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Pre-partum and Postpartum Supplementation Effect of *Moringa oleifera* Leaf Meal on Haematological and Biochemical Parameters of Murrah Buffaloes Reared under Loose Housing System

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ABSTRACT

The present investigation has been undertaken to study the effect of *Moringa oleifera* leaf meal supplementation on haematology and biochemical parameters of Murrah buffaloes reared under loose housing system. Eighteen lactating Murrah buffaloes were assigned to three dietary treatment groups (T_1, T_2 and T_3) resulting in six lactating buffaloes per treatment based on parity following factorial completely randomized design. The experiment was conducted for a period from 21 days before calving (pre-partum) to 5 months of lactation (postpartum) to find the effect of supplementation of moringa in following treatments, namely, T_1 (control) - Feeding as per ICAR standards (2013), T_2- 75 g MOLM/animal/day in addition to control feeding and T_3- Feeding as per ICAR standards (2013) + 150 g MOLM/animal/day in addition to control feeding. The findings of study revealed that haematological parameters viz. haemoglobin (g/dl) WBC, monocytes (%), basophils (%), eosinophils (%) levels of T_3 treatment group were higher than T_2 and T_3 but did not differ significantly among various treatment groups. Lymphocytes level of T_3 treatment group was significantly higher than T_1 and but did not differ significantly with T_2 treatment group. SGOT concentration (IU/L), SGPT concentration (IU/L), Plasma cholesterol concentration (mg/dl) and Plasma iron concentration (µg/dl) were better in supplemented groups but did not show any significant difference in different treatment groups.

HIGHLIGHTS

- Inclusion of MOLM did not show any significant effect on biochemical parameters.
- Lymphocytes level was found better with MOLM supplementation.

Keywords: *Moringa oleifera*, lactating, haematological, basophils and haemoglobin

India has the world's highest population of buffalo (109.7 million), which makes up roughly 20.45% of livestock and contributes about 45% of the nation's milk supply (DAHD, 2021). In comparison to indigenous cows (3.41 kg/day/animal), indigenous buffaloes had a greater average daily milk output per animal (6.41 kg) at the national level (DAHD Annual report, 2021-22). India is fortunate to be home to some of the greatest buffalo breeds in the world, with the Murrah being the most widely kept kind due to its high milk production and marketability. Indian Murrah is the most diffuse breed in the world which is about 42.8% of total buffalo population in India (Breed Report, DAHD; 2022). In light of the rapidly diminishing resources of feed and fodder, plant scientists and animal nutritionists have been studying underused fodder crops, shrubs, and trees during the past few years. The potential for phytogenic supplements in animal diet to replace antibiotic growth

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promoters has garnered interest during the past fifteen years (Puvača *et al.,* 2013). Researchers suggest that they stimulate digestion, fortify the immune system, and kill microorganisms. Herbal plant phytobiotics are becoming increasingly popular in animal nutrition for a variety of reasons, including their galactogogue, immunological modulating, highly antioxidant, and antibacterial qualities. Moringa's potential qualities, such as those of an antibacterial agent, antioxidant, anti-inflammatory, anti-ulcer, anti-hyperglycemic, anti-diabetic, and anti-cancer agent, among others, have led to its widespread use (Abdull Razis *et al.,* 2014; Arora *et al.,* 2013; Babu and Chaudhuri, 2005).

Moringa has powerful antibacterial and antifungal characteristics that make it a valuable therapeutic plant (Nickon *et al*., 2003). Kekana *et al.* (2020) studied that haematological parameters in cows were significantly influenced by MOLM supplementation during peripartum. Yusuf *et al.* (2018) found MOLM did not affect PCV, RBC, or Hb. High neutrophil numbers boost innate immunity due to their migratory and phagocytic activities (Malik *et al.,* 2013). Peripartum supplementation with MOLM demonstrated a substantial influence on haematological markers, consistent with previous observations (Fadiyimu *et al.,* 2010). Blood plasma transaminase enzymes activities (ALT and AST) are the most significant markers of liver cell activities, where an increase in the concentration of these enzymes indicates that tissue activity has been destroyed. Bashar *et al.* (2020) found that the levels of AST and ALT did not have any negative effect on animal health ($P > 0.05$) due to the feeding of Moringa supplements.

Various studies were conducted in different species to observe the effect of inclusion of only *Moringa oleifera* plant parts (leaves combined with flowers and shoots) leaves as on hematology and biochemical parameters but the effect of supplementation of *Moringa oleifera* leaf meal in Murrah buffaloes is scanty. The changes in haemato-biochemical values in blood are not investigated yet in Murrah buffaloes. Keeping in view the above facts, the present investigation has been undertaken to study the effect of *Moringa oleifera* supplementation on performance of lactating Murrah buffalo under loose housing system with the objective to examine the effect of Moringa on the haemato-biochemical parameters of experimental buffaloes.

