



Effect of Feeding Probiotics and Synbiotics on Hematological and Serum Biochemical Parameters in Murrah Buffaloes

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ABSTRACT

Background: The present study was carried to find the effect of probiotics and synbiotics supplementation on haematological and serum biochemical profile in Murrah buffaloes.

Methods: Eighteen Murrah buffaloes in their early lactation were randomly assigned into three groups. The control group viz Group-I was fed with basal diet (Hybrid napier, paddy straw and concentrate). The Group-II was supplemented with 30 gms of probiotic and Group-III was supplemented with 20 gms of synbiotic respectively, along with the basal diet for three months.

Result: No significant effect was found on haematological profile viz., Total leucocyte count, Total erythrocyte count, haemoglobin, Packed cell volume, Mean corpuscular volume, Mean corpuscular haemoglobin. Biochemical profiles viz. serum cholesterol levels, glucose levels, calcium levels, phosphorous levels were also not significantly different among the treatment and control groups.

Key words: Haemoglobin, Probiotic, Serum calcium, Serum cholesterol, Serum glucose, Serum phosphorous, Synbiotic, TLC.

INTRODUCTION

Buffaloes have been the mainstay of rural economy of small farmers in many developing countries. The total buffalo population in India is 109.85 million which showed an increase in population by 1% over the previous census (Livestock Census, 2019). There is a steady increase in the milk production from 55.6 million tons in 1991-1992 to 176.3 million tons in 2017-2018 with the per-capita availability of 375 gms (DAHD, 2017-18).

The Murrah buffalo is a breed of water buffalo (*Bubalus bubalis*) mainly kept for milk production. The colour is usually jet black colour with white markings on tail and faces and extremities sometimes found. The tightly curved horn is an important character of this breed. The body size is massive, neck and head are comparatively long. Butter fat content is 7%. Average lactation yield is vary from 1500-2500 kg.

Probiotics are defined as "live micro-organisms, which when administered in adequate amounts confer a health benefit on the host" (WHO). Synbiotics are dietary supplements combining both probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract. The studies on the use of these feed additives showed no significant effect on total leucocyte count (Gujjar *et al.*, 2018b; Aggazi *et al.*, 2014), total erythrocyte count (Gujjar *et al.*, 2018b; Kim *et al.*, 2011), packed cell volume (Kim *et al.*, 2011; Ghazanfaret *et al.*, 2015), hemoglobin (Kim *et al.*, 2011). The serum biochemical parameters like serum cholesterol (Gujjar *et al.*, 2018b; Morseyet *et al.*, 2014), serum glucose (Gujjar *et al.*, 2018b; El din 2015), serum calcium (Gujjar *et al.*, 2018b; Sretenovic *et al.*, 2008) and serum phosphorous (Gujjar *et al.*, 2018b; Zaworski *et al.*, 2014) also showed no significant effect with the use of probiotics and synbiotics.

In view of the above facts the present research was proposed with the following objectives to study the effect of

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probiotic and symbiotic supplementation on milk yield, milk composition, in lactating Murrah buffaloes.

MATERIALS AND METHODS

The present experiment was conducted to study the effect of Probiotic and Synbiotic supplementation on milk yield, milk constituents in Murrah buffaloes at Buffalo Research Station, Venkataramannagudem.

Selection of experimental animals

The study was conducted on eighteen Murrah buffaloes in their second month of lactation which were free from anatomical, physiological and infectious diseases. They were randomly allocated into three groups with each group consisting of six animals in completely randomized design (CRD). The animals were dewormed and kept in a separate

well ventilated shed from a week prior to the start of experiment. Animals are selected based on the previous lactation milk yield and yield in the preceding lactation months. All the three groups have an approximately similar average milk yield. Group-I is control; Group-II and Group-III are the treatment groups.

Management to experimental animals

Housing management

All the experimental animals were housed in a well ventilated shed with asbestos sheet covering over the closed area. The animals were left free all the day and tied during the time of probiotic and synbiotic supplementation. There is a provision of separate feeding trough and water tank for the experimental animals.

