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Rumen Degradability and *In Vitro* Fermentation Pattern in Concentrate Mixtures Containing Varying Levels of *Moringa oleifera* (Moringa) Leaves

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ABSTRACT

A study was conducted to evaluate the *in vitro* gas production (IVGP), nutrient degradabilities and fermentation characteristics of concentrate mixtures containing varying levels of *Moringao leifera* (moringa) leaves. The control concentrate mixture was prepared with maize grain, de-oiled rice bran, cotton seed cake and soybean meal as major ingredients. The other 7 iso-nitrogenous (20% CP) concentrate mixtures were prepared by partially replacing de-oiled rice bran and cotton seed cake with moringa (MOR) leaves at varying levels (5, 10, 15, 20, 25, 30 and 35%). The IVGP (ml/500mg DM) and *in vitro* dry matter degradability (%), *in vitro* organic matter degradability (%) and *in vitro* neutral detergent fibre degradability (%) gradually increased ($P<0.01$) from 0 to 30% and comparable at 35%. The partitioning factor and microbial biomass production (MBP) (mg/500mg) was highest at 5% and then decreased gradually with MOR leaves inclusion. The metabolizable energy (ME) (MJ/kg) was gradually increased from 0 to 25% inclusion of MOR and comparable at higher levels. Ammonia – N (mg/40 ml) and pH was highest at lower levels (0 to 10 and 0 to 15%) and then gradually decreased, while total volatile fatty acids (mmol/40 ml) increased and highest at 25% and then decreased with inclusion of MOR leaves in concentrate mixtures. It could be concluded that MOR leaves at higher levels (30-35%) can be included in concentrate mixtures as it improved IVGP and nutrient degradabilities.

KEYWORDS: Ammonia-N, Concentrate mixture, *In vitro g*as production, Moringa leaves

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INTRODUCTION

Fodder production in general is not enough to meet the nutritional requirements of animals around the year, especially during dry periods. The dry season causes nutritional stress and consequently decreases animal productivity. Concentrates supplementation during the dry season improves the productive performance of small ruminants, but it is generally not a profitable practice due to high feeding costs as the available ingredients for concentrate production are limited (Salem and Smith, 2008). A potential technique to increase the supply of high-quality feeds, particularly during the dry season, is to include fodder trees and shrub forages in concentrate mixtures for small ruminants. Moringa species are multipurpose trees from non-leguminous group. They are fast growing trees of economic and industrial importance and a potential source of animal feed. India is the largest producer of moringa with an annual production of 2.2 million tons of fruit pods (Sekhar et al., 2018). *Moringa oleifera* foliage has been reported to be a potential cheap source of protein for animal feeding (Sarwatt et al.,2004). Moringa was described as a protein

source by Kholif et al. (2015), including 241-277 g/kg of crude protein, 47% of which was bypass protein (Becker, 1995), a sufficient amino acid profile (Sanchez-Machado et al., 2010), and antioxidant polyphenolic contents (Sreelatha and Padma, 2009). .Compared to traditional protein feeds *viz.,* cotton seed cake, groundnut cake, sesame meal and soybean meal for ruminants *M. oleifera* is a cheaper protein ingredient (Kholif et al., 2015).

However, like other tree fodders, *M. oleifera* contains high content of naturally occurring antioxidants such as vitamin C, tocopherols, flavonoids and phenolic compounds and also negligible amount of anti-nutritive compounds (Soliva et al., 2005, Mendieta-Araica et al., 2011 and Nouman et al., 2014). Plants having bioactive products such as essential oils, saponins, and condensed tannins(Mendieta-Araica et al., 2011 and Nouman et al., 2014) with antimicrobial properties may be exploited in ruminant production for improving fermentation efficiency.Recent experiments that included morigna leaf meal as a protein feed are gaining increasing interests, with promising results such as enhanced feed utilization and milk production from goats (Kholif et al., 2015).Though much research was conducted on replacing moringa leaf meal as a protein supplement, little information is available regarding inclusion of moringa leaf meal at incremental levels in iso-nitrogeneous concentrate mixtures. Therefore, the present study was undertaken to evaluate inclusion of moringa leaves at varying levels in concentrate mixtures on *in vitro* gas production, nutrient degradabilities and rumen fermentation characteristics using glass syringes.

