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Effect of *Moringa oleifera* Leaves Supplementation via Urea Molasses Block on Milk Yield and Composition, Hematological and Blood Biochemical Profile in Azikheli Buffaloes

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ABSTRACT

This study was conducted to determine the effect of *Moringa oleifera* (MO) leaf supplementation via urea molasses block (UMB) on milk yield and composition, hematological and blood biochemical profile in Azikheli buffaloes. A total of sixteen Azikheli buffaloes aged 2-4 years were allotted to four equal groups that were placed in individual stalls rendered to the distribution of control and treatment groups. Control group MOS (0%) was provided with UMB without MO leaves, while the treatment groups MOS-10, MOS-20, and MOS-30 were offered UMB with 10%, 20%, and 30% of MO leaves respectively. The dry matter intake (DMI) and organic matter intake (OMI) were significantly different, with the highest unit increase in the MOS-30 group. The dry matter (DM) and organic matter (OM) digestibility were calculated as significantly the highest in the MOS-30 group. Similarly, the highest ($p<0.05$) unit increase in milk yield (MY) was observed in the MOS-30 group. The milk composition was also significantly different and the highest unit increase in protein, lactose, solid not fat (SNF), total solid (TS), and fat, and the lowest unit in fat was observed in the MOS-30 group. Blood content was significantly different, where the highest unit increase in RBCs (red blood cells), WBCs (white blood cells), HB (hemoglobin), TP (total protein), BUN (blood urea nitrogen), PCV (packed cell volume) but the lowest unit in TC (total cholesterol) was observed in the MOS-30 group. It is concluded that the addition of different levels of *Moringa oleifera* in the UMB to Azikheli buffaloes leads to an increase in DM and OMI, DM and OM digestibility, milk yield and composition, and better hematological parameters and can be fed to the buffaloes with UMB at the level of 30%.

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Key words
***Moringa oleifera*, UMB, Dry matter intake, Digestibility, Milk yield, Milk composition**

E INTRODUCTION

Livestock plays a vital role in the Agricultural section of Pakistan (Hashmi *et al*., 2021). In Pakistan, usually in the rural or underdeveloped urban sector, about 8.5 million people depend on livestock as the major source of their income (Rehman *et al*., 2017). Milk is an important and demanding product of the livestock sector and Pakistan is the fourth number in the world in milk production after China, India, and the USA (Chandio *et al*., 2017). Cattle and buffaloes are considered major dairy animals. The

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meat industry in Pakistan is evolving with unbelievable progress and the export meat industry has increased the country's revenue from US $ 108.54 million (2010-11) to US $ 123.61 million. This shows that livestock can increase the economic situation of their country. In Pakistan, dairy animals are used for meat purposes because of the very low number of meat breeds. The milk and meat of buffaloes, cows, goats, and sheep in Pakistan are similar to what they produced 59 years ago. Significant increases in milk and meat production lead to growth in export earnings or increase in the number of livestock over the years. Azikheli buffalo breed is mostly present in the province of Khyber Pakhtunkhwa and is used as a dairy breed and is resistant to particular diseases and temperatures. Azikheli buffaloes have a large body size, strong lean body, and need a lower amount of feed as compared to other breeds, which helps them to survive better than other cattle breeds present in the province of Khyber Pakhtunkhwa and are mostly present in Bajor, Swat, Dir, and Khyber and can also be seen in different affluent regions of Afghanistan (Khan *et al*., 2021). It is the best dairy breed in Pakistan and comparable with Nili Ravi in milk production as well as performance results (Khan *et al*., 2021).

