



Original article

Effects of dietary inclusion of *Moringa oleifera* leaf meal on nutrient digestibility, rumen fermentation, ruminal enzyme activities and growth performance of buffalo calvesSherief M. Abdel-Raheem^{a,b,*}, Ekramy H. Hassan^c^a Department of Public Health, College of Veterinary Medicine, King Faisal University, Saudi Arabia^b Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt^c Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt

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ABSTRACT

This study was conducted to investigate the impact of dietary inclusion of *Moringa oleifera* leaf meal (MLM) as a substitution for soybean meal on nutrient digestibility, rumen fermentation, rumen enzyme activity, blood metabolites, growth-related hormones, and growth performance of buffalo calves. Thirty buffalo calves eight to nine months of age with an average body weight of approximately 153.7 ± 0.97 kg were randomly distributed through three dietary treatments (ten calves/treatment). MLM inclusion rates were 15% (M15) and 20% (M20), replacing soybean meal by 50 and 75% in the concentrate mixture, respectively. The results indicated that, digestibility of dry matter, organic matter (OM), and crude fiber (CF) increased significantly ($p < 0.05$) with MLM inclusion, while the digestibility of crude protein (CP) and ether extract (EE) reduced significantly ($p < 0.05$) with MLM addition. Dietary supplementation with MLM significantly affected ($p < 0.001$) rumen fermentation by reducing ruminal enzymes, ruminal ammonia-N, total protozoa, and acetate/propionate ratio and increasing acetic, propionic, and butyric acids and total volatile fatty acid concentrations ($p < 0.001$). Furthermore, dietary inclusion of 15% MLM significantly improved ($p < 0.001$) final body weight, dry matter intake of feed, daily weight gain, feed conversion efficiency, blood metabolites, and plasma insulin growth factor-I (IGF-I). It can be concluded that MLM is a multi-purpose protein supplement that provides some nutritional and therapeutic advantages when replacing 50% of soybean meal. Dietary supplementation of 15% MLM improved rumen fermentation, growth performance, blood metabolites, plasma IGF-I and mitigated ammonia and methane without any adverse effects in growing buffalo calves.

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1. Introduction

One challenge facing animal producers is the scarcity and high cost of concentrates, especially protein sources, which force nutritionists to develop less expensive alternative protein supplements (Kholif et al., 2015). In Egypt and other developing countries, tree leaves as alternative foodstuffs are possible sources of nutrients

to increase animal production (Kholif et al., 2016). *Moringa oleifera* is a fast-growing softwood plant primarily distributed in tropical and subtropical areas. Due to its comprehensive nutritional, antioxidant, and medicinal qualities, *M. oleifera* has attracted increasing attention from animal nutrition researchers (Makkar and Becker, 1997). Leaves of *M. oleifera* are rich in crude protein, vitamins, minerals, fatty acids, and other secondary metabolites. The crude protein content in *M. oleifera* leaves on dry matter basis varies from 23 to 30.3% (Wu et al., 2013) with approximately 47% of rumen bypass protein (Becker, 1995), a sufficient amino acid profile (Sánchez-Machado et al., 2010), and polyphenolic antioxidants (Nouman et al., 2016). Leaves from *M. oleifera* are a low-cost protein supplement compared to other conventional protein supplements such as sesame and soybean meal (Kholif et al., 2015). The average crude fiber content of *M. oleifera* leaves is as low as 5.9%, nearly equal to that of soybean meal. There are many

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benefits from using *M. oleifera* foliage as a protein source, including the opportunity to harvest many times per season. Furthermore, dry moringa leaves can be stored for long periods without nutritive losses (Mendieta-Araica et al., 2011). The active natural antioxidants present in *M. oleifera* leaves include mainly polyphenols, polysaccharides, alkaloids, and vitamins, making them useful for various purposes as a feed additive (Moyo et al., 2012). The leaves are also rich in flavonoids and carotenoids (Pakade et al., 2013).

