

Original Research Article

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Effect of Probiotic, Prebiotic and Synbiotic Supplementation on Haematological and Serum Biochemical Profiles in Murrah Buffalo Calves

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ABSTRACT

Keywords

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Twenty four Murrah buffalo calves of about six months age were randomly divided into four groups of six calves each on the basis of body weight (BW) in a completely randomized design (CRD). Group I is control (C), Group-II was supplemented with multistrain probiotic containing 7 bacterial strains and 2 yeast strains (2×10^9 cfu/gm) at 5gm/calf/day (T1). Group-III was supplemented with prebiotic Mannonoligosaccharide + inulin at 5gm/calf/day (T2) and Group-IV was supplemented with synbiotic containing multistrain probiotic at 2.5gm and 2.5gms of prebiotic Mannonoligosaccharide + inulin per calf/day (T3) for a period of 90 days. The results showed no significant difference in haematological and serum biochemical parameters among the treatment and control groups. However, from 60th day onwards, significantly ($P < 0.05$) lower levels of serum cholesterol was observed in T1, T2 and T3 groups compared to the control. In conclusion, the dietary supplementation of the above said probiotic, prebiotic and synbiotics have no effect on haematological and serum biochemical values in in buffalo calves.

Introduction

Buffalo is the back bone of Indian dairy industry, however, the mortality rate in buffalo calves particularly during first few months of their postnatal life is high, predominantly due to calf scores, causing heavy loss to the buffalo producers in India. Therefore, to combat infection, antibiotics are commonly

being used as feed additives in the rations of calves. However indiscriminate and sub-therapeutic levels of antibiotics usage in calves results in development of drug-resistant microorganisms which are responsible for treatment failures (Jin *et al.*, 1997).

In an effort to replace antibiotics in animal feeds, Probiotic, prebiotics and combination of

probiotic and prebiotic (synbiotics) have been proposed as alternates to antibiotics. (Heinrichs *et al.*, 2003; Morill *et al.*, 1995). Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its microbial balance (Fuller, 1989). Rolfe (2000) suggested that multi-strain probiotic may be more useful than a single strain because they can proliferate more lactic acid bacteria than single strain probiotic. Prebiotics are non-digestible carbohydrates which are not metabolized in the small intestine and fermented in large intestine. Oligosaccharides such as fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and mannan-oligosaccharide (MOS) are among the most important prebiotics that have been studied as alternatives to antibiotics. Recently, prebiotics have been shown to have immune-enhancing characteristics (Okomato *et al.*, 2003; Fleige *et al.*, 2009). Synbiotics are dietary supplements combining both prebiotics and probiotics in a form of synergism that beneficially affects the host by improving the survival and implantation of microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of health-promoting bacteria, and thus improving host welfare. However, very scanty information is available on comparative study of benefits of probiotics, prebiotics and synbiotics in buffalo calves. So, this study was taken up to investigate the changes in haematological and serum biochemical parameters upon dietary supplementation of probiotics, prebiotics and synbiotics in buffalo calves.

Materials and Methods

Twenty four Murrah buffalo calves of approximately 6 months age were selected from LFC, NTR College of Veterinary Science, Gannavaram and divided into four groups of six calves each on the basis of body

weight (BW) in a completely randomized design (CRD). The average body weight (kg) in each of the groups was similar before the start of the experiment. All the experimental calves were housed in a well protected animal shed with good ventilation and provision for individual feeding and watering. All the calves were fed with Hybrid Napier green fodder and concentrate mixture as basal diet as per their nutrient requirements (ICAR 2013). The calves were divided into four groups i.e. Group I is control, Group-II was supplemented with multistrain probiotic containing 7 bacterial strains and 2 yeast strains (2×10^9 cfu/gm) @ 5gm/calf/day. Group-III was supplemented with prebiotic Mannon oligosaccharide + Inulin @ 5gm/calf/day and Group-IV was supplemented with synbiotic containing multistrain probiotic @ 2.5gm and 2.5gms of prebiotic Mannon oligosaccharide + Inulin per calf/day for a period of 90 days. All the experimental calves were identified using ear tags. Deworming and deticking was done using Fenbendazole @ 10mg/kg BW and Deltamethrin @ 4ml/L before the start of the experiment. All the calves were allowed to have adaptation period for 15 days before the actual trail, during which they were fed with basal diet. After the adaptation period calves were fed with their respective treatment diets for 90 days. The diet of the experimental animals included chopped Hybrid Napier as roughage source and concentrate mixture. Feeding of concentrates followed by fodder was done daily in the morning and evening at a fixed time. Feed offered and feed left in the individual mangers were noted to obtain feed intake of the calf. The leftover concentrate mixture, if any was weighed.

