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Effects of *Moringa oleifera* leaf meal supplementation on growth performance and blood profiles of cattle heifers

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Authors' Contribution	**Anwar, A.** & **A.A. Behan**, designed, conducted the experiment and draf the manuscript, **M.N. Rajput, S.U.A. Shah, & S. A Soomro** analysed the data and revised the manuscript.

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ABSTRACT

To evaluate the impact of dietary inclusion of *Moringa oleifera* leaf meal (MOLM) on the growth performance, body conformation, and blood biochemistry of intensively managed cattle heifers, a total of 12 heifers were randomly selected and divided into two groups viz. group-CON (basal diet) and group-MOLM (basal diet + 10% *M. oleifera* leaf meal). Results indicate that significantly higher feed intake was observed in the MOLM group. The heifers supplemented with MOLM were having significantly improved body weight, body height, body length and heart girth compared to the heifers in CON group. Blood parameters, including Red Blood Cells (RBCs), haemoglobin, and immunoglobulins, showed positive trends, with immunoglobulin G levels significantly higher in the MOLM group. In conclusion dietary *M. oleifera* leaf meal improved growth, body measurement and blood biochemistry of intensively managed cattle heifers.

Keywords: Moringa oleifera, heifer, growth, blood profile.

INTRODUCTION: Animal producers are facing great economical losses in livestock production due to critical shortage of protein feed. Thus researchers need to find alternative feed resources that could fulfil protein requirements (Kholif *et al.*, 2015). In many developing countries, the use of tree leaves has gained traction as a strategy to enhance nutrient supply and bolster animal production (Kholif *et al.*, 2016). Experts in animal nutrition are paying increasing attention to the fast-growing softwood plants amongst those *M. oleifera* is one that is mostly found in tropical and subtropical areas and has broad nutritional, anti-inflammatory, and medicinal characteristics (Makkar and Becker, 1997). *M. oleifera* leaves are an economical source of protein since they are high in crude protein (Kholif *et al.*, 2015). In addition to having an appropriate amino acid profile and 47% of rumen bypass protein *M. oleifera* leaves also contain polyphenolic antioxidants. With a crude protein concentration ranging from 23% to 30.3% on a dry matter basis (Wu *et al.*, 2013), *M. oleifera* emerges as a promising supplement for animal diets. Moreover, dried moringa leaves may be kept for a long time without losing any nutrients (Mendieta-Araica *et al.*, 2011). *M. oleifera* leaves contain polyphenols, polysaccharides, alkaloids, flavonoids, carotenoids, and other beneficial compounds, making them a viable feed ingredient for various applications (Moyo *et al.*, 2012). These leaves have also been demonstrated to reduce greenhouse gas emissions, alter rumen fermentation pathways, and enhance ruminant feed consumption and animal performance through their antibacterial and anthelmintic activities (Kholif *et al.*, 2016).

Moringa leaves are recognized for their high biological value and substantial potential as ruminant feed (Pradhan, 2016). They are rich in essential components including trace minerals, which play a crucial role in enhancing growth, function, and life processes by altering osmotic pressure, activating enzymes, growth hormones, and other organic compounds. However, the leaves may contain trace amounts of harmful nutrients, such as tannins, phenol, and saponins, which need to be examined for availability and nutritional potential (Sarwatt *et al.*, 2004). Depending on the state of development and the relative proportions of the leaves, petioles, and stems, but their biological value is high, making them a promising feed for ruminants (Pradhan, 2016). Therefore, the present study was conducted to examine the impact of dietary inclusion of *M. oleifera* leaf meal (MOLM) on the growth performance, body conformation, and blood biochemistry of intensively managed cattle heifers.

OBJECTIVES: The objectives of this study were as follows: (1) to evaluate the effect of dietary inclusion of *M. oleifera* leaf meal on the growth performance and body conformation of intensively managed cattle heifers (2) to determine the effect of dietary inclusion of *M. oleifera* leaf meal on blood biochemistry of intensively managed cattle heifers.