Although ruminants are considered a primary source of high-quality animal protein on the one hand, they are also responsible for substantial greenhouse gas emissions on the other hand. The leaves of *M. oleifera* are alternative natural feed additives that can alter the rumen fermentation pathway, inhibit methanogen efficiently and improve ruminant production (Soliva et al., 2005). The active compounds of *M. oleifera* with antimicrobial and anthelmintic properties have been shown to increase ruminant feed utilization and animal performance (Valdes et al., 2015; Cohen-Zinder et al., 2016; Kholif et al., 2016).

MLM increases the quantity of animal protein (red meat) in growing and fattening ruminants. Although multiple studies have recommended MLM as a cost-effective alternative dietary source of protein for ruminants, one which reduces methane emissions from ruminant farms, little information is available about the optimal concentration of *Moringa oleifera* leaves as a soybean meal alternative in feed for growing buffalo calves. Therefore, the objective of this study was to determine the effect of dietary inclusion of MLM on nutrient digestibility, rumen fermentation, rumen enzymes, blood metabolites, growth-related hormones, and growth performance in buffalo calves.

2. Materials and methods

2.1. Animals, diets, and management

This study was conducted at the research farm of the Faculty of Agriculture, Al-Azhar University Assiut, Egypt (27°2'N, 31°0'E). Thirty clinically healthy male buffalo calves, eight- to nine-months-old, with an average body weight of 153.7 ± 0.97 kg, were randomly distributed into three groups of 10 animals each for the six-month experimental period. Inside the farm, the ambient temperature was 25–33 °C, and relative humidity was 60–65%. The

experimental diet contained concentrate mixture and wheat straw, and Egyptian clover was fed as a roughage. The levels of concentrate and roughage were 2% and 1% of body weight, respectively. The experimental diets were formulated according to NRC guidelines (2001) to fulfill the nutritional requirements of growing calves. The composition and chemical analysis of the experimental diets are presented in Table 1. The diet was prepared and combined daily and given twice a day for the entire experimental period (6 months). Fresh water was available ad libitum. Before the start of the experiment, calves were dewormed. The control group was fed a basal diet (only soybean meal) without supplementation (M0). The other (treatment) groups were fed the basal diet supplemented with MLM at inclusion levels of 15% (M15) and 20% (M20), replacing soybean meal in the concentrated mixture by 50 and 75%, respectively. MLM was prepared as previously described (Kholif et al., 2015). Each calf was housed in a separate pen with a concrete floor and fitted with a locally made feed manger. Calf body weight was measured at the start of the experiment and every month thereafter. Feed intake was recorded weekly.

2.2. Digestibility trials

The indicator chromic oxide was used to estimate the digestibility of the feed nutrients. The digestibility trial lasted for 14 days until the completion of the experiment, with a 7-day preliminary period and 7-day collection period. For 14 days, each calf received exactly 10 g of powdered Cr₂O₃, hand-mixed into the concentrate mixture. Feed samples were collected daily during the collection period, and at the end of the collection process, the samples were mixed and ground via a 1 mm mesh screen. Approximately 200 g of fresh feces were collected twice daily on days 7–14 by fecal grabbing and placed in a refrigerator. The fecal samples from each animal were combined at the end of the digestibility trial, dried at 60 °C, and then ground via the 1 mm mesh screen for later chemical analysis. Chemical analyses of feed and feces were performed according to the protocols of the Association of Official Analytical Chemists (AOAC, 2005).

Atomic absorption spectrophotometry was used to estimate the chromium content of feces and feed using previously published methods (Williams et al., 1962). The following equation was used to calculate the digestibility of nutrients:

Table 1
Ingredients and chemical composition of experimental diets.

Items	M0	M15	M20	Egyptian clover	Wheat straw	MLM
Ingredient (%)						
Yellow Corn	57	50	45	–	–	–
Wheat bran	23	23	27	–	–	–
Soybean meal	16	8	4	–	–	–
MLM	0	15	20	–	–	–
Limestone	2	2	2	–	–	–
Salt	1	1	1	–	–	–
Premix*	1	1	1	–	–	–
Chemical composition, (% as fed basis)						
ME Mcal/kg	2.5	2.5	2.5	0.4	1.31	2.5
Crude protein (CP)	17	17	17	2.32	3.73	27
Dry matter (DM)	89.16	89.48	90.47	17.20	91	90.70
Organic matter (OM)	82.25	81.35	81.90	15	84	80.7
Crude fiber (CF)	1.68	5.70	6.78	4.01	37.41	12
Ether extract (EE)	3.09	3.18	3.36	0.3	1.46	3.2
Nitrogen free extract (NFE)	60.47	55.47	54.76	8.37	41.4	38.5
Ash	6.91	8.13	8.57	2.2	7	10
Calcium	0.84	1.16	1.26	0.48	0.28	2.32
Phosphorus	0.56	0.57	0.60	0.07	0.09	0.3

