



Effect of feeding concentrate mixtures containing varying levels of dried moringa (*Moringa oleifera*) leaves on haemato-biochemical and sex hormonal parameters in Murrah buffalo heifers[#]

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Abstract

A feeding trial was conducted for a period of 120 days for studying the haemato-biochemical and sex hormonal parameters in buffalo heifers fed with concentrate mixtures containing varying levels of dried moringa (*Moringa oleifera*) leaves. Fifteen Murrah buffalo heifers aged 18-26 months were selected and randomly allotted to three groups of five animals each and fed three dietary treatments, containing concentrate mixture (20 per cent CP and 68 per cent TDN) and ad libitum green grass. Dried moringa leaves were incorporated at 0, 10 and 20 per cent in the concentrate mixture for treatments T1 (control), T2 and T3 respectively. Comparison of serum biochemical values revealed higher serum glucose and oestrogen in buffalo heifers fed T3 when compared to those fed T1 and T2. The serum cholesterol values were significantly lower ($p < 0.05$) for buffalo heifers fed T2 and T3 when compared to T1. Evaluation of the results obtained in the present study revealed that moringa leaves can be effectively incorporated up to 20 per cent in the concentrate mixture of buffalo heifers without any adverse effects on haemato-biochemical parameters with improved oestrogen levels indicating better reproductive performance.

Keywords: Murrah buffalo heifers, dried moringa leaves, haemato-biochemical parameters, sex hormones

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Moringa trees are widely distributed in India, and the dried leaves of this can be incorporated as an ingredient in concentrate feed. It is a drought resistant tree fodder with high biological value and can be cultivated in hot, humid, tropical and subtropical regions. Being perennial in nature, it can be harvested several times in the same growing season and can tolerate dry seasons lasting up to six months (Sánchez *et al.*, 2006). Moringa leaves have high nutritional value, medicinal uses such as therapeutic and prophylactic properties and hepatoprotective effect (Rema and Liji, 2021). The crude protein (18.08 per cent) of moringa leaves is high (Priya *et al.*, 2020) and they are rich source of ascorbic acid, α -tocopherol, β -carotene, polyphenols, energy, amino acids (glutamic acid, arginine, aspartic acid, methionine and cysteine) and fatty acids such as linolenic acid, palmitic acid, linoleic acid, and oleic acid (Sánchez-Machado *et al.*, 2010), and various minerals such as Ca, K, Fe, Zn and vitamins (Gupta *et al.*, 1989). Moringa leaves are found to be a potential source of natural antioxidants and there are various flavonoids and phytoestrogens in them (Djais *et al.*, 2019).

Among livestock, buffaloes have been found to exhibit silent oestrus and therefore, under farm conditions there is difficulty in heat detection of buffaloes. Since moringa leaves are rich in phytoestrogens, its effect on the oestrus manifestation in buffaloes is worth studying. The level or variation of nutrients in feed affects the haematological and biochemical values. Various studies have been done in dairy cattle incorporating moringa leaves in feed, but in buffaloes studies are minimal. The studies on the effect of feeding moringa leaves on the sex hormone levels in buffalo heifers are also scanty. Hence, the present study was planned to formulate concentrate feed incorporated with dried moringa leaves at different levels for feeding Murrah buffalo heifers and to compare their haemato-biochemical parameters and sex hormonal status with those fed on conventional ration.

Materials and methods

The study was conducted at the Department of Animal Nutrition, College of

Veterinary and Animal Sciences and Buffalo farm of University Livestock Farm and Fodder Research and Development Scheme (ULF and FRDS), Mannuthy, Kerala, India.

Experimental animals, management and feeding

Fifteen Murrah buffalo heifers were selected from the buffalo farm of University Livestock Farm and Fodder Research and Development Scheme, Mannuthy for the experiment. All the animals were housed in a barn with sufficient ventilation and light. The barn was clean and dry with facilities for individual feeding and watering. The body weight of the experimental animals were taken and randomly allotted to three dietary treatments of five animals each. Experimental design used was CRD. All experimental animals were dewormed before the start of the experiment. Three dietary treatments *Viz.* T1, T2 and T3 containing concentrate mixture (20 per cent CP and 68 per cent TDN) were provided to experimental buffalo heifers and all dietary treatments were made isocaloric and isonitrogenous. Dried moringa leaves was incorporated at 0, 10 and 20 per cent in the concentrate mixture for treatments T1 (control), T2 and T3 respectively. They were kept under identical environmental and management conditions prevailing in the farm and were fed as per ICAR (2013) feeding standards throughout the experimental period of 120 days. Green grass and water were offered *ad libitum* to all experimental buffalo heifers. The moringa leaves used in the present study were procured from Sree Shiva and Co, Madurai, Tamil Nadu, India.