Moringa oleifera belongs to the family Moringaceae and is commonly known as *Moringa* is very important in livestock rearing due to its extraordinary abilities (Su and Chen, 2020). *M. oleifera* is incredibly rich in various types of vitamins, amino acids as well and minerals (Islam *et al*., 2021). *Moringa* and its products are very helpful in different agriculture systems to obtain high-quality crops. The most important features of *Moringa* are that it has high biological standards and is used as medicine or bio-pesticide. *Moringa* green leaves can be used as a vegetable that contains carotene, different proteins containing different amino acid concentrations, several vitamins, and minerals, and contain phytochemicals such as kaempferitrin, rhamnetin, quercetin, isoquercitrin, and kaempferol (Hassan *et al*., 2021). The leafy part of the *M. oleifera* is a good source of protein and calcium and is used as a nutritional diet treatment for the malnutrition young, especially pregnant and lactating animals because of its high nutritional value (Meireles *et al*., 2020). *M. oleifera* leaves also contain flavonoids in all stages of maturity that can be used as therapeutic agents against cancer-causing cells and may be able to prevent tumor invasion in animals (Meireles *et al*., 2020). *Moringa* leaves contain saponins, which are very beneficial in maintaining low cholesterol and have negative (cytotoxic) properties (Padayachee and Baijnath, 2020). The *Moringa* leaves flour as an ingredient is used in the preparation of urea-molasses block (UMB), which can be fed to malnutrition animals because of the scarcity of premium feed in the dry season (Zhao *et al*., 2022). Feed made with various ingredients such as urea and molasses, is the best energy source, and may lead to increased nutritional value as well, as an increase in the quality of feed that balances the nutritional needs of animals (Zhao *et al*., 2022). Therefore, this study was designed to test the nutritional composition of *M. oleifera* leaves along with their nutritional value in supplementing with UMB on the production performance (milk production and composition), nutrient digestibility, hematological and blood biochemical profile of Azikheli buffaloes.

MATERIALS AND METHODS

Study location and experimental animals

This study was conducted at the Buffaloes Conservation Development Farm and Research Center, Lower Dir, Munda Khyber Pakhtunkhwa, Pakistan. Sixteen Azikheli lactating buffaloes were selected under the same nursing category and were divided into four experimental groups consisting of four randomly constructed designs. The experimental study was continued for 40 days. All the animals were fed properly and they were allowed to drink water adlibitum.

Feed source and nutrition

Experimental feed supply i.e., local market was used to buy *Moringa*. After the grinding of *Moringa* leaves, they were added to UMB which was prepared in the Department of Animal Nutrition, the University of Agriculture, Peshawar. *Moringa* leaves diets were prepared in four experimental diet groups i.e., one group was kept as control (MOS-0), whereas the other three groups were treatment groups e.g., MOS-10, MOS-20, and MOS-30 as shown in Table I. The formulations for tests were according to the NRC Standard (2005) which was used for large breeds of Holstein Friesian HF lactating buffaloes. After the adaptation and gradual changes to the pre-test, diet was provided three times a day. One was given at 08:00 am, the second was given at 01:00 pm and the third was given at 06:00 pm. The animal was provided an average amount of diet in which daily feed provided as well as rejected on the previous day by the animal, was calculated accordingly. Manufacturing the size of each UMB depends on the amount of various ingredients that were going to be added to it. Commonly used methods for the production of blocks are as follows.

Preparation of urea molasses blocks (UMB)

The best way to create a better block is a good mixing of the ingredients together. First of all, the lumps were separated from the ingredients properly and then weighed properly to avoid over weight of the bags that may lead to

Table I. Experimental diets.

Ingredients	MOS-0	MOS-10	MOS-20	MOS-30
Moringa oleifera	0.00	10.0	20.0	30.0
Wheat bran	30.0	20.0	10.0	0.00
Molasses	43.0	43.0	43.0	43.0
Urea	7.00	7.00	7.00	7.00
Clay	6.00	6.00	6.00	6.00
Limestone	7.00	7.00	7.00	7.00
DCP	4.00	4.00	4.00	4.00
Salt	3.00	3.00	3.00	3.00