MATERIAL AND METHODS: **Experimental design:** The cattle heifers in this study were raised under a stall-feeding management system at the Livestock Experiment Station, Department of Livestock Management, Sindh Agriculture University Tandojam. Twelve (16-18 months old) heifers were selected for the study and divided into two distinct groups: group CON (fed on self-prepared concentrate mixture (crushed maize, wheat bran, cotton seed cake and rice polish and the roughages including wheat and rice straw) + green fodder basal diet only), group MOLM (fed on basal diet + 10% MOLM), with six heifers in each group. The heifers were housed in an animal shed and given a 15-day acclimatization period before being kept for 120 days. Each heifer was marked with a number, and they were fed self-prepared basal diet and additional 10% *M. oleifera* leaf meal to group MOLM. Water was provided ad libitum to all heifers in both groups. The feed was given to all cattle heifers two times a day (morning and evening). The basal diet was formulated according to NRC (2001) guideline.

Preparation of MOLM: The preparation of *M. oleifera* leaves (MOLM) involved harvesting plant samples from the field using soft twigs and leafy branches. The tender twigs were subjected to sun-drying for two to three days, after which the partly dried leaves were separated through threshing. The leaves were then sun-dried once more for approximately 48 hours on black plastic sheets. Once fully dried, the leaves were placed in bags, finely pulverized in a hammer mill, and stored in a well-ventilated area (Aharwal *et al.*, 2018).

Growth performance: The daily feed intake was recorded by offering pre-weighed feed, the left-over feed was subtracted from the amount of feed offered. It was calculated as; feed intake = offered feed – remaining feed. The feed was offered @ 1% of body weight of heifer to each group. The animals of both groups were weighed initially, by using weighing machine (Yameto made in China) available at the Livestock Experiment Station then weight was recorded fortnightly in kilograms prior to feeding in the morning. Final body weight was recorded at the end of the trial period. All variations in the body weight and feed intake of all experimental animals were recorded on fortnightly basis. The feed efficiency for each group was calculated as total feed consumed divided by total weight gain on fortnightly basis (Koch *et al.*, 1963). Body conformation was measured every 15-days interval using a measuring tape, with measurements taken in centimeters.

Blood hematology: Blood samples were aseptically collected from the jugular vein of female heifers using a 5mL sterile syringe and then carefully transferred into 1.5mL tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) to prevent blood clotting. Subsequently, the collected samples were promptly transported to Animal Medical Center (AMC) for determination of complete blood count by Hematological analyzer. The samples were collected twice (before start of experiment and at the end of trial) from both groups for determination of complete blood profile including White Blood Cell count (WBC), Red Blood Cell count (RBC), Hemoglobin (HB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Platelet count.

Hematology-analyzer was used for the calculation of normal values of blood constituents. The blood glucose was determined by the glucose oxidase method through glucometer at Animal Medical Center (AMC) (Abdel-Raheem and Hassan, 2021).

Immunoglobulin G (Ig) levels: The concentration of Immunoglobulin G (IgG) was assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) kit for bovine IgG. Blood samples were sent to Karachi Lab for analysis (Kekana *et al.*, 2021).

Statistical Analysis: The acquired data were statistically evaluated using Analysis of Variance ANOVA in a Completely Randomized Deisgn (Steel *et al.*, 1960), and Duncan's multiple range test was used to assess mean differences for significance (Duncan, 1955). The statistical analyses were performed using JMP software.

RESULTS AND DISCUSSION:

Feed intake of cattle heifers (kg) in 120 days: The results for feed intake revealed significantly ($P<0.05$) higher feed intake (with the average 242 kg) in group MOLM (fed on basal diet + 10% MOLM), compared to CON (fed on basal diet only), with the average value of 234.8 kg. The observed differences were found to be statistically significant at a confidence level of $P<0.05$ (Figure 1).