Haematological and serum biochemical studies

The blood samples were collected from the experimental animals at the end of feeding trial and analysed for various haemato-biochemical parameters such as haemoglobin (cyanmethemoglobin method), serum glucose (GOD-PAP method), cholesterol (CHOD-PAP method), total protein (Jong and Vegeter, 1950), albumin (Bromocresol green method), blood urea nitrogen (modified Berthelot method), alanine transaminase (ALT), aspartate aminotransferase (AST), and serum creatinine

(AOAC, 2016), using available standard kits. Sex hormones such as progesterone and oestrogen were analysed (Radioimmunoassay technique), using available standard kits.

Statistical analysis

Data obtained on different parameters during the course of experiment were analysed statistically as per (Snedecor and Cochran, 2014) by analysis of variance (ANOVA) technique, using the software Statistical Product and Service Solutions (SPSS) version 24.0.

Results and discussion

Haematological and serum biochemical parameters

The data on haemato-biochemical parameters in blood samples collected from buffalo heifers at the end of trial are given in Table 1.

The values for haemato-biochemical parameters such as haemoglobin (11.37-12.28 g/dL), serum glucose (68.66-76.79 mg/dL), cholesterol (64.66-78.90 mg/dL), total protein (7.41-7.86g/dL), albumin (3.28-3.51 g/dL), blood urea nitrogen (26.69-28.21 mg/dL), creatinine (1.42-1.45 mg/dL), ALT (42.23-44.85 U/L) and AST (60.90-65.26 U/L) of experimental buffalo heifers recorded in the present study were within the normal range reported for the species by Abd-Ellah *et al.* (2014), which indicated that the experimental animals were apparently

healthy. Statistical analysis of the data revealed that there was no significant difference in the haemato-biochemical parameters among three treatment groups except for serum glucose and cholesterol. The buffalo heifers maintained on dietary treatment T3 had significantly higher ($p < 0.05$) blood glucose than T1, whereas the values obtained for T2 was comparable to that of T1 and T3. The buffalo heifers maintained on dietary treatments T2 and T3 had significantly lower ($p < 0.05$) serum cholesterol compared to T1.

Elaidy *et al.* (2017) reported that buffalo calves fed with concentrate mixture containing dried moringa leaves at a rate of 0, 5, 10, 15, and 20 per cent had serum values for total protein as 6.30, 6.72, 6.15, 6.27 and 6.37 g/dL, creatinine as 0.85, 0.77, 0.83, 0.73 and 0.92 mg/dL and ALT as 61.67, 55.33, 56.67, 52.33 and 51.67 U/L respectively, and were not significantly different ($p > 0.05$). The total protein and creatinine values were lower and ALT higher compared to the values obtained in the present study. The serum albumin values (3.47, 3.23, 3.12, 3.15, 2.97 g/dL) of buffalo calves fed with concentrate containing dried moringa leaves at rates of 0, 5, 10, 15 and 20 per cent respectively, were significantly lower ($p < 0.05$) in all moringa incorporated ration compared with control, the values being comparable with those obtained in the present study. Wafa *et al.* (2017) observed that buffalo bulls fed dried moringa leaves incorporated at 4 and 8 per cent in concentrate feed mixture had significantly lower values for cholesterol (156.18, 148.39 mg/dL), creatinine

Table 1. Haematological and serum biochemical parameters¹ of buffalo heifers maintained on three experimental rations

Parameters	T1	T2	T3	p-value
Haemoglobin (g/dL)	11.37±0.63	12.28±0.65	11.45±0.66	0.562 ^{ns}
Serum glucose (mg/dL)	68.66 ^b ±2.22	71.99 ^{ab} ±1.46	76.79 ^a ±1.94	0.032*
Cholesterol (mg/dL)	78.90 ^a ±2.06	70.05 ^b ±1.79	64.66 ^b ±1.86	<.001*
Total protein (g/dL)	7.47±0.42	7.86±0.43	7.41±0.56	0.778 ^{ns}
Albumin (g/dL)	3.51±0.09	3.28±0.14	3.48±0.06	0.285 ^{ns}
Blood urea nitrogen (mg/dL)	28.21±1.23	27.02±1.62	26.69±1.27	0.725 ^{ns}
Serum creatinine (mg/dL)	1.45±0.059	1.43±0.06	1.42±0.09	0.943 ^{ns}
Alanine transaminase (U/L)	44.15±1.31	44.85±1.42	42.23±0.44	0.285 ^{ns}
Aspartate aminotransferase (U/L)	60.90±4.61	63.69±5.61	65.26±4.38	0.819 ^{ns}

¹ Mean values are based on five replicates; ns-non significant at $p > 0.05$

*Means bearing different superscripts within a row differ significantly at $p < 0.05$

Table 2. Sex hormonal parameters¹ of buffalo heifers maintained on three experimental rations