MATERIALS AND METHODS

The *Moringa oleifera* (moringa) leaves were procured from Indian Agri Farm, Chettikulam near Kanyakumari, Tamil Nadu (India). The concentrate feed ingredients (maize grain, de-oiled rice bran, cotton seed cake, soybean meal, salt, calcite powder and mineral mixture) were procured from the local market in Hyderabad (India). The experimental diets were prepared to be iso-nitrogenous and iso-caloric by including moringa leaves at varying levels (0, 5, 10, 15, 20, 25, 30 and 35%) in the concentre mixtures. A basal concentrate mixture (control, CON) was formulated with maize grain, de-oiled rice bran, cotton seed cake and soybean meal as major ingredients and concentrate mixtures with moringa leaves (MOR) were prepared by partially replacing cotton seed cake and de-oiled rice bran in basal diet. Concentrate mixtures were prepared by thoroughly mixing and ground through 1 mm sieve in Wiley mill to reduce sampling errors. The moringa leaves and concentrate mixtures were analyzed for proximate constituents (AOAC, 2019) and neutral detergent fibre (Van Soest et al., 1991).

Rumen liquor (RL) was obtained from four adult sheep using stomach tube fed on sorghum stover based complete diet (50R:50C in equal proportions at 10.00 am and 4.00 pm) before morning feeding and then was pooled and strained with 4 layered muslin cloth. Then RL was kept in water bath (39^{o}C) flushed with carbon dioxide to support the liveability of anaerobic organisms. The incubations were carried out in 100 ml glass syringes (Haberle Labortechnik, Lonsee-Ettenchieâ, Germany) kept in water bath maintained at 39±0.5^{o}C as described by Menke et al., (1979) and Menke and Steingass (1988). The amount of concentrate mixtures and the volume of incubation medium were 500 mg and 40 ml, respectively.The medium mixture was prepared using double strength rumen buffer (Blummel et al., 1997) and the ratio of medium mixture to rumen liquor was kept at 2:1.The concentrate mixtures were incubated in triplicate and blank was set comprised of rumen fluid-medium mixture alone and was run simultaneously. The gas production was recorded at 0 h, ½ h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 24 h. After removing the syringes at 24 h, the pH was recorded immediately and the contents were transferred to 600 ml capacity spoutless beaker, shaken and allowed to settle. After 15 minutes, 10 ml supernatant was carefully pipetted for ammonia-N (NH_3-N) estimation and 5 ml supernatant was stored with saturated mercuric chloride for total volatile fatty acids (TVFA) estimation. Then the syringes were rinsed twice with 25 ml of neutral detergent solution

(double strength; Van Soest et al., 1991) and contents were transferred through narrow outlet into the respective beakers and NDF was estimated (Blummel et al., 1997).

The i*n vitro* dry matter degradability (IVDMD) (%), organic matter degradability (IVOMD) (%) and neutral detergent fibre degradability (IVNDFD) (%) were determined by

IVDMD% = DM% of the substrate – DM % of the residue

IVOMD % = OM% of substrate incubated on DM basis – OM % of the residue

IVNDFD % = NDF% of substrate incubated on DM basis – NDF % of the residue

The partitioning factor (PF), Microbial biomass protein (MBP) and metabolizable energy (ME) were estimated as follows:

$$\text{Partitioning factor} = \frac{\textit{In vitro} \text{ true DM Digested (mg)}}{\text{Total gas produced (ml)}}$$

MBP (mg) = IVOMD (mg) – (Net gas volume × 2.20)

Where 2.20 is the stoichiometric factor for mixed diets (Blummel and Lebzien, 2001).

In vitro ME (MJ/kg DM) = 2.20 + 0.1356 GP + 0.057 × CPDM/kg (Menke and Steingass, 1988)

Where, GP = Net gas production at 24 h fermentation (ml/0.5 g DM);

CPDM/kg = Crude protein on DM basis × 10

The data was subjected to statistical analysis using software (SPSS, Version 17). One way analysis of variance through generalized linear model was used to analyse all the results. The treatment means were ranked using Duncan's multiple range test with a significance at $P<0.05$ (Duncan, 1955). All the statistical procedures were done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The ingredient composition of concentrate mixtures containing varying levels of moringa leaves are presented in Table 1. The organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen free extract (NFE), total ash and neutral detergent fibre (NDF) content in moringa leaves was 88.2, 17.9, 8.66, 6.71, 54.9, 11.7 and 39.1 %, respectively (Table 2). The observed CP was in agreement with values (17.1%) reported by He et al.(2020). Su et al. (2020) reported almost similar EE (6.4 to 7.2%) and CF (10.5%) in moringa leaves, but higher CP (23.0 to 28.0%). However, lower CP (8.75%), CF (2.91%), and NDF (31.7%) were reported by Belhi et al. (2018) compared to present study values, while Yusuf et al. (2018) reported similar NDF (35.5%) content. These variations observed in present and past findings might be due to different stages of maturity of leaves or differences in plant variety and agro-climatic conditions.