Two blood samples from each calf were collected aseptically at fortnightly intervals before morning feeding via a single jugular vein puncture with the help of sterilized needles. One sample was collected in EDTA

vacuutainer (AcCuvet-PLUS) for haematological analysis and the other sample was collected into an anti-coagulant free centrifuge tube (Tarsons) to obtain serum. Whole blood was collected into EDTA vacuutainer for analysis of haematological constituents like haemoglobin (Hb), RBC, WBC, PCV using automatic haematology analyser (Mindray BC-2800). Serum samples were obtained after centrifuging whole blood at 2000 x 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 analysed for serum glucose, total protein (TP), albumin, globulin, cholesterol. The commercially available kits procured from Coral Clinical System-Tulip Diagnostics India were used for assessment of glucose, total protein, albumin, globulin, cholesterol. The concentrations of the said biochemical parameters in serum were determined using a spectrophotometer (Thermo fisher scientific-Multiskan® Go). The analysis was carried out according to the manufacturer's recommendations. The data obtained was analyzed according to statistic computer program SPSS version (15.01) (SPSS, 2006). The data pertaining to hematological and serum biochemical profiles were analyzed using ANOVA and Post-hoc tests.

Results and Discussion

Heamotological parameters

Heamoglobin

The results on the effect of dietary supplementation of synbiotics on haemoglobin (Hb) concentration in Murrah calves are represented in the Table 1. The Hb concentration was within the normal range in all the experimental calves. Statistical analysis revealed that there was no significant difference observed among the groups

throughout the experimental study. Present results were consistent with the findings of Kim *et al.*, (2011) who observed no significant effect on hemoglobin levels in Holstein calves supplemented with probiotics. Contrary to the present results, Dar *et al.*, (2017) observed increased Hb concentration in calves supplemented with probiotic, prebiotic and synbiotic compared to the control.

Total erythrocyte count

The effect of probiotic, prebiotic and synbiotic supplementation on total erythrocyte count in Murrah calves is represented in the Table 1. The results revealed no significant difference in total erythrocyte count among the treatment and control groups which was in conformity with the results of Kim *et al.*, (2011) and Dimova *et al.*, (2013) who reported that erythrocyte count remained unaffected by probiotic supplementation in calves. Dar *et al.*, (2017) and Adams *et al.*, (2008) also reported no significant effect of probiotic supplementation on erythrocyte count. Contrary to the present findings, Abdel-Fattah and Fararh (2009) observed a significant increase in RBC when broiler chicks were supplemented with probiotics, prebiotics and synbiotics.

Total leucocyte count

The effect of probiotic, prebiotic and synbiotic supplementation on total leucocyte count in Murrah calves is represented in the Table 1. The results of the present study indicates that there was no statistically significant difference in total leucocyte count among the groups upon supplementation of probiotics, prebiotics and synbiotics in Murrah calves. Chen *et al.*, (2005) and Agazzi *et al.*, (2014) also reported that there was no effect of supplementing probiotic on white blood cell count of growing pigs and calves, respectively. Contrary to the present findings, Dar *et al.*, (2017) reported

that supplementation of probiotics, prebiotics and synbiotics in calves significantly ($P < 0.05$) increased total leucocyte count compared to the control. Al-Siaidy *et al.*, (2010) also reported significantly higher white blood cell count in calves fed with both commercial grade and culture of *Lactobacillus acidophilus* than control group.

Packed cell volume

The effect of probiotic, prebiotic and synbiotic supplementation on packed cell volume (PCV %) in Murrah calves is represented in the Table 1. The results revealed no significant difference in PCV among the treatment and control groups. The present results were consistent with the findings of Kim *et al.*, (2011) who observed no significant difference between probiotic supplemented group and antibiotic treated group in Holstein calves. Similarly no significant effect was observed by Gazanfar *et al.*, (2015) on packed cell volume values by probiotic supplementation in dairy heifers. Contrary to the present findings, Dar *et al.*, (2017) reported that supplementation of probiotics, prebiotics and synbiotics in calves significantly ($P < 0.05$) increased PCV compared to the control.