MOS-0, *Moringa* supplemented with 0% UMB; MOS-20, *Moringa* supplemented with 10% UMB; MOS-20, *Moringa* supplemented with 20% UMB; MOS-30, *Moringa* supplemented with 30% UMB. Wheat bran (for protein purposes and helps to clench the blocks together), Molasses (for energy purposes), Urea (for nitrogen purposes), Limestone (for minerals purposes), Salt, and DCP (Dicalcium phosphate) (for minerals purposes).

the injury of the animals while taking them to the required place. Now, all the ingredients were mixed according to the measured quantities in the prepared formula of the block. After this, urea was gradually added to molasses in small amounts keep stirring the mixture gently for about 20 min. Then the molasses was heated by placing it under the sun to improve mixing and proper handling of the UMB. Then the bran and other fiber contents just like a cake and clay were added to improve the mixing of other ingredients or by combining the salts with cement to improve the hardening process. After carefully mixing the ingredients, the mixture was added into the mold or small buckets for molding purposes. Then the mold remained there until the ingredient mixture was properly dry as well as stiff for handling. After twenty-four hours, the block was taken out from the molds and put on a rack to let them dry.

Production performance parameters

The daily dietary intake of each test buffalo was calculated by removing the rejected feed from the supplied feed. The milk yield (MY) of all the animals was recorded daily. To test the composition of the milk, 100 ml milk samples were taken in plastic bottles from each of the buffaloes daily. Elko's milk (lacto-analyzer) technique was used in the laboratory for the determination of total solids (TS), solid not fat (SNF), fats, milk protein, and lactose accumulation at the Dairy Technology Center Lab at VRI (Veterinary Research Institute), Peshawar.

Hematology and serum biochemistry

A blood test was conducted by collecting 5ml of blood sample of each animal from the jugular vein in the EDTA tube. The samples were taken to the lab immediately. A hematological examination was performed at the VRI, Peshawar. To analyze the serum concentration of blood samples, the tubes were centrifuged for about 10 min under 3000 rpm and were preserved in the refrigerator at 4°C until the blood metabolites were analyzed. Commercial kits (Stabio Diagnostic Company) were used to analyze the total protein (TP), glucose, red blood cells (RBCs), white blood cells (WBCs), hemoglobin (HB), and packed cell volume (PCV) (Bedenicki *et al*., 2014). Ultraviolet (UV) spectrophotometric technique (Optizen 3220 UV, Mecasys Co. Ltd., Korea) was used to determine the serum TP, total cholesterol (TC), and blood urea by using Spinreact kits (Spinreact, GIRONA, Spain).

Chemical analysis of feed

After the collection of the feed samples, they were dried in air properly then they were ground in 1mm particle size in a Thomas-Willey milling machine. These samples were stored at room temperature in labeled bottles to close the analysis of all the fractions by a standard laboratory. Association of Official Agricultural Chemists (AOAC, 2007) methods were followed for dry matter (DM), ash, ether extract (EE), crude protein (CP), and crude fiber (CF) analysis. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the methods of Goering and Van Soest *et al*. (1991).

In vitro *dry matter digestibility*

Digestibility of the DM was determined by the technique called in vitro dry matter digestibility (IVDMD) (Tilley and Terry, 1963). Firstly, the sample was digested for 48h with the help of a shaking incubator where 30ml of buffer solution and 10 ml of rumen liquor were added in the IVDMD tubes with the sample along with the flushing of carbon dioxide under anaerobic (without oxygen) situation at the temperature of 39°C, which give an environment for digestion just like the rumen of buffaloes. The liquor was obtained from the buffaloes, who were fed with experimental feed for at least one week and then the liquor was collected from rumen via rumen fistulation. Buffer solution with a pH of 6.9 was prepared in the laboratory according to the IVDMD method. After 48 h, IVDMD tubes were taken out of the incubator and tube were centrifuged for about 4 min at 3000 rpm and supernatant was discarded from each tube. The residues in IVDMD tubes were dried at 70 °C in an oven till they all came to the constant weight. After that, for cooling IVDMD tubes were placed in a desiccator for 5 min and the tubes were weighed again. Liquor with buffer solution was added to six IVDMD tubes and six empty IVDMD tubes were centrifuged in each run, just to determine the blank reading. The IVDMD of feed samples was calculated according to the given formula:

$$\text{IVDMD (gm/kg)} = \frac{T1 - (T2 - T3)}{T1} \times 1000$$

Where T1 is dried sample weight, T2 is dried and undigested residues weight and T3 is Blank reading.