MLM, *Moringa oleifera* leaf meal

* Minerals and vitamins (premix) composition: each Kg contains 40,000 I.U Vit. A; 100,000 I.U Vit. D; 233.3 mg vitamin E; 166.7 mg vitamin K3; 166.6 mg Vit. B1, 66.7 mg Vit. B2; 200 mg Vit. B6; 1 mg Vit. B12; 150 mg Vit. C; 1000 mg niacin; 3333.3 mg choline chloride; 100 mg folic acid; 2 mg biotin; 223.3 mg pantothenic acid; 1000 mg magnesium sulfate; 333.3 mg iron sulfate; 500 mg zinc sulfate; 100 mg cobalt sulfate.

Digestibility of nutrient (%) = $100 - (100 \times \% \text{ marker in feed} / \% \text{ marker in feces} \times \% \text{ nutrient in feces} / \% \text{ nutrient in feed})$ (Maynard and Loosli, 1969).

2.3. Rumen liquor parameters

Rumen liquor was collected from each calf using a stomach tube three hours after the morning feeding at the end of the digestibility trial. The samples were split into two sections using plastic tubing (50 mL). The first portion was filtered through 1 layer of cheesecloth and used to calculate the total number of protozoa (Elghandour et al., 2017). The second portion was filtered through 4 layers of cheesecloth and used directly to measure pH using a digital pH meter (Beckman, Model 45, USA).

The concentration and molar proportions of individual short-chain fatty acids were detected by gas-liquid chromatography (HP, model 5890, USA). Fatty acid separation was performed using a capillary column (30 m × 0.25 mm internal diameter, 1-m film thickness, Supelco Nukol; Sigma-Aldrich, ON, Canada) and flame ionization detection. The column temperature was adjusted to 100 °C for 1 min, and then raised by 20 °C/min to 140 °C, then by 8 °C/min to 200 °C, and held at this temperature for 5 min. The temperature of the injector and detector were maintained at 200 °C and 250 °C, respectively. Helium was used as the carrier gas. Rumen concentration of ammonia nitrogen (NH₃-N) (mg/dL) was measured according to AOAC method 973.49 (AOAC, 2005).

2.4. Rumen enzyme activity

Enzymatic activity in the rumen fluid was estimated spectrophotometrically (Unico, USA). The activities were measured according to the following previously published protocols: cellulase and alpha-amylase were measured following Miller (1959), lipase was measured as in Peled and Krenz (1981), protease as in Folin and Ciocalteu (1927), and urease as in Weatherburn (1967). Extracellular protein concentrations in the crude enzyme were assayed according to Lowry et al. (1951).

2.5. Blood sampling

At the end of the experiment, 6 h after morning feeding, blood samples were collected by jugular vein puncture. Blood samples were centrifuged at 3000 rpm at 4 °C for 20 min to collect both serum and plasma, which were then saved at -20 °C until blood parameters were analyzed. Blood metabolites (cholesterol, glucose, total protein, and albumin) were analyzed by spectrophotometry

using commercial test kits (Spinreact, Girona, Spain) as described by the manufacturer. Plasma growth hormones and insulin-like growth factor-I were determined using the Bovine GH ELISA Test Kit (Endocrine Technologies, Newark, CA, USA) per the manufacturer's directions. Intra- and inter-assay variance coefficients were 5.53% and 7.45%, respectively.

The total plasma IGF-I concentration was determined according to the manufacturer's directions using an IGF-I-specific ELISA kit (Active IGF-I ELISA; Beckman Coulter, USA). Analytical sensitivity was 0.03 ng/ml. The coefficients of variation in the intra- and inter-assay were 3.5% and 8.5%, respectively.