Serum biochemical profiles

Serum glucose level

The effect of probiotic, prebiotic and synbiotic supplementation on serum glucose level in Murrah calves is represented in the Table 2. Statistical analysis revealed that there was no significant difference in the serum glucose levels among the treatments. The present results were consistent with the findings of Dar *et al.*, (2017) who reported non-significant difference in glucose levels in probiotic, prebiotic and synbiotic supplemented calves throughout the experimental period. The results were in accordance with the findings of Adams *et al.*, (2008) and Frizzo *et al.*, (2010)

who reported no significant difference in serum glucose levels in probiotic and control group calves. Further, Rekiel *et al.*, (2007) and Silva *et al.*, (2012) reported that prebiotic supplementation had no effect on plasma glucose levels in pigs and calves respectively. Gujar (2017) also reported no significant difference in the serum glucose levels among probiotic, prebiotic and synbiotic supplementation in Sahiwal cattle compared to the control group cattle. Chaudhary *et al.*, (2008) reported that the dietary supplementation of direct fed microbial had no significant effect on serum glucose levels of crossbred cattle calves. Similar to the present findings El-Din (2015), also reported no significant effects of probiotic on serum glucose levels in cows.

Albumin

The effect of probiotic, prebiotic and synbiotic supplementation on albumin level in Murrah calves is represented in the Table 2. The results revealed that there was no significant difference in the albumin levels among treatments which were in agreement with the findings of Galip (2006) who observed that the albumin concentration was not significantly modified in rams supplemented with yeast. Chaudhary *et al.*, (2008) also observed no significant effect on albumin concentration in crossbred cattle calves when supplemented with direct fed microbials.

Globulin

The effect of probiotic, prebiotic and synbiotic supplementation on serum globulin levels in Murrah calves is represented in the Table 2.

Statistical analysis revealed that there was no significant difference in the serum globulin levels among the groups. In accordance to the present study Galip (2006) observed that the dietary supplemental yeast has no effect on serum globulin concentration in rams.

Table.1 Effect of probiotic, prebiotic and symbiotic supplementation on haematological profiles in murrah calves

Parameters	Days	C	T-1	T- 2	T-3
Hb (g/dL)	0 th Day	9.80±0.34	9.41±0.27	8.98±0.32	8.91±0.23
	90 th Day	8.98±0.18	9.41±0.37	9.38±0.53	9.35±0.37
RBC(x10 ⁶ /μL)	0 th Day	8.93±0.22	8.86±0.32	9.14±0.19	8.6±0.31
	90 th Day	9.31±0.26	9.04±0.27	8.96±0.18	9.05±.18
WBC(x10 ³ /μL)	0 th Day	12.38±0.94	12.4±0.46	12.03±0.62	10.56±0.38
	90 th Day	12.23±0.45	14.31±1.43	14.28±1.83	13.08±.71
PCV (%)	0 th Day	31.25±0.59	32.15±0.52	31.15±0.58	31.18±0.91
	90 th Day	29.91±0.68	32.21±0.67	31.20±1.34	30.68±1.38

Table.2 Effect of probiotic, prebiotic and symbiotic supplementation on serum profiles in murrah calves

Parameter	Days	C	T-1	T- 2	T-3
Glucose (mg/dL)	0 th Day	79.87±3.63	82.85±2.78	76.08±2.61	77.58±3.18
	90 th Day	87.99±0.77	88.28±1.16	90.57±1.32	89.51±1.66
Albumin (g/dL)	0 th Day	3.23±0.13	3.37±0.16	2.72±0.15	3.15±0.2
	90 th Day	2.10±0.04	2.19±0.06	2.21±0.07	2.11±0.04
Globulin (g/dL)	0 th Day	1.69±0.12	1.67±0.10	1.94±0.15	2.02 ±0.17
	90 th Day	1.45±0.41	1.39±0.09	1.30±0.06	1.37±0.05
(A/G) ratio	0 th Day	1.91±0.12	2.01±0.10	1.40±0.15	1.48±0.17
	90 th Day	1.44±0.04	1.57±0.09	1.7±0.06	1.54±0.05
Totalprotein (g/dL)	0 th Day	4.92±0.31	5.04±0.23	4.66±0.11	5.02±0.46
	90 th Day	3.55±0.06	3.58±0.03	3.51±0.07	3.48±0.08
Cholesterol (mg/dl)	0 th Day	90.17±7.23	97.26±6.29	96.06±8.34	98.68±2.70
	90 th Day*	110.32±1.31 ^a	80.78±1.76 ^b	83.02±1.36 ^b	75.64±0.26 ^b