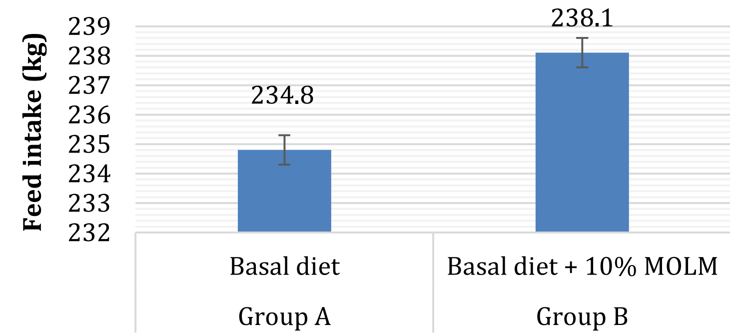

Figure 1: Feed intake (kg) of intensively managed cattle heifers fed on MOLM

Body weight of cattle heifers (kg): The initial weight on day 0 displayed no significant difference ($p>0.05$) among both groups. However, from the 2nd to the 8th week significant difference ($P<0.05$) was observed, in which MOLM (fed on basal diet + 10% MOLM) showed higher body weight then CON (fed on basal diet only) from week 2nd to 8th. The final weight was also seen significantly higher in ($P<0.05$) in MOLM, than CON which showed 226.37±0.31 kg final body weight (table 1).

Fortnights	CON	MOLM	P-Value
	Basal diet	Basal diet + 10% MOLM	
Initial	184.12±0.20^{a}	183.82±0.22^{a}	0.3525
1st	189.85±0.26^{a}	190.62±0.21^{a}	0.0568
2nd	194.69±0.21^{b}	196.72±0.24^{a}	0.0002
3rd	199.60±0.27^{b}	201.81±0.59^{a}	0.0004
4th	204.22±0.33^{b}	209.06±0.22^{a}	0.0001
5th	209.21±0.25^{b}	216.22±0.22^{a}	0.0001
6th	216.09±0.32^{b}	223.37±0.25^{a}	0.0001
7th	220.97±0.17^{b}	230.37±0.32^{a}	0.0001
8th	226.37±0.31^{b}	237.19±0.32^{a}	0.0001

Table 1: Body weight (kg) of intensively managed cattle heifers fed on MOLM on fortnight basis.

Daily weight gain and total weight gain in 120 days: The greater daily gain was recorded from MOLM (fed on basal diet + 10% MOLM) with the average value of 0.44g compared to CON with the average value of 0.352g, similarly total gain in BW was also found high in MOLM as 53.37kg compared to CON as 42.25kg (table 2).

Variables	CON	MOLM	P-value
	Basal diet	Basal diet + 10% MOLM	
Average daily gain (g)	0.352	0.444	0.0001
Total gain (kg)	42.25	53.37	0.0001

Table 2: Average daily gain (g) and total gain (kg) of cattle heifers fed on MOLM.

Feed efficiency of cattle heifers: The feed efficiency was 5.55 and 4.46 in MOLM and CON respectively, in which high feed efficiency was observed in MOLM (figure 2).

Body height of cattle heifers (cm): No significant ($p>0.05$) difference was observed in the initial fortnight between group MOLM (fed on basal diet + 10% MOLM) and CON (fed on basal diet only), both the groups showed linearly increasing body height, but the results revealed from 2nd to 8th fortnight that MOLM presented greater ($P<0.05$) values than CON group. The final body height of cattle heifers also showed significant ($P<0.05$) difference between MOLM and CON with the values presented as 125.83±0.24 cm and 119.93±0.18 cm in MOLM and CON respectively (table 3).

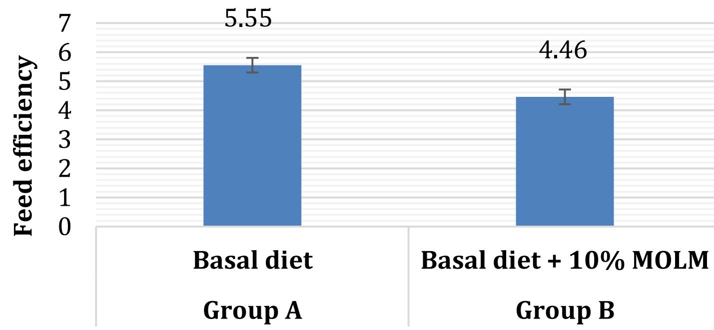

Figure 2: Feed efficiency of intensively managed cattle heifers fed on MOLM

Fortnights	CON	MOLM	P-Value
	Basal diet	Basal diet + 10% MOLM	
Initial	110.89±0.17^{a}	111.15±0.21^{a}	0.3708
1st	111.78±0.17^{a}	112.28±0.22^{a}	0.1172
2nd	112.91±0.17^{b}	114.92±0.22^{a}	0.0001
3rd	114.05±0.17^{b}	117.87±0.23^{a}	0.0001
4th	115.21±0.17^{b}	119.42±0.23^{a}	0.0001
5th	116.37±0.17^{b}	120.99±0.23^{a}	0.0001
6th	117.54±0.18^{b}	122.58±0.24^{a}	0.0001
7th	118.73±0.18^{b}	124.20±0.24^{a}	0.0001
8th	119.93±0.18^{b}	125.83±0.24^{a}	0.0001

Table 3: Body height (cm) of intensively managed cattle heifers fed on MOLM on fortnight basis.