2.6. Statistical analyses

Statistical analyses were conducted using SPSS (version 19). The Kolmogorov-Smirnov normality test was applied to examine if variables were normally distributed. Differences between group means were performed by one-way analysis of variance (ANOVA). The Duncan multiple range test (Steel and Torrie, 1980) was applied to test the effects of treatments on studied parameters. The data are presented as mean ± S.E., and statistical significance was assigned at $p < 0.05$.

3. Results

3.1. Nutrient digestibility and feeding value

The findings in Table 2 show that, relative to the control group, dietary supplementation of MLM to buffalo calves substantially improved ($p < 0.05$) the digestibility of dry matter (DM), organic matter (OM), and crude fiber (CF) (Table 2). However, with the addition of MLM to the feed, the digestibility of crude protein (CP) and ether extract (EE) decreased significantly ($p < 0.05$) relative to the control group.

At 15% MLM supplementation, the increase in DM, OM, and CF digestibility was greater. With dietary supplementation of MLM, the feeding value in terms of total digestible nutrients (TDN %) and digestible crude protein (DCP %) was significantly reduced ($p < 0.05$), especially when MLM supplementation was 20%. The improvement in DM, OM, and CF digestibility was greater at 15% than at 20% MLM.

3.2. Rumen fermentation activities

Dietary supplementation of MLM induced a significant ($p < 0.001$) effect on rumen fermentation kinetics (Table 3). The

Table 2
Effect of dietary supplementation of MLM on nutrient digestibility and feeding value of buffalo calves.

Item	Treatment			P-value
	M0	M15	M20	
Nutrient Digestibility				
DM	61.32 ^c ± 0.14	66.42 ^a ± 0.13	62.03 ^b ± 0.13	<0.001
OM	62.68 ^c ± 0.12	68.57 ^a ± 0.16	63.85 ^b ± 0.11	<0.001
CP	66.06 ^a ± 0.44	60.91 ^b ± 0.27	57.73 ^c ± 0.17	<0.001
CF	58.51 ^b ± 0.29	64.57 ^a ± 0.59	59.22 ^b ± 0.23	<0.001
EE	64.04 ^a ± 0.18	62.32 ^b ± 0.15	61.79 ^c ± 0.13	<0.001
NFE	58.21 ^a ± 0.09	56.90 ^b ± 0.40	55.11 ^c ± 0.16	<0.001
Feeding value				
TDN	56.85 ^a ± 0.10	55.56 ^b ± 0.28	53.3 ^c ± 0.11	<0.001
DCP	10.53 ^a ± 0.07	9.71 ^b ± 0.04	9.20 ^c ± 0.02	<0.001

^{a,b,c}Means within the same raw in each item with different superscripts are significantly different ($P < 0.05$).

DM, dry matter; OM, organic matter; CP, crude protein; CF, Crude fiber; EE, ether extract; NFE, Nitrogen free extract; TDN, total digestible nutrients; DCP, Digestible crude protein.

MLM, *Moringa oleifera* leaf meal

M0, M15 and M20, *Moringa oleifera* leaf meal at 0, 15 and 20% of the concentrate mixture.

Table 3
Effect of dietary supplementation of MLM on rumen parameters of buffalo calves.