MATERIALS AND METHODS

Eighteen lactating Murrah buffaloes of different lactation stages were selected from the herd of buffalo farm of the Department of Livestock Production Management, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar on the basis of same parity (1st to 5th parity). These buffaloes were assigned to three dietary treatment groups (T_1, T_2 and T_3) resulting in six lactating buffaloes per treatment based on parity following factorial completely randomized design (FCRD). The experiment was conducted for a period from 21 days postpartum to 5 months of lactation (171 days) to find the effect of supplementation of MOLM in following treatments presented in Table 1, namely, T_1 (control)- Feeding of lactating buffaloes with wheat straw, green fodder and concentrate mixture (on DM basis) to meet out the nutrients requirements as per ICAR standards (2013), T_2- Feeding as per ICAR standards (2013) + 75 g MOLM/animal/day, T_3- Feeding as per ICAR standards (2013) + 150 g MOLM/animal/day. MOLM was procured from the local market. MOLM was given in concentrate mixture to buffaloes from 21 day prepartum to 150 days in milk (DIM) postpartum. The chemical composition (% DM basis) and cost of different feed ingredients fed to the experimental buffaloes are presented in Table 2. Blood samples were collected at the beginning of the experiments *i.e*. 21 days before calving, at the day of calving and thereafter at end of experiment *i.e*. 150 days after calving. Haematological parameters such as Haemoglobin (Hb), Red blood cells (RBC), White Blood Cells (WBC), neutrophils, basophils, eosinophils, monocytes etc. were analyzed with the Automated Haematology Analyser MS45 blood cell counter. Biochemical parameters in the plasma and serum samples were determined using an Automated Random Access Clinical Chemistry Analyzer (EM Destiny 200TM, Erba Diagnostics Mannheim GmbH). The experiment was started with prior approval taken to conduct the present investigation in the 21st meeting of Institutional Animal Ethics Committee, LUVAS held on February 12th, 2021.

STATISTICAL ANALYSIS

Data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) using Completely Randomized Design (CRD). All the data were subjected to ANOVA using the General Linear Models procedure

Table 1: Details of feeding regimen of buffaloes under different dietary treatments

Treatments	Feeding Regime
T_1 (Control)	Feeding of lactating buffaloes with wheat straw, green fodder and concentrate mixture (on DM basis) to meet out the nutrients requirements as per ICAR standards (2013)
T_2	Feeding as per ICAR standards (2013) +75 g MOLM/animal/day
T_3	Feeding as per ICAR standards (2013) +150 g MOLM/animal/day

Table 2: Chemical composition (% DM basis) and cost of different feed ingredients fed to the experimental buffaloes

Ingredients	DM	CP	CF	EE	Ash	OM	NFE	₹/kg
Fodder								
Wheat straw	94.68	2.87	35.20	1.20	9.3	85.74	46.47	3.69
Green Berseem	22.31	15.2	25.70	1.80	11.08	94.84	52.14	1.50
Green Maize	24.93	9.1	24.70	3.41	8.31	84.03	46.82	1.50
Concentrate								
Maize	88.92	9.10	2.40	3.40	2.25	91.04	76.14	20.25
Barley	92.91	11.9	5.20	2.32	5.21	93.26	73.84	16.50
Groundnut cake	95.12	41.5	7.4	9.53	7.30	96.38	37.95	32.13
Soyabean meal	88.95	46.80	4.80	1.51	7.31	97.25	44.14	46.44
Mustard cake	93.77	31.71	7.56	6.19	7.27	91.51	46.05	27.88
Moringa oleifera leaf meal (MOLM)	90.12	23.12	8.95	4.67	10.13	83.21	46.47	160.00
Mineral mixture	96.45	—	—	—	—	—	—	68.00
Common salt	98.46	—	—	—	—	—	—	2.91

* DM- dry matter, CP- crude protein, CF- crude fibre, EE- ether extract, OM- organic matter, NFE- nitrogen free extract and Rs.- Rupees.

of SPSS-23 software (SPSS, 2019). The mean differences among different treatments were separated by Duncan's multiple range tests. Consequently, a level of ($P<0.05$) was used as the criterion for statistical significance (Duncan, 1955).