Body length of cattle heifers (cm): The Body length was observed and recorded from 1st to 8th fortnight. The initial length was observed non-significantly different ($p>0.05$) among MOLM (fed on basal diet + 10% MOLM) and CON (fed on basal diet only), similarly the body length on 3rd fortnight was also observed non-significantly different ($p>0.05$) between both groups, while from fortnight 3rd to 8th the group MOLM showed significantly ($P<0.05$) increased results than CON group. The final body length of cattle heifers was observed as 121.49±0.52cm in MOLM and 113.55±0.42cm in CON with the significant difference ($P<0.05$), determined through statistical analysis (table 4).

Fortnights	CON	MOLM	P-Value
	Basal diet	Basal diet + 10% MOLM	
Initial	105.29±0.52^{a}	105.69±0.46^{a}	0.5845
1st	106.14±0.52^{a}	106.75±0.46^{a}	0.4071
2nd	107.17±0.50^{a}	108.71±0.47^{a}	0.0569
3rd	108.20±0.48^{b}	110.93±0.48^{a}	0.0041
4th	109.25±0.46^{b}	114.36±0.49^{a}	0.0001
5th	110.31±0.45^{b}	116.69±0.50^{a}	0.0001
6th	111.38±0.44^{b}	119.08±0.51^{a}	0.0001
7th	112.46±0.43^{b}	120.28±0.52^{a}	0.0001
8th	113.55±0.42^{b}	121.49±0.52^{a}	0.0001

Table 4: Body length (cm) of intensively managed cattle heifers fed on MOLM on fortnight basis

Body girth of cattle heifers (cm): The Body girth measurements was taken on fortnightly basis, the initial average body girth of cattle heifers was observed with no significant ($p>0.05$) difference, however from 2nd to 8th fortnight the measurements showed significant difference ($P<0.05$) in between MOLM and CON among. The MOLM group showed better results, the linear increase in the body girth was observed in both groups, The final body girth of cattle heifers was observed as 151.73±0.32 cm in MOLM and 145.05±0.26 cm in CON group with significant difference ($P<0.05$) (table 5).

Blood parameters of cattle heifers: The results revealed a significant difference ($P<0.05$) in the Red Blood Cell (RBC) count with values of 4.09±0.377 in the CON group and 6.75±0.377 in the MOLM group. The MOLM group showed a higher RBC count then CON. Similarly the Haemoglobin concentration in g/dL was also found significantly ($P<0.05$) higher in MOLM than CON with the values presented as 5.57±0.738 in CON and 9.05±0.738 in MOLM. The average Hematocrit (HCT) percentage was observed as 32.79±0.607 and 37.62±0.607 in CON and MOLM respectively. The comparison revealed a significant difference ($P<0.05$), with MOLM

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displaying higher HCT values than CON. The Mean Corpuscular Volume (MCV) was found 44.82±1.07 in CON and 46.22±1.07 in MOLM with non-significant difference (p>0.05), similarly to that the Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH) values were found non-significantly different (p>0.05) with the values of 32.68±0.984 in CON and 40.312±2.13 in MOLM for MCHC and 32.68±0.984 and 32.03±0.984 for MCH in CON and MOLM respectively (table 6).

Fortnights	CON Basal diet	MOLM Basal diet + 10% MOLM	P-Value
Initial	134.12±0.24ᵃ	134.03±0.28ᵃ	0.8140
1st	135.20±0.24ᵃ	135.38±0.29ᵃ	0.6534
2nd	136.57±0.25ᵇ	138.57±0.29ᵃ	0.0009
3rd	137.94±0.25ᵇ	142.12±0.30ᵃ	0.0001
4th	139.34±0.25ᵇ	143.99±0.30ᵃ	0.0001
5th	140.74±0.25ᵇ	145.89±0.31ᵃ	0.0001
6th	142.17±0.25ᵇ	147.81±0.31ᵃ	0.0001
7th	143.60±0.26ᵇ	149.76±0.32ᵃ	0.0001
8th	145.05±0.26ᵇ	151.73±0.32ᵃ	0.0001

Table 5: Body girth (cm) of intensively managed cattle heifers fed on MOLM on fortnight basis.