Item	Treatment			P-value
	M0	M15	T20	
pH	6.41 ^b ± 0.003	6.54 ^a ± 0.02	6.40 ^b ± 0.004	<0.001
NH ₃ -N (mg/dl)	15.75 ^a ± 0.04	12.76 ^b ± 0.03	10.75 ^c ± 0.04	<0.001
Total VFAs (mmole/L)	79.51 ^a ± 0.11	85.42 ± 0.31	80.74 ± 0.15	<0.001
Acetate (C2) (mmol/L)	52.37 ^c ± 0.11	56.27 ^a ± 0.19	52.96 ^b ± 0.13	<0.001
Propionate (C3) (mmol/L)	15.29 ^c ± 0.02	17.11 ^a ± 0.08	15.95 ^b ± 0.13	<0.001
n-Butyrate (mmol/L)	7.69 ± 0.03 ^a	7.37 ^b ± 0.02	7.71 ^a ± 0.03	<0.001
Iso-Butyrate (mmol/L)	1.13 ^b ± 0.07	1.41 ^a ± 0.06	1.2 ^b ± 0.06	<0.05
n-Valerate (mmol/L)	1.21 ^c ± 0.01	1.47 ^a ± 0.04	1.31 ^b ± 0.01	<0.001
iso-Valerate (mmol/L)	1.81 ^a ± 0.02	1.77 ^a ± 0.01	1.6 ^b ± 0.06	<0.01
Acetate/ Propionate ratio	3.42 ^a ± 0.01	3.28 ^b ± 0.02	3.32 ^b ± 0.03	<0.001
Molar proportion, (mol/100 mol)				
Acetate (C2)	65.86 ± 0.10	65.87 ± 0.09	65.59 ± 0.19	0.3
Propionate (C3)	19.24 ^b ± 0.04	20.03 ^a ± 0.08	19.75 ^b ± 0.14	<0.001
Butyrate (C4)	9.67 ^a ± 0.04	8.63 ^c ± 0.02	9.54 ^b ± 0.04	<0.001
Total Protozoa, x 10 ⁵	6.87 ^a ± 0.03	4.37 ^b ± 0.04	3.40 ^c ± 0.05	<0.001

^{a,b,c}Means within the same row in each item with different superscripts are significantly different (P < 0.05).

MLM, *Moringa oleifera* leaf meal

NH₃-N, Ammonia nitrogen; VFAs, Volatile fatty acids

MLM, *Moringa oleifera* leaf meal

M0, M15 and M20, *Moringa oleifera* leaf meal at 0, 15 and 20% of the concentrate mixture.

Table 4
Effect of dietary supplementation of MLM on ruminal enzymes activity as well as rumen protein concentration in buffalo calves.

Items	Treatments*			P-value
	M0	M15	M20	
α-amylase activity (μg glucose/min/ml)	5.43 ^a ± 0.02	4.84 ^b ± 0.11	3.94 ^c ± 0.16	<0.001
Cellulase activity (μg glucose/min/ml)	5.22 ^a ± 0.12	4.45 ^b ± 0.19	3.22 ^c ± 0.11	<0.001
Lipase activity (μg p- nitrophenol/min/ml)	7.67 ^a ± 0.21	4.73 ^b ± 0.32	3.53 ^c ± 0.11	<0.001
Urease activity (μg NH ₃ /min/ml)	48.31 ^a ± 0.47	33.4 ^b ± 0.32	30.38 ^c ± 0.6	<0.001
Protease activity (μmol of tyrosine/min/ml)	27.44 ^a ± 0.6	21.61 ^b ± 0.42	18.32 ^c ± 0.26	<0.001
Rumen protein concentration (mg/ml)	2.35 ^c ± 0.04	3.59 ^b ± 0.02	3.98 ^a ± 0.06	<0.001

^{a,b,c}Means within the same row in each item with different superscripts are significantly different (P < 0.05).

MLM, *Moringa oleifera* leaf meal

M0, M15 and M20, *Moringa oleifera* leaf meal at 0, 15 and 20% of the concentrate mixture.

ruminal pH 3 h after feeding was increased significantly (p < 0.001) in calves fed a diet with 15% MLM. However, relative to the control group, the concentration of ruminal NH₃-N, the total protozoa count, and the acetate to propionate ratio (C2/C3) were significantly reduced (p < 0.001) in both M15 and M20 inclusion levels. Calves in the M15 group had the highest (p < 0.001) concentrations of total short-chain VFA (acetic, propionic, and isobutyric) and relative molar proportion of propionic compared to the control (M0) and M20 groups.

3.3. Rumen enzyme activities

Ruminal cellulase, α-amylase, lipase, urease and protease activity were significantly (p < 0.001) decreased in treatment groups M15 and M20 compared to the control group M0 (Table 4). Moreover, the concentration of rumen protein was significantly higher (p < 0.001) in both treatment groups relative to the control group.

3.3.1. Growth performance

Final body weight, the total and daily weight gain were significantly increased (p < 0.001) for calves fed M15 and M20 relative to those fed the control diet (M0) (Table 5). Concentrate and roughage intake and total DM intake were significantly (p < 0.001) improved by 15% MLM supplementation compared to the control group. Furthermore, the addition of both 15 and 20% MLM significantly decreased (p < 0.001) the feed conversion ratio compared to M0.