RESULTS AND DISCUSSION

The overall mean hemoglobin (g/dl) concentrations were 11.61, 12.31 and 12.83 in treatment groups T_1, T_2 and T_3, respectively presented in table 3. The statistical analysis of the data revealed that hemoglobin levels of T_3 treatment group was higher than T_1 and T_2 but did not differ significantly among various treatment groups during different intervals of experiment. These findings are consistent with those of Haridas (2018), who found no statistically significant increase in hemoglobin concentration in goats and sheep fed Moringa compared to those fed a control diet. The overall mean WBC (M/mm3) concentrations were 7.92, 9.79 and 9.25 in treatment groups T_1, T_2 and T_3, respectively. The statistical analysis of the data revealed that white blood cells levels did not differ significantly among various treatment groups. The overall mean lymphocytes (%) concentrations were 45.46, 47.81 and 51.16 in treatment groups T_1, T_2 and T_3, respectively. Contrarily to present finding, Babeker and Abdalbagi (2015) studied the effect of feeding different levels of *M. oleifera* leaves in goats and found that erythrocytic indices showed significant variations among the groups but analogues to present finding that total WBCs which increased significantly ($P<0.05$) in moringa fed group. When given in peripartum period, MOLM supplementation in cattle improved haematological values (Fadiyimu *et al.,* 2010).

The statistical analysis of the data revealed that lymphocytes level of T_3 treatment group was significantly higher than T_1 and but did not differ significantly with T_2 treatment group. The enhanced haematology and cell-

Table 3: Average haematological parameters in blood of experimental buffaloes under different dietary treatments

Parameters	Period	Treatments		
		T1	T_2	T_3
Blood Haemoglobin concentration (g/dl)	At start of experiment	10.98±1.6	11.5±0.98	12.65±1.12
	At time of calving	10.83±0.72	11±1.18	12.25±0.71
	At end of experiment	13.03±1	14.42±1.51	13.58±1.39
	Overall mean	11.61±0.71	12.31±1.07	12.83±0.39
White blood cells concentration (M/mm^3)	At start of experiment	7.35±0.67	10.24±1	8.83±1.34
	At time of calving	7.4±0.27	11.7±1.92	10.51±1.34
	At end of experiment	9.02±0.83	7.44±0.49	8.41±0.82
	Overall mean	7.92±0.55	9.79±1.25	9.25±0.64
Red blood cells concentration (M/mm^3)	At start of experiment	7.14±0.58	7.26±0.26	8.85±1.18
	At time of calving	7.46±1.00	7.73±0.80	9.6±1.05
	At end of experiment	9.68±0.64	9.81±0.30	10.16±0.26
	Overall mean	8.09±0.80	8.27±0.78	9.54±0.38
Blood lymphocytes concentration (%)	At start of experiment	45.77±2.16	43.85±2.67	48.97±2.34
	At time of calving	44.60±1.66	49.45±1.53	50.87±3.12
	At end of experiment	46.00±1.74	50.12±3.89	53.63±3.07
	Overall mean	45.46±0.43^a	47.81±1.99^{ab}	51.16±1.35^b
Blood monocytes concentration (%)	At start of experiment	2.32±0.49	2.55±0.45	2.68±0.29
	At time of calving	2.12±0.51^a	3.63±0.33^b	3.12±0.22^{ab}
	At end of experiment	2.55±0.72	3.07±0.46	3.13±0.52
	Overall mean	2.33± 0.12	3.08±0.31	2.98±0.15
Blood neutrophils concentration (%)	At start of experiment	45.62±2.79	50.53±3.36	38.77±6.49
	At time of calving	47.10±3.16	43.33±1.68	43.58±4.38
	At end of experiment	50.45±4.33	51.73±2.1	56.2±3.64
	Overall mean	47.72±1.43	48.53±2.62	46.18±5.2
Blood basophils concentration (%)	At start of experiment	0.53±0.1	0.47±0.15	0.35±0.06
	At time of calving	0.48±0.09	0.55±0.1	0.45±0.1
	At end of experiment	0.57±0.11	0.42±0.06	0.57±0.12
	Overall mean	0.53±0.03	0.48±0.04	0.46±0.06
Blood eosinophils concentration (%)	At start of experiment	3.58±0.24	4.18±0.94	3.43±0.5
	At time of calving	3.78±0.63	2.23±0.56	4.27±0.97
	At end of experiment	3.52±0.47	2.88±0.1	4.05±1.17
	Overall mean	3.63±0.08	3.10±0.57	3.92±0.25

*The mean values with different superscripts in a row differ significantly ($P<0.05$).

mediated immunity following MOLM supplementation is consistent with the findings of Kafilzadeh *et al.* (2014), who advocated for oral supplementation as the optimum strategy compared to injection and feed mixing for supplementation of antioxidants. The overall mean monocytes (%) concentrations were 2.33, 3.08 and 2.98 in treatment groups T_1, T_2 and T_3, respectively. The overall mean neutrophils concentrations (%) were 47.72, 48.53 and 46.18 in treatment groups T_1, T_2 and T_3, respectively. According to Umar *et al.* (2017), a rise in neutrophils is linked to a reduction in lymphocytes, and vice versa; similar values are seen in present study. An increased number of neutrophils is beneficial because their migratory and phagocytic activities in the bloodstream help to maintain a healthy innate immune response (Malik *et al.,* 2013). The overall mean basophils concentrations

(%) were 0.53, 0.48 and 0.46 in treatment groups T_1, T_2 and T_3, respectively. Kekana *et al.* (2020) studied that peripartum supplementation of MOLM had a positive effect on haematological parameters. The statistical analysis of the data revealed that most of hematological parameters viz. hemoglobin levels (g/dl), RBC (M/mm^3), WBC (M/mm^3), monocytes concentration (%), neutrophils concentration (%), basophils concentration (%), eosinophils concentration (%) did not differ significantly among various treatment groups during different intervals of experiment.