Statistical analysis

The data was analyzed through the statistical software SPSS by using the following model in statistics:

$Y_{(ij)} = \mu_{(GM)} + \beta_{(j)} + \epsilon_{(ijklm)}$

Where; $Y_{(ij)}$ = Milk Yield (MY) or observation for ith at j^{th} ration or treatment, $\mu_{(GM)}$ = Grand mean or overall mean, $\beta_{(j)}$ = Effect of the j^{th} treatment; treatment comprises (diets MOS 0%, MOS 10%, MOS 20% and MOS 30%), $\epsilon_{(ijklm)}$= Random error for the $i^{th,}$ jth, k^{th}, l^{th}, m^{th}(etc.) treatments, treatment comprises (diets MOS 0%, MOS 10%, MOS 20% and MOS 30%).

RESULTS AND DISCUSSION

Table II shows the results of the nutrient intake of Azikheli buffaloes offered *M. oleifera* supplemented with UMB. The highest significant values of DMI, OMI, CPI, CFI, NDFI, and ADFI were noted in the MOS-30 diet group than in the MOS-0 group. *M. oleifera* is a rich source of protein and contains most of the minerals that increase the palatability. The DMI and OMI results are supported by Dorothy *et al*. (2018) who revealed that the addition of *M. oleifera* leaves in the diet of cattle increases the intake of feed because Moringa is a source of protein and is more palatable to the animals. Malik *et al*. (2019) supported these results, and described that the addition of *M. oleifera* leaves to the diet can increase the consumption of feed with an increase in weight. *M. oleifera* consists of diverse vitamins, and minerals and contains all essential amino acids, therefore, CPI was higher in buffaloes (Gopalakrishnan *et al*., 2016). The increase in CFI was due to the increase in microbial flora in the rumen (Shiriki *et al*., 2015). The results for the nutrient digestibility and milk yield are presented in Table III. The highest DM, OM, CP, CF, EE, NDFD, ADF, digestibility and MY were calculated in the MOS-30 diet group as compared to the control (MOS-0) group. In terms of nutrient digestibility increase, it can be ranked as MOS-30>MOS-20 >MOS-10 >MOS-0. This is because of the increase in the population of microbial flora due to the supplementation of Moringa, which is a complete nutritional diet that leads to increased digestibility of nutrients consumed by the Azikheli buffaloes. Malik *et al*. (2019) supported these results and stated that the addition of *M. oleifera* in UMB can lead to higher digestibility of fibrous feed as well as feed intake. Saidu *et al*. (2021) also support our results, stating that the *Moringa* leads to an increase in feed digestibility and an increase in the feed flow rate within the gastrointestinal tract (GIT). Asaolu *et al*. (2012) stated that *Moringa* leads to an increase in the digestibility of dry matter. Rika *et al*. (2020) revealed that feeding *M. oleifera* leaves powder block leads to an increase in the production of milk in the cattle. This was due to lactogogum and phytosterols (consisting of campesterol, stigmasterol, and B-sitosterol present in the leaves of *M. oleifera* leaves) which stimulate the prolactin hormones that lead to increased milk production. Brar *et al*. (2022) stated that *M. oleifera* leaves supplementation to animals increased

Table II. Nutrient intake of Azikheli buffaloes offered *Moringa oleifera* supplemented with urea molasses block.