Parameters	T1	T2	T3	p-value
Progesterone (ng/mL)	2.72±.94	3.68±1.20	4.98±1.18	0.386 ^{ns}
Oestrogen (pg/mL)	38.29 ^b ±12.66	71.09 ^{ab} ±15.59	100.46 ^a ±6.47	0.012 *

¹ Mean values are based on five replicates; ns-non significant at p> 0.05

*Means bearing different superscripts within a row differ significantly at p<0.05

(0.71, 0.66 mg/dL) and AST (55.20, 54.28 U/L) respectively when compared those fed moringa-free diet. The values of serum cholesterol were higher than those obtained in the present study, whereas the creatinine and AST values were lower. They also observed that buffalo bulls fed diet with 8 per cent moringa had significantly higher (p<0.05) serum glucose (65.89 mg/dl), total protein (8.74 g/dl) and albumin (4.73 g/dl) than those fed diet with 4 per cent moringa and moringa free diet. Aharwal *et al.* (2019) reported that Murrah buffalo calves fed with moringa leaf meal at 0, 10 and 15 per cent levels in the concentrate mixture had haemoglobin of 11.7, 12.12 and 12.48 g/dL, total protein of 6.50, 6.38 and 6.33 g/dL, albumin of 3.56, 3.52 and 3.49 g/dL and blood urea nitrogen of 39.63, 39.51 and 38.97 mg/dL respectively, and were not significantly different (p>0.05). The values of haemoglobin and albumin were comparable to those of the present study, but the total protein value was lower and the blood urea nitrogen were higher. According to Abdel-Raheem and Hassan (2021), buffalo calves fed concentrate mixture with moringa leaf meal at inclusion rates of 15 per cent and 20 per cent, replacing soya bean meal by 50 per cent and 75 per cent respectively, had significantly higher (p<0.05) serum glucose (89.32, 95.99 mg/dL), total protein (8.33, 9.06 g/dL) and albumin (4.21, 4.51 g/dL) values but lower total cholesterol (121.97, 117.27 mg/dL) and BUN (26.43, 23.17 mg/dL) values respectively, than those fed moringa-free diet. The values of serum glucose, total protein, albumin, and total cholesterol were higher than those obtained in the present study, whereas the values for blood urea nitrogen were comparable.

Sex hormonal parameters

The data on sex hormonal parameters estimated at the end of the experiment for progesterone and oestrogen, as well as number of animals that exhibited oestrus symptoms are presented in Table 2 and 3, respectively.

The average progesterone concentration in the serum of buffalo heifers fed on the three experimental rations T1, T2 and T3 at the end of the trial was 2.72±0.94, 3.68±1.20 and 4.98±1.18 (ng/mL) and the average oestrogen concentration was 38.29±12.66, 71.09±15.59 and 100.46±6.47 (pg/mL) respectively. Statistical analysis of the data revealed that there was no significant difference in progesterone concentration of buffalo heifers maintained on three dietary treatments, whereas oestrogen concentration was significantly higher (p<0.05) in T3, compared to T1 and the values of T2 was comparable to T1 and T3. The number of buffalo heifers exhibited oestrous signs for treatment T1, T2, and T3 were 2, 4 and 3 respectively.

The levels of progesterone obtained in the present study are in the normal physiological range (0.360 to 4.888 ng/ml) as reported by Takkar *et al.* (1982). Ahmed *et al.* (2010) found that serum progesterone levels in buffaloes were 3.66 ng/ml at mid-luteal phase of the oestrus cycle, which is comparable to the values obtained in the present study. The values obtained in the present study for oestrogen was higher than those obtained by Sharma *et al.* (2015), in cycling buffaloes on the day of oestrus being 25.41 pg/ml of oestradiol 17-β. Dhakad *et al.* (2016) observed the normal serum oestrogen value of non-pregnant buffaloes as 103.57 pg/ml which is comparable to values obtained in present study. Abdoon *et al.* (2020) stated that serum progesterone and oestrogen values of buffaloes ranged between 0.37 to 1.76 ng/mL and 57.99 and 242.46 pg/ml respectively during the luteal and follicular phases of the hot and cold seasons, the progesterone values being slightly lower than those obtained with the present study whereas the values for oestrogen obtained in this study fit into the range, except that for the control group.

Likewise, Prasad *et al.* (2020) also

observed that oestrus induction, conception rate, and follicular development of Murrah buffaloes consuming green grass along with curry leaves and moringa leaves were higher when compared to the ones fed untreated rations. Similar results were observed by Kumar *et al.* (2021) in lactating cows and Dutta *et al.* (2022) in cattle and buffaloes fed diet incorporated with moringa leaves.

Conclusion

On summarising the results, it could be concluded that dried moringa leaves can be incorporated at 10 and 20 per cent in the rations of buffalo heifers. The haemato-biochemical parameters among treatment groups were similar except for serum glucose and cholesterol. The higher oestrogen levels in buffalo heifers fed moringa leaves lead to more animals manifesting oestrus signs, indicative of better reproductive performance.

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