The overall mean SGOT levels (IU/L) were 70.34, 64.78 and 68.66 in treatment groups T_1, T_2 and T_3, respectively, presented in table 4. The statistical analysis of the data revealed that SGOT levels of all treatment groups were found similar, although values were in normal range. Analogous to the findings of the present study, Zeng *et al.* (2018) revealed that Moringa silage had no significant effects on the serum concentrations of AST (SGOT). Therefore, MO had no effects on hepatic metabolism of lactating cows. El-Esawy *et al.* (2018) found similar results in lactating cows. Contrarily to present finding, Damor *et al.* (2017) found that SGOT levels were found to be significantly (P<0.05) higher in Moringa fed group as compared to control group in Mehsana goat kids. The overall mean SGPT levels (IU/L) were 25.90, 22.25 and 22.79 in treatment groups T_1, T_2 and T_3, respectively. The statistical analysis of the data revealed that SGPT levels were found similar all treatment groups. Present results are in accordance with the findings of El-Esawy *et al.* (2018) who concluded that introducing *Moringa oleifera* stems at the level of 20% in replacing all berseem hay in the ration of lactating cows recorded the best results concerning some blood plasma parameters. The activities of liver ALT (SGPT) enzyme were nearly similar for the different groups. Similarly, Zeng *et al.* (2018) and Damor *et al.* (2017) revealed that Moringa silage had no significant effects on the serum concentrations of ALT (P<0.05). Analogous to the findings of the present study, Zaher *et al.* (2020) who concluded that iron (Fe) levels were not statistically affected by Moringa foliage feeding at different levels. The present results are in agreement with the findings of earlier researcher. The study shows that supplementation of MOLM in ration did not affect iron levels in blood of animals. The present results are in agreement with the findings of Zaher *et al.* (2020)

Table 4: Average haemato-biochemical parameters in blood of experimental buffaloes under different dietary treatments

Parameters	Period	Treatments		
		T1	T_2	T_3
Serum Glutamic-oxaloacetic Transaminase (SGOT) concentration (IU/L)	At start of experiment	75.36±14.59	72.67±4.39	71.95±3.3
	At time of calving	65.52±9.45	57.85±5.33	71.33±4.07
	At end of experiment	70.15±8.64	63.82±6.27	62.7±4.55
	Overall mean	70.34±6.21	64.78±3.96	68.66±2.42
Serum glutamic pyruvic transaminase (SGPT) concentration (IU/L)	At start of experiment	28.73±2.61	25.22±2.05	25.88±3.46
	At time of calving	26.57±1.38	23.50±3.56	23.85±3.45
	At end of experiment	22.40±3.31	18.03±3.75	18.63±2.87
	Overall mean	25.90±1.66	22.25±1.99	22.79±2.21
Plasma copper concentration (mg/dl)	At start of experiment	71.17±6.18	72.00±5.52	70.17±5.99
	At time of calving	81.5±13.82	69.00±7.87	67.5±3.85
	At end of experiment	71.33±7.60	71.33±7.85	64.67±3.53
	Overall mean	74.83±3.63	70.83±5.02	67.50±2.11
Plasma iron concentration (µg/dl)	At start of experiment	7.24±2.25	5.76±2.12	11.57±3.77
	At time of calving	13.44±4.07	17.08±3.75	16.95±3.06
	At end of experiment	18.6±2.69^a	24.58±1.82^{ab}	25.43±1.18^b
	Overall mean	13.09±3.28	15.81±5.47	17.98±4.03

*The mean values with different superscripts in a row differ significantly (P<0.05).

concluded that copper (Cu) levels were not statistically affected by Moringa foliage feeding at different levels. These results indicated that plasma copper level was not affected by supplementation of MOLM in ration of Murrah buffaloes.

CONCLUSION

These results indicated that most of hematological parameters were found in normal range and have no harmful effect on health of animals but not affected by supplementation of *Moringa oleifera* leaf meal in ration of lactating Murrah buffaloes. But significantly ($P<0.05$) increased lymphocytes were observed in the study may be beneficial to animals in fighting against infections. Plasma iron concentration of animals was affected on longer supplementation at end of experiment. It is inferred from study that MOLM supplementation does not have harmful effects on heamato-biochemical parameters.

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