Feeding management

All the experimental animals were fed with green fodder, wheat straw and concentrate feed according to guidelines of ICAR, 2013. The control group viz. Group-I is fed with basal diet (Hybrid Napier, paddy straw and concentrate). The Group-II and Group-III animals were fed with 30 gms of pro biotic and 20 gms of synbiotics respectively along with the basal diet.

Probiotic

Commercial preparation of probiotic is supplemented at a dose of 30 gms/animal/day.

Composition

Lactobacillus acidophilus, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus bifidum*, *Lactobacillus lactis*, *Lactobacillus fermentum*, *Streptococcus faecium*, *Aspergillus oryzae*.

Strength

Each 100 gms. Contains minimum of 132 billion c.f.u.

Synbiotic

Commercial preparation of synbiotic is supplemented at a dose of 20 gms/animal/day.

Composition

Probiotic (Lactoplus Vet)+Inulin+Mannan oligosaccharide.

Feeding of the experimental animals

The description of experimental feeding to the animals was presented in Table 1.

Milking management

The animals were milked twice a day at 4:00 A.M and 4:00 P.M. The milking was done at milking parlour and done by full hand milking method. The milk yield was recorded by using a digital weighing machine.

Table 1: Description of experimental feeding to the animals.

Groups	Feed supplement offered	Dose/animal/day
Group-I	Nil	Control
Group-II	Probiotic	30 g/animal/day
Group-III	Synbiotic	20 g/animal/day

Methodology of the parameters studied

Hematological parameters

Collection of blood samples

The blood samples were collected into EDTA vacutainers using sterile two way needle from the jugular vein. The collected blood samples were taken to the laboratory and are analyzed for hemoglobin, total leucocyte count, total erythrocyte count, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin using automatic hematology analyzer (Mindray BC-2800).

Serum biochemical parameters

Serum samples were obtained after centrifuging whole blood at $2000 \times g$ for 10 min; 4°C in refrigerated centrifuge (Thermo Fisher scientific). The serum so obtained was collected into eppendorf tubes. Serum stored in eppendorf tubes was analyzed for serum cholesterol, serum glucose, serum calcium and serum phosphorous. The commercially available kits [Transasia (ERBA) biomedical test kits] were used for assessment of serum cholesterol, serum glucose, serum calcium and serum phosphorous. The concentrations of the biochemical parameters in serum were determined using a spectrophotometer (Thermo Fisher Scientific Multiskan $\text{\textcircled{R}}$ Go). The analysis was carried out according to the manufacturer's recommendations.

Statistical analysis

The milk yield and milk composition at weekly intervals, hematological and biochemical parameters on initial and final day of the experiment period among the three groups were compared using one way ANOVA, with Tukey's posthoc analysis as implemented in SPSS v17.

RESULTS AND DISCUSSION

Blood parameters

Total leucocyte count

The mean leucocyte count ($\times 10^3/\mu\text{L}$) of lactating Murrah buffaloes in Group-1, Group-II and Group-III groups was presented in Table 2. At the beginning of the experiment were 10.07 ± 0.338 , 10.50 ± 0.292 and 10.13 ± 0.468 and at the end of experiment were 9.92 ± 0.369 , 10.7 ± 0.245 and 10.28 ± 0.550 respectively. The mean leucocyte count ($\times 10^3/\mu\text{L}$) was not significantly different among the experimental groups during the experimental period.

Similar to our findings, Gujjar *et al.* (2018b), Aggazi *et al.* (2014) also reported no effect of supplementing probiotic on white blood cell count of Sahiwal cattle and Holstein calves, respectively.

Total erythrocyte count

The mean erythrocyte count ($\times 10^3/\mu\text{L}$) of lactating Murrah buffaloes in Group-1, Group-II and Group-III at the beginning of the experiment were 8.37 ± 0.287 , 8.55 ± 0.268 and 8.616 ± 0.36 and at the end of experiment were 9.92 ± 0.36 , 10.70 ± 0.24 and 10.28 ± 0.55 , respectively. Significant difference was not observed in the mean erythrocyte count ($\times 10^6/\mu\text{L}$) among the treatment and control groups.

Parallel to the present findings, Kim *et al.* (2011) and Dimova *et al.* (2013) also reported no effects on the erythrocyte count by probiotic supplementation in calves. Dar *et al.* (2017) and Adams *et al.* (2008) also reported no significant effect on probiotic supplementation on erythrocyte count.