3.3.2. Blood metabolites

Dietary supplementation of MLM to growing calves significantly influenced all studied blood metabolites (Table 6). Serum glucose, total protein, albumin, and globulin concentrations in M15 and M20 calves were significantly greater (p < 0.001) than M0. However, blood urea nitrogen, serum albumin/globulin ratio, and total cholesterol were significantly reduced (p < 0.001) in M15 and M20 compared with the control group. The concentration of serum growth hormone was not statistically affected (p > 0.05) by the experimental diets. Plasma IGF-I was significantly increased in calves supplemented by 15% MLM relative to calves in the M0 and M20 treated groups.

4. Discussion

MLM serves as a healthy and inexpensive source of protein and micronutrients. As a novel protein supplement with high biological value, *M. oleifera* may help mitigate the feeding crisis as an unconventional protein source for ruminants. In the current study, the improvement in DM, OM, and CF digestibility with MLM supplementation was consistent with the results of previous studies (Kholif et al., 2015; Kholif et al., 2018; Kholif et al., 2019; Parra-Garcia et al., 2019; Dhanasekaran et al., 2020). Previous experiments have shown that *M. oleifera* extract improved (p < 0.01) digestibility of organic matter (OM), dry matter, and neutral detergent fiber (NDF) in Nubian goats (Kholif et al., 2018, 2019). Further-

Table 5
Effect of dietary supplementation of MLM on performance of growing buffalo calves.

Item	Treatments			P-value
	M0	M15	M20	
Initial body weight (kg)	153.700 ± 0.97	153.00 ± 2.04	154.7 ± 0.56	0.67
Final body weight (kg)	305.40 ^c ± 0.72	318.10 ^a ± 0.99	310.10 ^b ± 0.97	<0.001
BW gain (kg)	151.7 ^b ± 1.22	165.1 ^a ± 2.65	155.4 ^b ± 1.24	<0.001
Daily gain (kg)	0.842 ^b ± 0.006	0.917 ^a ± 0.01	0.863 ^b ± 0.006	<0.001
DMI of concentrate	4.86 ^a ± 0.06	4.97 ^a ± 0.03	4.44 ^b ± 0.05	<0.001
DMI of roughage	2.08 ^a ± 0.03	2.13 ^a ± 0.01	1.91 ^b ± 0.02	<0.001
Total DM intake (kg/day)	6.94 ^a ± 0.08	7.10 ^a ± 0.04	6.35 ^b ± 0.07	<0.001
Feed conversion ratio	8.24 ^a ± 0.13	7.74 ^b ± 0.15	7.36 ^c ± 0.08	<0.001

^{a,b}Means within the same row in each item with different superscripts are significantly different (P < 0.05).

DMI : Dry matter intake

Feed conversion ratio = Total dry matter intake (kg)/daily weight gain (kg)

MLM, *Moringa oleifera* leaf meal

M0, M15 and M20, *Moringa oleifera* leaf meal at 0, 15 and 20% of the concentrate mixture

Table 6
Effect of dietary supplementation of MLM on some blood parameters of growing buffalo calves.

Item	Treatment			P-value
	M0	M15	M20	
Glucose, mg/dl	78.19 ^c ± 0.28	89.32 ^b ± 0.19	95.99 ^a ± 0.42	<0.001
Total protein, g/dl	7.26 ^c ± 0.02	8.33 ^b ± 0.07	9.06 ^a ± 0.05	<0.001
Albumin (g/dl)	4.12 ^c ± 0.01	4.21 ^b ± 0.02	4.51 ^a ± 0.02	<0.001
Globulin (g/dl)	3.13 ^c ± 0.03	4.12 ^b ± 0.06	4.54 ^a ± 0.04	<0.001
A/G ratio	1.31 ^a ± 0.02	1.02 ^b ± 0.01	0.99 ^b ± 0.07	<0.001
BUN (mg/dl)	31.49 ^a ± 0.21	26.43 ^b ± 0.20	23.17 ^c ± 0.15	<0.001
Cholesterol, mg/dl	132.87 ^a ± 1.36	121.97 ^b ± 0.23	117.27 ^c ± 0.34	<0.001
GH ng/ml	2.27 ± 0.02	2.28 ± 0.01	2.25 ± 0.02	0.39
IGF-I ng/ml	124.07 ^b ± 0.048	127.88 ^a ± 0.41	123.38 ^b ± 0.11	<0.001

^{a,b,c}Means within the same row in each item with different superscripts are significantly different (P < 0.05).