The chemical composition of concentrate mixtures containing varying levels of moringa leaves (0 to 35%) were similar for most of the nutrients (Table 2) since only cotton seed cake and de-oiled rice bran of control concentrate mixtures were replaced with moringa leaves in experimental concentrate mixtures. Incorporation of moringa leaves, did not affect CP and OM content of the concentrate mixtures as all the concentrate mixtures were formulated to be iso-nitrogenous. The EE content increased, while CF and NDF content decreased gradually with increase in inclusion level of moringa leaves in concentrate mixtures, which was due to higher EE and lower CF and NDF contents in moringa leaves (8.66, 6.71 and 39.16%, respectively) compared to cotton seed cake (3.14, 32.0 and 67.9%, respectively) and de-oiled rice bran (1.06, 18.2 and 49.1%, respectively).

Table 1. Ingredient composition (%) of concentrate mixtures containing varying levels of *Moringa oleifera* leaves (MOR)

Ingredient	CON[2]	MOR5[3]	MOR10[3]	MOR15[3]	MOR20[3]	MOR25[3]	MOR30[3]	MOR35[3]
Maize	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
De-oiled rice bran	37.00	34.00	30.00	27.00	24.00	20.00	17.00	14.00
Cotton seed cake	21.00	19.00	18.00	16.00	14.00	13.00	11.00	9.00
Moringa oleifera leaves	0.00	5.00	10.00	15.00	20.00	25.00	30.00	35.00
Mineral and vitamin mixture[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcite powder	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Mineral and vitamin mixture provided per kg diet: Calcium 2.5 g, Phosphorus 1.275 g, Magnesium 0.065 g, Iron 0.0175 g, Sulphur 0.092 g, Zinc 0.096 g, Copper 0.042g, Manganese 0.015 g, Potassium 1.5 mg, Sodium 0.2 mg, Iodine 3.5 mg, Cobalt 1.5 mg, Vitamin B_6 0.2 mg, Vitamin A 7500 IU, Vitamin D_3 750 IU, Vitamin E 3 mg, Niacinamide 0.012 g.
[2]Control diet [3]*Moringa oleifera* (Moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.

Table 2.Chemical composition (% DM basis) of concentrate mixtures containing varying levels of *Moringa oleifera* (moringa) leaves (MOR)

Constituent	Concentrate mixture								MOR[3]
	CON[1]	MOR5[2]	MOR10[2]	MOR15[2]	MOR20[2]	MOR25[2]	MOR30[2]	MOR35[2]	
Dry matter	93.6	93.5	93.4	93.2	93.3	92.9	93.0	93.3	91.5
Organic matter	90.0	89.6	90.3	90.3	90.7	90.8	91.3	91.2	88.2
Crude protein	20.0	20.1	20.0	20.1	19.8	19.9	20.0	20.1	17.9
Ether extract	1.86	2.23	2.49	2.65	2.69	2.77	2.85	3.26	8.66
Crude fiber	17.2	16.1	15.2	14.8	14.2	13.1	11.2	10.1	6.71
Nitrogen free extract	50.8	51.1	52.5	52.7	53.9	55.0	57.2	57.7	54.9
Total ash	9.96	10.3	9.61	9.67	9.29	9.16	8.62	8.80	11.7
Neutral detergent fiber	60.7	58.7	57.1	56.2	54.4	53.7	52.3	51.6	39.1

Each value is the average of duplicate analysis
[1]Control diet (0% moringa leaves)
[2]*Moringa oleifera* (moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.
[3]*Moringa oleifera* (moringa) leaves