Means with different superscripts in a row differ significantly (*P<0.05)

Similarly Chaudhary *et al.*, (2008) also observed no significant effect on globulin concentration when supplemented with direct fed microbial in crossbred cattle calves. However, contrary to our findings Singh *et al.*, (2016) found an increase in serum globulin levels in the group fed with probiotic in Murrah buffalo calves. Singh *et al.*, (2016a) also observed significant difference (P<0.01) in serum globulin levels between control and probiotic treatment group in Barbari kids.

Albumin to globulin (A/G) ratio

The effect of probiotic, prebiotic and synbiotic supplementation on albumin to globulin ratio

is presented in Table 2. The present findings revealed that there was no significant difference in albumin to globulin ratio among the groups. In accordance to our findings Chaudhary *et al.*, (2008) observed no significant effect on albumin to globulin ratio in crossbred calves supplemented with direct fed microbial. Galip (2006) also observed that the serum albumin to globulin ratio were not significantly modified in rams.

Contrary to our findings Singh *et al.*, (2016) observed higher albumin to globulin ratio in probiotic supplemented groups when compared to control in Murrah buffalo calves. Similarly Singh *et al.*, (2016a) found higher

albumin to globulin ratio in supplemented groups in Barbari kids.

Total protein

The effect of probiotic, prebiotic and synbiotic supplementation on total protein is presented in Table 2. The results of the present study revealed that there was no significant difference in the total protein levels among the groups. In accordance to our findings Chaudhary *et al.*, (2008) reported that the dietary supplementation of direct fed microbial had no significant effect on total protein in crossbred calves. This indicates that synbiotic supplementation did not affect utilization of dietary proteins or protein synthesis in liver.

Contrary to our findings Singh *et al.*, (2016) observed higher total protein ($P < 0.05$) in probiotic supplemented groups in Murrah buffalo calves. Singh *et al.*, (2016a) also observed higher total protein level at monthly interval in probiotics supplemented group as compared to unsupplemented group in Barbari kids.

Cholesterol

The effect of probiotic, prebiotic and synbiotic supplementation on cholesterol in Murrah calves is represented in the Table 2. Statistical analysis revealed that there was no significant difference in the serum cholesterol up to 45th day. From 60 to 90 days, there was significantly ($P < 0.05$) lower levels of serum cholesterol was observed in probiotic, prebiotic and synbiotic supplemented groups. The results of present study are accordance with the findings of Dar *et al.*, (2017) who indicated that probiotic (*Lactobacillus acidophilus*), prebiotic (MOS) and synbiotic supplementation were effective in reduction of serum cholesterol and triglycerides at 60th and 90th day. The reduction in serum cholesterol

might be due to the assimilation of cholesterol into the *Lactobacilli acidophilus* cells (Drouault *et al.*, 2002). Lower cholesterol values observed in synbiotic supplementation might be due to the production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics as described by De Preter *et al.*, (2007) in humans. The results are in accordance with the results of Al-Saiady (2010) who also reported a significant decrease in serum cholesterol levels in probiotic administered calves. Tang *et al.*, (2005) reported significantly lower values of cholesterol in pigs that were supplemented with chitosan and galacto-mannan oligosaccharide as compared to control. Contrary with the present results, Takagi *et al.*, (2011) reported significantly higher cholesterol concentration at 14th day and 30th day of experimental study in probiotic treated calves than control calf group. Frizzo *et al.*, (2010) also reported no significant effect of feeding probiotic on serum total cholesterol levels in calves. No significant difference was observed in serum cholesterol levels between prebiotic group and control group in pigs (Rekiel *et al.*, 2007).

The dietary supplementation of the above said probiotic, prebiotic and synbiotics have no effect on haematological and serum biochemical values in buffalo calves.

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