Parameter	CON Basal diet	MOLM Basal diet + 10% MOLM	p-value
RBC (10^9 /L)	4.09±0.377ᵇ	6.75±0.377ᵃ	0.0002
Haemoglobin (g/dL)	5.57±0.738ᵇ	9.05±0.738ᵃ	0.005
HCT %	32.79±0.607ᵇ	37.62±0.607ᵃ	0.0001
MCV (fl)	44.82±1.07ᵃ	46.22±1.07ᵃ	0.373
MCHC (g/L)	37.93±2.13ᵃ	40.312±2.13ᵃ	0.444
MCH (g/dL)	32.68±0.984ᵃ	32.03±0.984ᵃ	0.644
WBC (10^9 /L)	10.77±0.475ᵃ	6.38±0.475ᵇ	0.0001
Neutrophils (10^9 /L)	5.28±0.416ᵃ	4.09±0.416ᵃ	0.063
Lymphocytes (10^9 /L)	5.41±0.465ᵃ	3.683±0.465ᵇ	0.0195
Monocytes (10^9 /L)	2.30±0.171ᵃ	1.78±0.171ᵃ	0.052
Eosinophils (10^9 /L)	1.995±0.183ᵃ	1.492±0.183ᵃ	0.073
Basophils (10^9 /L)	1.532±0.128ᵃ	1.043±0.128ᵇ	0.017
Platelets (10^9 /L)	338±39.5ᵃ	376±39.5ᵃ	0.507
Glucose (mg/dl)	68.5±1.27ᵃ	60.87±1.27ᵇ	0.0008
Immunoglobulins (g/L)	9.25±0.34ᵃ	9.37±0.34ᵃ	0.801

Table 6: Blood parameters of intensively managed cattle heifers fed on MOLM

The WBC count was found significantly (P<0.05) higher in CON as 10.77±0.475 and MOLM as 6.38±0.475, the differential leukocyte count (DLC) exhibited the neutrophils values as 5.28±0.416 and 3.683±0.465 in CON and MOLM respectively, in which CON found with significantly (P<0.05) higher count. Similarly, monocytes count was also found significantly (P<0.05) greater in CON than MOLM with the values represented as 2.30±0.171 and 1.78±0.171 respectively. The eosinophils count was observed as 1.995±0.183 in CON and 1.492±0.183 in MOLM with no significant difference (p>0.05). The basophils were found significantly lesser in MOLM as 1.043±0.128 than CON as 1.532±0.128. The average blood glucose was found significantly greater in CON than MOLM as the values represented as 68.5±1.27 and 9.37±0.34 respectively. The levels of Immunoglobulins were found in the blood as 9.25±0.34 and 9.37±0.34 in CON and MOLM respectively.

The utilization of *M. oleifera* leaf meal (MOLM) as a dietary supplement presents a promising and cost-effective solution, offering a rich source of protein and minerals along with various medicinal health benefits. It has a high biological value as a novel protein supplement, which could potentially alleviate the feeding crisis by offering an alternative protein source for ruminants (Kekana *et al.*, 2019). The growth of cattle heifers primarily occurs in their skeletal and muscular systems, with protein deposition and mineralization being major factors. The rate of growth is typically indicated as the percentage increase in body size/weight (Kertz *et al.*, 1998). Body weight plays a significant role in the lifetime performance of animals, including growth, production, reproduction, and is often used to evaluate nutritional status and development (Chimonyo *et al.*, 2000).