The amount of gas produced in *in vitro* gas production technique is either directly from the fermentation or indirectly from the buffering of short chain fatty acids. In the present study, *in vitro* gas production (ml/500mg DM) increased (P<0.01) with increase in inclusion levels (0-35%) of moringa leaves in concentrate mixtures (Table 3), which indicated a higher content of fermentable soluble components. The feeds rich in NDF are less degradable than soluble carbohydrates and therefore reduce gas production (Parissi et al., 2005). As the inclusion levels of moringa leaves increased in concentrate mixture, it lowered the NDF content, therefore, produced higher volume of gas. In contrast, control concentrate mixture was high in structural carbohydrates (CF and NDF) compared to moringa containing diets (Table 2) therefore, contributed to less gas production due to low fermentation. The digestive interactions between the substrates, named associative effects, can modify the metabolic processes in the rumen. The associative effect of moringa leaves on acceleration of the gas production of concentrate mixture could be related to fulfilment of nutritional adequacy of fermentation due to supplementation of carotenoids, essential amino acids and minerals through moringa leaves, resulting in stimulation of the fibrolytic activity and also due to the presence of bioactive secondary compounds like saponins, flavanoids and growth promoting factors (Makkar and Baker, 1997 and Bamishaiye et al., 2011). Our results are supported by the findings of Keshri et al. (2022) and Leitanthem et al. (2022), who found that adding moringa to concentrate mixtures at incremental amounts to total mixed rations significantly improved gas production and digestibility.

Table 3. Effect of inclusion of *Moringa oleifera* (moringa) leaves at varying levels in concentrate mixture on *in vitro* gas production, *in vitro* degradability of nutrients and partitioning factor assessed by *in vitro* gas production technique

Diet	IVGP (ml/500mg DM)	IVDMD %	IVOMD %	IVNDFD %	PF
CON[1]	62.2^{d} ±1.48	68.7^{e} ±0.63	71.9^{d} ±0.70	48.01^{c} ±1.04	5.23^{b} ±0.12
MOR5[2]	59.3^{d} ±1.77	71.1^{d} ±0.37	72.9^{d} ±0.38	48.7^{c} ±1.09	5.59^{a} ±0.17
MOR10[2]	83.5^{c} ±1.57	71.0^{d} ±0.15	73.3^{d} ±0.49	47.1^{c} ±0.27	4.01^{cd} ±0.08
MOR15[2]	86.4^{c} ±1.20	75.6^{c} ±0.85	77.6^{c} ±0.58	54.8^{b} ±1.58	4.12^{c} ±0.04
MOR20[2]	86.1^{c} ±1.30	75.4^{c} ±0.49	77.7^{c} ±0.50	52.9^{b} ±0.98	4.13^{c} ±0.04
MOR25[2]	97.1^{ab} ±2.15	77.8^{b} ±0.84	80.6^{b} ±0.87	58.3^{a} ±1.58	3.81^{d} ±0.07
MOR30[2]	93.5^{b} ±0.95	79.3^{ab} ±0.07	82.7^{a} ±0.39	60.1^{a} ±0.23	4.09^{cd} ±0.02
MOR35[2]	98.4^{a} ±0.58	79.5^{a} ±0.17	83.4^{a} ±0.12	60.0^{a} ±0.43	3.93^{cd} ±0.04
SEM	2.940	0.820	0.890	1.100	0.130
P value	0.0001	0.0001	0.0001	0.0001	0.0001

Each value is an average of three observations

[abcd]Means with different superscripts in a column differ significantly: Pd”0.01

IVGP - *in vitro* gas production, IVDMD - *in vitro* dry matter degradability, IVOMD - *in vitro* organic matter degradability, IVNDFD - *in vitro* neutral detergent fibre degradability, PF - Partitioning factor

[1]Control diet (0% moringa leaves)

[2]*Moringa oleifera* (moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.

SEM: Standard Error Mean; P value: Probability value.

Inclusion of moringa leaves significantly (P<0.01) improved *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD) and *in vitro* neutral detergent fibre degradability (IVNDFD) when compared with control (Table 3). The observed values for IVDMD, IVOMD (up to 30% inclusion) and IVNDFD (up to 25% inclusion) increased (P<0.01) linearly

compared with control. At 35% inclusion levels of moringa leaves, the DM, OM and NDF digestibility was comparable to that of 30% inclusion. Similar to the present study, Keshri et al. (2022) and Leitanthem et al. (2022), showed significant improvement in IVDMD and IVOMD of total mixed ration with incremental supplementation of moringa leaves in concentrate mixture of toral mixed ration. Kholif et al. (2015 and 2018) reported increased NDF digestibility with increased inclusion of moringa leaves *in vivo*. The higher digestibility of DM, OM and NDF could be attributed to increased stimulation of microbial activity in rumen due to high content of micro minerals, polyphenols and flavonoids in moringa leaves (Hove et al. 2001).