A/G : albumen/ globulin ratio

GH, growth hormone, IGF-I, insulin growth factor-I

MLM, *Moringa oleifera* leaf meal

M0, M15 and M20, *Moringa oleifera* leaf meal at 0, 15 and 20% of the concentrate mixture.

more, the results of the current study supported the findings of Aregheore (2002), which suggested that the substitution of 20 and 50% batiki grass with fresh *M. oleifera* improved weight gain and the digestibility of nutrients (DM, OM, CP, NDF) in growing goats. Our results are also consistent with Fadiyimu et al. (2010), who found that feeding a concentrate with 25% *M. oleifera* leaves significantly improved CP intake, DM and nutrient digestibility, nitrogen retention, blood profiles, and weight gain in sheep.

There are several possible explanations for 15% MLM improvement of nutrient digestibility. Rumen microflora can utilize low levels of secondary metabolites (e.g., tannins, essential oils, phenolics, and saponins) from *Moringa oleifera* and use them as energy sources without any negative impact on rumen fermentation (Frutos et al., 2004; Hart et al. 2008; Bodas et al. 2012). Although higher levels of MLM seem to negatively affect rumen microorganisms due to their antimicrobial properties (Bodas et al. 2012),

The rumen pH values obtained in the current study were significantly affected by dietary MLM supplementation, ranging from 6.4 to 6.54. These values are within the suitable range for fiber digestion, as proposed by Ørskov and Ryle (1990). Furthermore, Kholif et al. (2015) detected that ruminal pH was not influenced by feeding *M. oleifera* leaves to lactating goats. Notably, we also observed a significant decrease in rumen urea nitrogen in calves fed an MLM-supplemented diet. These results are consistent with those observed in previous studies (Jelali et al., 2014; Kholif et al., 2015, 2018). There are several possible explanations for this result. Tannins, saponin, and polysaccharides present in *Moringa oleifera* leaves decrease ruminal protozoal numbers. Tannins are known to be alternative feed additives to preserve dietary proteins from

ruminal degradation. Rumen protozoa are considered the main source of rumen ammonia through ingestion and proteolysis of bacterial protein (Wallace et al., 1994; Cheeke, 2000). MLM may have a role in controlling the release of ammonia in the digestive tract of ruminants by reducing the degradation and deamination of ruminal protein and decrease rumen ammonia (Bhatta et al., 2012; Jelali and Salem, 2014).

The observed decrease in the acetate/propionate ratio and the increase in propionate and total VFA concentration with MLM inclusion were consistent with the results of Lins et al. (2019) and Ebeid et al. (2020). These results imply that MLM can shift rumen fermentation to increase propionate and decrease total protozoa count, leading to methanogenesis limitation by reducing hydrogen supply (Polyorach et al., 2014) as protozoa are considered the main producer of rumen methane (Iqbal et al., 2008; Hook et al., 2010).

Various mechanisms have been proposed to explain why *M. oleifera* decreases the production of methane: (a) decrease of crude fiber digestion, (b) inhibition of methanogens, and (c) decreased degradation of proteins (Carulla et al., 2005; Tiemann et al., 2008; Salem et al., 2013).

The significant decrease in total protozoa count in calves fed MLM (M15 and M20) in this study could be ascribed to the active substances in MLM such as saponin, a well-documented dose-dependent antiprotozoal agent (Wallace et al., 1994; Jayanegara et al. 2014; Ebeid et al., 2020). Rumen ciliate protozoa are significantly reduced by the unsaturated fatty acids present in MLM, which are toxic to ciliated protozoa (Sánchez-Machado et al., 2010; Moyo et al., 2011).

Rumen microbes secrete enzymes (e.g., carboxymethylcellulase, xylanases, amylases, and proteases) that allow them to obtain energy through the degradation of complex carbohydrates (cellulose, hemicellulose, and pectin) into volatile fatty acids in ruminants (Li et al., 2017).