Haemoglobin

The Hb concentration (g/dL) of lactating Murrah buffaloes in Group-I, Group-II and Group-III was presented in Table 3. At the start of the experiment were 12.26 ± 0.28 , 12.40 ± 0.81 and 12.40 ± 0.97 and at the end of experiment were 12.03 ± 0.36 , 12.25 ± 0.38 and 12.12 ± 0.38 , respectively. Statistical analysis revealed that there was no significant difference in mean Hb concentration (g/dL) among the groups.

Similar results were reported by Kim *et al.* (2011) who found no significant effect on haemoglobin levels in Holstein calves supplemented with probiotics.

Packed cell volume

The mean PCV (%) of lactating Murrah buffaloes in Group-I, Group-II and Group-III was presented in Table 4. At the beginning of the experiment were 36.75 ± 0.85 , 37.25 ± 0.99 and 37.16 ± 1.19 and at the end of experiment were 35.98 ± 1.11 , 36.75 ± 1.10 and 36.43 ± 1.10 , respectively. There was no statistically significant difference observed in PCV (%) among the groups during the experiment.

The results presented were consistent with the findings of Kim *et al.* (2011), who found no significant difference in Holstein calves between the probiotic supplemented group and the antibiotic treated group. Similarly, Ghazanfar *et al.* (2015) did not find any significant effect on packed cell volume values with probiotic supplementation in dairy heifers.

Serum parameters

Serum cholesterol

The mean serum cholesterol (mg/dL) values of lactating Murrah buffalo's in Group-I, Group-II and Group-III was presented in Table 5. At the beginning of experiment were 95.23 ± 0.56 , 95.47 ± 0.37 and 95.44 ± 0.25 and at the end of the experiment were 96.82 ± 0.57 , 97.96 ± 0.27 and 97.43 ± 0.25 , respectively. There was no statistically significant difference observed in the serum cholesterol (mg/dL) among the groups during the experiment.

The results of present study are in accordance with the findings of Gujjar *et al.* (2018b) who found no significant difference on supplementing probiotics and synbiotics to Sahiwal cattle. Also, the findings of Morsey *et al.* (2014) are in accordance with our results.

Serum glucose

The mean serum glucose (mg/dL) level in lactating Murrah buffaloes in Group-I, Group-II and Group-III was presented in Table 6. At the beginning of the experimental trial were 47.02 ± 0.54 , 47.79 ± 0.29 and 47.66 ± 0.33 and at the end of the experiment were 49.01 ± 0.48 , 50.08 ± 0.35 and 50.08 ± 0.34 , respectively. There was no statistically significant difference in the serum glucose (mg/dL) observed among the groups during the experiment.

The present results were consistent with the findings of Gujjar *et al.* (2018b), Morsy *et al.* (2014), El-Din (2015), Yalcinet *et al.* (2011) and Bruno *et al.* (2009). Paralelly, Dar *et al.* (2017) reported a non-significant difference in glucose levels in probiotic, prebiotic and synbiotic supplemented calves throughout the experimental period.

Serum calcium

The mean serum calcium (mg/dL) level in lactating Murrah buffaloes was presented in Table 7. At the beginning of the experimental trial were 8.53 ± 0.10 , 8.93 ± 0.15 , 8.62 ± 0.16 and at the end of the experiment were 8.84 ± 0.08 , 9.17 ± 0.16 and 8.87 ± 0.20 , respectively in Group-I, Group-II and Group-III. There was no statistically significant difference in the serum calcium (mg/dL) observed among the groups during the experiment.

Table 2: Effect of probiotic and synbiotic supplementation on total leucocyte count ($\times 10^3/\mu\text{L}$) in Murrah buffaloes.

Day	Total leucocyte count ($\times 10^3/\mu\text{L}$)		
	Group-I	Group-II	Group-III
Before	10.07 ± 0.338	10.50 ± 0.292	10.13 ± 0.468
After	9.92 ± 0.369	10.70 ± 0.245	10.28 ± 0.554
Overall mean	9.99 ± 0.0769	10.60 ± 0.1	10.21 ± 0.074

Table 3: Effect of probiotic and synbiotic supplementation on haemoglobin(g/dL) in murrah buffaloes.