Partitioning factor (PF) is the partitioning of nutrients between gases and microbial cells (Blummel et al.,1997) and the high PF value (low gas production per unit of substrate degraded) is indicative of proportionally higher microbial yield. Partitioning factor (PF) and microbial biomass production (MBP) were significantly ($P<0.01$) higher up to 5% inclusion of moringa leaves in concentrate mixture which decreased with further increase in inclusion of moringa leaves compared to control (Table 3 and 4). Similar CP content but varying tannins (Kholif et al., 2015), in conjunction with greater ruminal degradability of protein (Makkar and Becker 1997) and high proportion of ammonia N (Soliva et al., 2005) are the factors for increased microbial protein synthesis. The lower PF and MBP observed in concentrate mixtures with inclusion of moringa at higher levels compared to control could be either due to lower NH_3-N production (Table 5) and higher gas production (Table 3) from these concentrate mixtures. In contrast to our study, Dey et al. (2014) reported significantly ($P<0.05$) increased MBP production with wheat straw and moringa leaves mixtures at 10% moringa inclusion and further increase at 20% inclusion in diet. Whereas, replacing soybean meal from 0 to 100% with moringa leaves in TMR did not affect ($P>0.05$) PF and MBP as reported by Elghandour et al. (2017).

Table 4. Effect of inclusion of *Moringa oleifera* (moringa) leaves at varying levels in concentrate mixture on microbial biomass production and metabolizable energy assessed by *in vitro* gas production technique

Diet	MBP (mg/500mg)	ME (MJ/kg)
CON[1]	188.6^{b} ±3.59	9.39^{c}±0.11
MOR5[2]	200.5^{a} ±4.33	9.15^{c}±0.16
MOR10[2]	150.6^{f} ±3.81	10.5^{b}±0.07
MOR15[2]	165.9^{de} ±2.19	10.7^{b}±0.02
MOR20[2]	166.2^{de} ±1.88	10.7^{b}±0.05
MOR25[2]	156.7^{ef} ±3.92	11.4^{a}±0.09
MOR30[2]	176.5^{c} ±0.47	11.1^{a}±0.04
MOR35[2]	170.5^{cd} ±3.54	11.3^{a}±0.09
SEM	3.330	0.17
P value	0.0001	0.0001

Each value is an average of three observations

[abcd]Means with different superscripts in a column differ significantly: Pd"0.01

MBP (mg/500 mg) - Microbial biomass production, ME (MJ/Kg) - Metabolizable energy

[1]Control diet (0% moringa leaves)

[2]*Moringa oleifera* (moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.

SEM: Standard Error Mean; P value: Probability value.

The metabolizable energy (ME) value (MJ/kg) indicates the chemical composition as well as the rate at which the carbohydrates ferment in the rumen as it is calculated from the gas produced at 24 h incubation period and the CP level of the feed. The ME content gradually increased P<0.01) with increase in moringa leaves inclusion in concentrate mixtures up to 25% and no further improvement was observed at 30 and 35% inclusion of moringa leaves (Table 4). The increased ME value in present study may be due increased gas production with increased inclusion of moringa leaves in concentrate mixtures which were iso-nitrogenous. Melesse et al., (2013) reported metabolizable energy ranging from 9.31 to 9.94 MJ/kg when moringa leaves alone were incubated compared to other components (twigs, seed, seed pods) of moringa which was nearly comparable to our values at higher level of moringa inclusion.

Table 5. Effect of inclusion of *Moringa oleifera* (moringa) leaves at varying levels in concentrate mixture on rumen metabolites assessed by *in vitro* gas production technique

Diet	pH	TVFA (mmol/40ml)	NH_3-N (mg/40ml)
CON[1]	6.83^{a} ±0.03	2.06^{d} ±0.03	12.5^{a} ±0.26
MOR5[2]	6.80^{ab} ±0.00	2.21^{cd} ±0.04	12.5^{a} ±0.04
MOR10[2]	6.80^{ab} ±0.00	2.38^{bc} ±0.04	12.4^{a} ±0.36
MOR15[2]	6.77^{abc} ±0.03	2.48^{b} ±0.11	11.2^{b} ±0.32
MOR20[2]	6.73^{bc} ±0.03	2.53^{b} ±0.08	11.5^{b} ±0.20
MOR25[2]	6.70^{c} ±0.00	2.80^{a} ±0.05	11.1^{b} ±0.20
MOR30[2]	6.73^{bc} ±0.03	2.41^{b} ±0.04	10.3^{c} ±0.14
MOR35[2]	6.70^{c} ±0.00	2.45^{b} ±0.02	10.0^{c} ±0.04
SEM	0.010	0.050	0.200
P value	0.007	0.0001	0.0001

Each value is an average of three observations
[abcd]Means with different superscripts in a column differ significantly: Pd"0.01
TVFA- Total volatile fatty acid, NH_3-N- Ammonia nitrogen
[1]Control diet (0% moringa leaves)
[2]*Moringa oleifera* (moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.
SEM: Standard Error Mean; P value: Probability value.