The significant decrease in α -amylase activity with the addition of MLM is consistent with the findings of Hoffmann et al. (2003), which indicated that MLM decreased total amylolytic activity and starch digestion. In contrast, Jadhav et al. (2018) found that feeding *Moringa oleifera* did not affect rumen enzymes or microbial populations. The authors proposed that lower tannins and saponin contents of MLM fail to induce harmful effects on rumen enzymes. The significant increase in the concentration of rumen protein with the inclusion of MLM (M15 and M20) was consistent with the results of previous studies (Kholif et al., 2016; Ebeid et al., 2020). A possible explanation for these results may be the decrease in rumen $\text{NH}_3\text{-N}$ in treatment groups (M15 and M20), indicating the improved conversion of amino acids, peptides, and rumen $\text{NH}_3\text{-N}$ into microbial protein. High rumen protein contents also support this improved conversion.

The improvement in the final body weight and average daily weight gain with MLM dietary supplementation in calves is consistent with previous studies (Aregheore, 2002; Fadiyimu et al., 2010; Adegun et al., 2011; Moyo et al., 2012; Babiker et al., 2017; Kholif et al. 2018; Zeng et al. 2018). These studies revealed that the inclusion of *M. oleifera* leaves in different farm animal diets improved growth performance and feed conversion rate, therefore supporting its use as a natural alternatives protein supplement to boost ruminant production. The significant increase in total body weight gain by calves fed MLM diets could be attributed to the improvement in utilization of MLM rumen bypass protein, high nutrient profile, palatability (feed intake), and high nutrient utilization efficiency (Jiwuba et al., 2016).

Usually, blood metabolite measurements are used to assess the overall health and vitality of animals. All estimated values of blood parameters in this study were within the normal reference ranges (Boyd, 2011). The significant increase in serum glucose accompanied by a significant decrease in serum cholesterol was consistent with other studies (Kholif et al., 2016; Kholif et al., 2018; Kekana et al., 2019). Dietary inclusion of *M. oleifera* significantly decreased the concentrations of serum triglycerides and cholesterol but increased ($p < 0.05$) the concentration of serum glucose in lactating goats (Kholif et al., 2016) and lactating dairy cows (Zeng et al., 2018). In contrast, Jelali et al. (2014) illustrated that MLM diet decreased ($p < 0.05$) serum total proteins and urea concentrations but had no impact on serum glucose concentration ($p > 0.05$).

Insulin-like growth factor-1 (IGF-1) and growth hormone (GH) have been applied as indicators of growth potential, alterations in body composition and average daily gain (ADG) in beef cattle. The significant increase in insulin growth factor-I (IGF-I) in calves fed 15% MLM was consistent with the findings of previous studies. These studies found a positive correlation between high level of nutrition, high average daily gain and body weight with the concentrations of plasma IGF-1 in growing steers (Röpke et al., 1994; Davis and Simmen, 1997; Torrentera et al., 2009). From the obtained results in the current study, it is more obvious that, dietary supplementation of 15% MLM improved mostly all studied parameters in growing buffalo calves.

5. Conclusion

Dietary inclusion of MLM improved rumen fermentation in terms of reducing ($p < 0.001$) ruminal enzymes, ruminal ammonia-N, total protozoa, and acetate/propionate ratio, however it increased acetic, propionic, butyric, and total volatile fatty acid

concentrations. In addition, supplemental MLM significantly improved ($p < 0.001$) final body weight, dry matter intake of feed, daily weight gain, feed conversion efficiency, blood metabolites, and IGF-I. It can be concluded that MLM (specifically, at 15% substitution) is a multi-purpose protein supplement with the potential to replace 50% of soybean meal with nutritional and medicinal advantages similar to the previous generation of natural feed additives. Dietary supplementation of 15% MLM improved rumen fermentation, growth performance, blood metabolites, plasma IGF-I and mitigate ammonia and methane in growing buffalo calves.

Statement of animal right

All institutional and national guidelines for care and use of animals were followed according to the Egyptian Medical Research Ethics Committee (no. 14–126).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Further Reading

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