Day	Haemoglobin (g/dL)		
	Group-I	Group-II	Group-III
Before	12.26 ± 0.28	12.40 ± 0.81	12.40 ± 0.97
After	12.03 ± 0.363	12.25 ± 0.381	12.18 ± 0.381
Overall mean	12.15 ± 0.114	12.33 ± 0.074	12.29 ± 0.109

Table 4: Effect of probiotic and synbiotic supplementation on packed cell volume in murrah buffaloes.

Day	Packed cell volume (%)		
	Group-I	Group-II	Group-III
Before	36.75 ± 0.853	37.25 ± 0.997	37.16 ± 1.19
After	35.98 ± 1.11	36.75 ± 1.10	36.43 ± 1.10
Overall mean	36.37 ± 0.385	37.00 ± 0.25	36.79 ± 0.364

Table 5: Effect of probiotic and synbiotic supplementation on cholesterol (mg/dl) in Murrah buffaloes.

Day	Serum cholesterol (mg/dl)		
	Group-I	Group-II	Group-III
Before	95.23 ± 0.56	95.47 ± 0.38	95.44 ± 0.25
After	96.82 ± 0.57	97.96 ± 0.28	97.43 ± 0.25
Overall mean	96.03 ± 0.77	96.72 ± 1.24	96.44 ± 1.00

Table 6: Effect of probiotic and synbiotic supplementation on Serum glucose (mg/dl) in Murrah buffaloes.

Day	Serum glucose (mg/dL)		
	Group-I	Group-II	Group-III
Before	47.02 ± 0.54	47.79 ± 0.29	47.66 ± 0.33
After	49.01 ± 0.48	50.08 ± 0.35	50.08 ± 0.346
Overall mean	48.02 ± 1.00	48.94 ± 1.14	48.87 ± 1.21

Table 7: Effect of probiotic and synbiotic supplementation on serum calcium (mg/dl) in Murrah buffaloes.

Day	Serum calcium (mg/dL)		
	Group-I	Group-II	Group-III
Before	8.53±0.10	8.93±0.15	8.62±0.16
After	8.84±0.08	9.17±0.16	8.87±0.20
Overall mean	8.69±0.16	9.05±0.12	8.75±0.13

Table 8: Effect of probiotic and synbiotic supplementation on serum phosphorous (mg/dl) in murrah buffaloes.

Day	Serum phosphorous (mg/dL)		
	Group-I	Group-II	Group-III
Before	4.56±0.054	4.68±0.060	4.57±0.053
After	4.66±0.069	4.76±0.056	4.68±0.041
Overall mean	4.61±0.05	4.72±0.40	4.63±0.05

Similar results were reported by Gujjar *et al.* (2018b), who found no significant difference in serum calcium level when probiotic and synbiotics are supplemented to Sahiwal cattle. Sretenovic *et al.* (2008) reported no significant difference in Holstein Friesian cows on feeding yeastier containing *saccharomyces cerevisiae* and probiotic bacteria.

Serum phosphorous

The mean serum phosphorous (mg/dL) level in lactating Murrah buffaloes was presented in Table 8. At the beginning of the experimental trial were 4.56±0.05, 4.68±0.06, 4.57±0.05 and at the end of the experiment were 4.66±0.06, 4.76±0.05, 4.68±0.04, respectively in Group-I, Group-II and Group-III. There was no statistically significant difference in the serum phosphorous (mg/dL) observed among the groups during the experiment.

Similar results were reported by Gujjar *et al.* (2018b), who found no significant difference in serum phosphorous level when probiotic and synbiotics are supplemented to Sahiwal cattle. In contrary, Zaworski *et al.* (2014) reported a significant increase in the serum phosphorous levels.

CONCLUSION

The present study was conducted to study the effect of probiotic and synbiotic supplementation on haematological and serum biochemical parameters in Murrah buffaloes. It was concluded that there was no significant effect on these parameters with the supplementation of probiotics and synbiotics.

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Conflict of interest

The authors declare no conflict of interest regarding the submission of this manuscript.

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