The rumen pH an indicative of internal homeostasis of rumen environment and a stable rumen pH is vital for the efficient rumen fermentation. In our study, the mean pH among concentrate mixtures containing varying levels of moringa leaves differed significantly (P<0.01) and ranged from 6.70 to 6.83. The higher pH observed with control and moringa leaves inclusion up to 15% in concentrate mixtures could be correlated with higher NH_3-N and lower VFA content (Table 5). In contrast to our study, Kholif et al. (2015) reported increased rumen pH when sesame meal was replaced with moringa at 10, 15 and 20% (7.02 to 7.06) compared to control (6.88), while in our study, no effect on ruminal pH was observed up to 15% inclusion of moringa leaves.. Ruminal pH values in control and all moringa containing concentrate mixtures ranged between 6.70 and 6.83, and fell within the range considered acceptable for fiber digestion (Orskov and Ryle, 1990). Greatest ruminal pH was observed in control and moringa included concentrate mixtures up to 15% moringa inclusion. The decreased ruminal pH with increased inclusion of moringa leaves in concentrate mixtures in our study could be due to decrease in fiber levels and increase in NFE content (Table 2) resulting in

gradual increase in TVFA concentration. Kholif et al., (2015) also observed increase in pH with diets containing higher fiber fractions (NDF: 33.75, 34.33, 34.62 and 34.90%) and (ADF: 20.64, 21.88, 22.49 and 23.11%, respectively) with gradual replacement of sesame cake with moringa leaves at 0, 10, 15 and 20% in concentrate mixtures.

In the present study, TVFA (mmol/40ml) increased ($P<0.01$) gradually with linear increase in inclusion of moringa leaves up to 25% and no improvement was observed with higher levels of replacement (Table 5). Higher DM, OM and NDF degradabilities observed with increase in moringa inclusion (Table 3) resulted in higher ($P<0.01$) TVFA concentration. Kholif et al. (2015 and 2018) reported increased TVFA concentration with moringa leaf meal diets compared to control. In contrast, Elghandour et al. (2017) when replaced soybean meal with moringa leaves gradually from 0 to 100%, reported lowered TVFA concentration with increase in moringa levels in diet and attributed it to high fibre levels and low CP content in moringa leaves than soybean meal, thus had negative effect on ruminal fermentation resulting in decreased TVFA concentration. Similarly, Elghandour et al. (2017) and Olafadehan and Okunade (2016) attributed decreased ruminal TVFA concentration to reduced fiber degradability of moringa leaf containing ration in the rumen compared to control diet. Increased TVFA concentration in present study could be interpreted as a result of improved degradability of moringa leaves containing concentrate mixtures compared to control.

The NH_3-N concentration (mg/40ml) in the present study ranged from 10.04 to 12.54 and decreased ($P<0.01$) with increased inclusion of moringa leaves in concentrate mixtures beyond 10% (Table 5). Similar to our results, Elghandour et al. (2017) reported gradual decrease in NH_3-N concentration when soybean meal was replaced with moringa leaves from 0 to 100% in TMR. While, Kholif et al. (2015 and 2018) reported no effect on NH_3-N concentration in rumen when alfalfa hay was replaced with moringa leaves at 25%.The decreased ruminal NH_3-N with morigna leaf meal could be a result of the reported low degradability of morigna leaf meal protein in the rumen due to its tannins and phenolic compounds (Kholif et al., 2015). Tannins in feeds may reduce ruminal protein degradation as it binds with dietary protein and protects it from ruminal degradation (Frutos et al., 2004). Also, secondary metabolites like saponins and tannins have the ability to decrease ruminal protozoa that play a major role in ruminal feed protein degradation (Newbold et al., 1997).

CONCLUSIONS

In vitro screening of various inclusion levels of moringa leaves in concentrate mixtures for their effects on rumen fermentation efficiency suggests that moringa leaves at higher levels (30 and 35%) of inclusion, may favour partitioning of rumen fermentation end products towards higher ME and/or VFA production in concentrate mixtures. Inclusion of moringa leaves in concentrate mixtures at higher levels (30 and 35%) is having higher IVGP and improved DM, OM and NDF degradability thus may help in economizing andbalancing the ration.

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