease in protozoa number in the Moringa-supplemented diet compared to the control diet, and this can be attributed to the active substances in Moringa leaves such as saponin, which has been documented as an antiprotozoal agent (Ebeid et al., 2020). This decrease can also result from the unsaturated fatty acids present in Moringa leaves, as they are toxic to rumen ciliate protozoa (Moyo et al., 2011) and cause defaunation. This result is consistent with the result of Sultana et al. (2018), Ebeid et al. (2020), and Abdel-Raheem & Hassan (2021), who reported that Moringa supplementation decreases the amount of rumen ciliate protozoa in goats, water buffaloes, and buffalo calves respectively.

As a consequence of the decrease in the protozoa numbers, an increase in the total bacterial population is expected as there is no predation of bacteria by the protozoa, and this will decrease the energy loss through methanogenesis by 5.5–7.9 % of gross energy intake (Mathieu et al., 1996). The decrease in the estimated methane production in the Moringa-supplemented diet can also result from the secondary metabolites present in the leaves because they have an inhibitory effect on ruminal methanogenic bacteria (Bodas et al., 2012). This result agrees with the report from Kholif et al. (2017) and Li et al. (2019), who used lactating goats and cows as their model animals to investigate the effect of Moringa on methane emission.

In this study, Moringa leaves generally had a positive effect on rumen fermentation, and studies have shown that low or medium levels of secondary metabolites in ruminant diets have a positive effect on rumen fermentation and productivity compared to high levels (Salem et al., 2014). The variation among the results of studies might be related to the nature and concentration of secondary metabolites in different leaves.

Conclusion

Based on the result of this study, it can be concluded that Moringa leaves can serve as a substitute for Lucerne hay in the diet of dairy cows to improve rumen fermentation as they enhanced fermentation kinetics and decreased methane production through effective modulation of the rumen microbiome. Moringa leaves can be used as supplements for dairy cows subsiding on low-quality basal diets during the period of feed scarcity in arid and semi-arid regions and agro-pastoral areas and therefore help to mitigate the feeding crisis as a non-conventional protein source for ruminants.

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