Parameters (%)	MOS-0	MOS-10	MOS-20	MOS-30	SEM	P-value
DMI	12.35^{d}	12.74^{c}	13.12^{b}	14.42^{a}	0.311	0.007
OMI	10.91^{d}	11.75^{c}	12.21^{b}	13.26^{a}	0.286	0.008
CPI	1.030^{d}	1.210^{c}	1.610^{b}	2.600^{a}	0.039	0.005
CFI	3.510^{d}	3.780^{bc}	3.970^{b}	4.060^{a}	0.121	0.002
EEI	1.010^{cd}	1.370^{c}	1.670^{b}	1.890^{a}	0.023	0.003
NDFI	5.310^{d}	5.820^{c}	6.070^{b}	6.870^{a}	0.151	0.001
ADFI	2.820^{d}	3.130^{c}	3.410^{b}	3.870^{a}	0.091	0.001

Mean values having different superscripts within the same row are significantly different (P<0.05). For group description, see Table I. DMI, Dry matter intake; OMI, Organic matter intake; CPI, Crude protein intake; CFI, Crude fiber intake; EEI, Ether extract intake; NDFI, Neutral detergent fiber intake; ADFI, Acid detergent fiber intake.

Table III. Nutrient digestibility and milk yield of Azikheli buffaloes offered *Moringa oleifera* supplemented with urea molasses block.

Parameters (%)	MOS-0	MOS-10	MOS-20	MOS-30	SEM	P value
DMD	42.30^{d}	51.18^{c}	59.80^{b}	65.28^{a}	0.311	0.001
OMD	60.12^{d}	64.63^{c}	70.02^{b}	75.88^{a}	0.286	0.001
CPD	68.32^{d}	72.65^{c}	77.23^{b}	82.57^{a}	0.039	0.002
CFD	44.25^{d}	51.89^{c}	56.15^{b}	61.35^{a}	0.153	0.005
EED	58.04^{cd}	62.34^{c}	67.72^{b}	72.01^{a}	0.023	0.004
NDFD	48.37^{d}	52.30^{c}	56.21^{b}	61.24^{a}	0.151	0.002
ADFD	38.22^{c}	42.51^{b}	45.11^{b}	50.02^{a}	0.091	0.003
MY	4.350^{d}	4.810^{c}	5.470^{b}	5.870^{a}	0.139	0.002

Mean values having different superscripts within the same row are significantly different (P<0.05). For group description, see Table I. DMD, Dry matter digestibility; OMD, Organic matter digestibility; CPD, Crude protein digestibility; CFD, Crude fiber digestibility; EED, Ether extract digestibility; NDFD, Neutral detergent fiber digestibility; ADFD, Acid detergent fiber digestibility; MY milk yield (liter/day).

milk production. Table IV demonstrates the results of the milk composition of Azikheli buffaloes. Protein, lactose, SNF, TS, and ash were calculated significantly higher in the *M. oleifera* supplemented groups (MOS-30, 20, and 10) as compared to the control group but the fat was calculated significantly higher in the control group than in the *M. oleifera* supplemented groups. Mendieta *et al*. (2011) supported these results by the supplementation of *M. oleifera* leaves to the pure breed of Holstein Friesian which led to an increase in protein concentration. Utari and Warly (2021) also support our results and state that the digested amino acids from feeding *M. oleifera* leaves are absorbed by blood from the intestine and brought to the alveoli of the mammary gland, where these amino acids are used to make milk protein. Rika *et al*. (2020) revealed that feeding *M. oleifera* leaves powder block to the cattle led to more non-fiber carbohydrates (NFC) than the NDF, brought a decrease in fat concentration in milk and increased concentration of protein in the milk. The results regarding the hematology and serum biochemistry parameters of the Azikheli buffaloes are shown in Table V. RBC, WBCs, Hb, TP, BUN and PCV were calculated significantly higher in the MOS groups, while total cholesterol in the control group. In terms of hematology and serum biochemistry parameters increase, it can be ranked as MOS-30>MOS-20>MOS-10>MOS-0. Utari and Warly (2021) reported that *M. oleifera* is responsible for the production of digested amino acids that are absorbed by blood circulation from the intestine which leads to an increase in total protein concentration in the blood. There was a slight change in cholesterol level in the blood. Similarly, Babiker *et al*. (2021) stated that feeding *M. oleifera* pelleted diet brought no changes in cholesterol levels. This effect of *M. oleifera* could have been attributed to the production of high phenolic content along with antioxidants, phytochemicals, and antioxidants, which helps to reduce the production as well as the absorption of cholesterol (Saxena *et al*., 2013). PCV values were slightly higher which could be due to the high plane of *M. oleifera* supplementation, which increases the nutrition contents (Moyo *et al*., 2013).