The current study showed the improvement in the final body weight and average daily weight gain in cattle fed with *M. oleifera* leaf meal (MOLM) supplementation. Previous studies have shown that *M. oleifera* leaf meal (MOLM) supplementation can improve the final body weight and average daily weight gain in cattle heifers and diversify farm animal diets (Zeng *et al.*, 2018). These studies suggest that MOLM is a natural alternative protein supplement that can boost growth performance and feed conversion rate in ruminants. The observed improvement in growth performance may be attributed to MOLM's rumen bypass protein as well as its high nutritional content and antibacterial characteristics (Fahey et al., 2001; Jiwuba et al., 2016). The improvement in average daily weight gain in the group that consumed MOLM may be a result of enhanced nutritional digestion. Abdel-Raheem and Hassan (2021) stated that MOLM supplementation in calves brings a significant improvement in growth. The improvement in body weight increase may be related to the high amount of nutrients in MOLM and its antibacterial properties (Fahey *et al.*, 2001). Overall, the evidence suggests that MOLM could be a valuable natural protein supplement for promoting ruminant output, and further research is warranted to investigate its full potential.

The finding of the experiment exhibited maximum feed intake in cattle heifers fed on basal diet + 10% *M. oleifera* leaf meal (MOLM), similar to the findings of Aharwal *et al.* (2018) which reported that the feed intake was significantly higher in dried *M. oleifera* leaves supplemented rations than control group. This led to the moringa supplemented group gaining more body weight throughout the trial. Abdel-Raheem and Hassan (2021) also observed that calves' feed intake increased with the addition of moringa leaf meal to their diet. The effectiveness of feed utilization influences how well feed is used for the calves' development.

The feed efficiency was recorded significantly greater in group MOLM than control. The best chance for enhancing daily development is when the recommended quantity of feed is ingested each day. More feed intake results in increased growth, which is often accompanied by an improvement in production efficiency as a whole. As maintenance expenses decline proportionally as growth increases throughout a certain time. The body conformation including body height, length and chest girth was found better in with significantly improved values in cattle's fed with *M. oleifera* leaf meal supplement. Results are supported with the findings of Kekana *et al.* (2019) the body measurements of heart girth and body length increased significantly with *M. oleifera* leaf meal levels. Typically, blood metabolite measurements are used to evaluate an animal's general health and vitality. In this investigation, all calculated blood parameter values were within the usual reference limits (Boyd, 1984).

Our results indicated the difference in blood parameters of both groups, the red blood cells (RBCs) and other related RBCs indices were found better in MOLM, could be the possible cause of greater weight gain, the white blood cells (WBCs) along with differential leukocyte count were found significantly higher in control group whereas found lower in group MOLM, may be due to the antimicrobial properties of *M. oleifera*. No significant difference was found in platelets and antibodies among both groups; however, the blood glucose level was found lower in MOLM. The findings were supported by Aharwal *et al.* (2018) *M. oleifera* showed a significantly better effect on blood metabolite concentrations. The findings of Abdel-Raheem and Hassan (2021) were consistent with previous research studies such as Kholif *et al.* (2016), Kekana *et al.* (2019), which demonstrated that consumption of M. oleifera led to a significant increase in blood glucose levels contrary to our findings. Similarly Kholif *et al.* (2016) found that the intake of M. oleifera as a supplement to goats increased serum glucose levels (P < 0.05), Wafa *et al.* (2017) found no significant increase or decrease among groups control and MOLM treated (in small quantities) group, which indicates the effect of dosage in altering the blood glucose level in animals when supplemented with MOLM. Furthermore, in contrast to our findings Mandal *et al.* (2015) found that supplementing cow calves with sun-dried MOLM boosted IgG levels. In contrast, (Elaidy *et al.*, 2017) found low IgG levels in buffalo calves after supplementing with oven dried MOLM. This variation could be due to differences in nutrition quantity, production stage, and breed (Falowo *et al.*, 2018).

CONCLUSION: It is concluded that *M. oleifera* leaf meal supplementation can successfully be used as a growth promoter which is economical and nutritive. Furthermore *M. oleifera* leaf meal supplementation has a significantly superior effect on growth performance and blood profiles of cattle heifers. Better results were obtained in the experimental study with 10% *M. oleifera* leaf meal supplementation with the basal diet, showed a greater weight gain and body conformation in cattle heifers. Further research should be conducted to determine the best amount/dose of *M. oleifera* leaf meal supplementation and its effect on various parameters in large ruminants as well as small ruminants.

CONFLICT OF INTEREST: The authors declared no conflict of interest.

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