CONCLUSIONS

Nutrient intake and digestibility, milk yield, milk composition, and blood hematology improved in the various *M. oleifera* supplemented urea molasses block (UMB) diet groups as compared to the control group. Therefore, *M. oleifera* supplemented with urea molasses block up to 30% can be used for Azikheli buffaloes, which can easily be transported between hilly areas as well as can easily be accessible in scarcity periods.

Table IV. Milk composition of Azikheli buffaloes offered *Moringa oleifera* supplemented with urea molasses block.

Parameters (%)	MOS-0	MOS-10	MOS-20	MOS-30	SEM	P value
Protein	3.230^{d}	4.150^{c}	4.340^{b}	4.48^{a}	0.203	0.001
Fat	4.960^{a}	4.630^{b}	4.460^{c}	4.21^{d}	0.122	0.005
Lactose	4.470^{d}	4.630^{c}	4.810^{b}	4.98^{a}	0.213	0.002
SNF	11.39^{d}	11.54^{c}	11.76^{b}	11.97^{a}	0.103	0.005
TS	16.98^{c}	17.29^{bc}	17.64^{b}	17.93^{a}	0.131	0.004
Ash	0.640^{cd}	0.720^{c}	0.850^{b}	0.920^{a}	0.251	0.002

Mean values having different superscripts within the same row are significantly different (P<0.05). For group description, see Table I. SNF solid not fat; TS, total solid.

Table V. Hematology of Azikheli buffaloes offered *Moringa oleifera* supplemented with urea molasses block.

Parameters	MOS-0	MOS-10	MOS-20	MOS-30	SEM	P value
RBCs ($10^{6}\mu l^{-1}$)	8.520^{d}	8.870^{c}	9.040^{b}	9.430^{a}	0.043	0.001
WBC ($10^{3}\mu l$-^{1})	17.26^{d}	17.43^{c}	17.65^{b}	17.93^{a}	0.047	0.004
HB (g^{-dl})	11.25^{b}	11.38^{b}	11.61^{b}	11.82^{a}	0.143	0.028
PCV (%)	27.47^{d}	28.60^{c}	29.67^{b}	30.07^{a}	0.219	0.005
Glucose (mg^{-dl})	82.31^{d}	82.58^{c}	82.92^{b}	83.36^{a}	0.152	0.004
TP (g^{-dl})	5.220^{d}	5.650^{c}	6.190^{b}	6.490^{a}	0.120	0.002
TC (mg^{-dl})	136.7^{a}	116.1^{b}	89.01^{c}	74.28^{d}	0.062	0.004
BUN (mmol/L)	3.830^{d}	4.150^{c}	4.310^{b}	4.550^{a}	0.211	0.006

Mean values having different superscripts within the same row are significantly different (P<0.05). For group description, see Table I. RBCs, Red blood cells; WBCs, White blood cells; HB, Hemoglobin; TP, Total protein; TC, Total Cholesterol; BUN, Blood urea nitrogen: PCV, Packed cell volume.

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IRB approval

The study was approved by the Advanced Studies and Research Board (ASRB), The University of Agriculture Peshawar (No. 422PS/UAP, dated January 04, 2023).

Ethical statement

This study was approved by the animal welfare and care committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, and all the measures and tools were considered to minimize the pain and discomfort of birds during the conduction of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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