



Effect of dietary supplementation of *Lactobacillus acidophilus* on blood biochemical profile, antioxidant activity and plasma immunoglobulin level in neonatal Murrah buffalo calves

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ABSTRACT

An experiment was designed to evaluate the effect of dietary supplementation of *Lactobacillus acidophilus* on blood biochemical profile, antioxidant activity and plasma immunoglobulin level in neonatal Murrah buffalo calves. The 90 day trial was conducted on 24 neonatal Murrah buffalo calves randomly divided into 4 dietary treatments, viz. CON (basal diet alone), T1 (basal diet + *L. acidophilus* as a fermented milk @ 100 mL/calf/day having 10⁸ CFU/mL), T2 (basal diet + *L. acidophilus* as a fermented milk @ 200 mL/calf/day having 10⁸ CFU/mL) and T3 (basal diet + *L. acidophilus* as a fermented milk @ 300 mL/calf/day having 10⁸ CFU/mL). Supplementation of probiotics improved the plasma glucose level in T2 and T3 as compared to CON. Total protein (TP), plasma albumin (A), plasma globulin (G) and A:G ratio did not change with the supplementation of probiotic in calves. Total cholesterol and HDL cholesterol levels in plasma remained same in all the 4 groups. Total antioxidant (TA) activity was higher in T2 and T3 as compared to CON, whereas it was intermediate in T1. Super oxide dismutase (SOD) activity was significantly higher in T1, T2 and T3 groups as compared to CON whereas catalase and glutathione peroxidase (GPx) activity remained same in all groups throughout experimental period. The total plasma immunoglobulin and plasma IgG remained uninfluenced in all the groups. In conclusion, supplementation of *L. acidophilus* improved energy metabolism and antioxidant capacity in neonatal Murrah buffalo calves.

Key words: Antioxidant activity, *Lactobacillus acidophilus*, Murrah, Neonatal calves, Probiotic

India has 111.30 million buffalo is which is 57.3% of the total world buffalo population (194.2 million). Presently milk production in India is 127.3 million tonnes milk, out of which the buffalo contributes about 62.35% of the total milk produced (BAHS 2012). Buffalo husbandry in our country is playing a pivotal role in augmenting income and uplifting livelihood of the disadvantaged section of Indian society. The name “Black Gold” has emerged as a synonym for the most popular breed of buffaloes in our country, i.e. Murrah, which serve as a capital reserve or cash crop to the rural society by providing economic stability, livelihood security and social status to the farmers (Balbhadra 2013). Calves are the future for successful dairy enterprise in India. Therefore, calf health status is not only essential for sustenance of the livestock industry but is also indispensable in the wake of preserving and maintaining good quality germplasm. Sudden changes in diet or environment, disease or other stress can cause alteration in microbial ecosystem (Krehbiel *et al.* 2003). Newborn calves exposed to high levels of stress during the first to fourteen day of life because

they suffer from change in environment, diet, feeding condition, handling stress and low immunity. The two causative pathogens such as *Salmonella* spp. (Gill *et al.* 2001) and *Escherichia coli* (Shu and Gill 2002) are the most frequent etiologic agents in neonatal calf scours during the first week of life (Barrington *et al.* 2002).

Since 1940, administration of antibiotics in both therapeutic and sub-therapeutic levels has been the standard practice for dealing with pathogenic bacteria problems in dairy animals in addition to obtain economic benefits in terms of improved calf performance and reduced medicinal costs (Masucci *et al.* 2011). The widespread usage of antibiotics in dairy animal has led to antibiotic residues found in animal products and increased the emergence of drug-resistant bacteria in human beings (Abu-Tarboush *et al.* 1996). So, use of all antibiotics as additives in animal feed has been banned in EU (Commission Regulation EC No 2200/2001) since 2006.

Probiotics came up as a good adjuvant to promote the overall health in general and stabilization of gastrointestinal microbiota in particular as an alternative to antibiotics and becoming widely popular in this regards. It is well known that feeding calves with probiotics, especially lactic acid bacteria (Sharma *et al.* 2016), improves gut health of neonate with a subsequent increase in digestion efficiency

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and it lead to better growth performance (Frizzo *et al.* 2010). Scanty information is available regarding *L. acidophilus* action on blood biochemical profile, antioxidant modulation and plasma immunoglobulin level, particularly in neonatal Murrah buffalo calves. Keeping the above background in view, we hypothesized that dietary supplementation of *L. acidophilus* could improve blood biochemical profile, antioxidant activity and plasma immunoglobulin level in neonatal Murrah buffalo calves.

MATERIALS AND METHODS

Animals, experimental design and diet: The present experiment was conducted in the Livestock Research Centre of the Institute. The animal experimental design was approved by the Institutional Animal Ethics Committee (IAEC) and conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

A freeze-dried pure culture of *L. acidophilus* NCDC-15 strain was taken from the National Centre for Dairy Culture (NCDC), Department of Dairy Microbiology of the Institute for preparation of probiotic culture. The freeze-dried form of *L. acidophilus* was revived in MRS broth and, then one loop of *L. acidophilus* inoculums was inoculated in milk and kept for 24 h at 37–39°C for fermentation milk. Next day, the same fermented of milk was used as an inoculum for the next coming day and such process was continued for 7 days. After 7 day, glycerol stock was used to prepare fermented milk. The colony forming unit (CFU) / gram of fermented feed was counted at every alternate day to check the viability of bacterial cells and was maintained at the level of 10^8 CFU/mL throughout the feeding period.

Neonatal Murrah buffalo calves (24) of 5–7 d old and 36 ± 2.0 kg of body weight (BW) were randomly assigned into 4 groups (CON, T1, T2 and T3) with 6 animals in each group. Murrah buffalo calves were removed from their dams after completion of colostrum feeding and housed individually in well-ventilated calf pens. The calves were housed in pens with separate feeder and waterer, calves had *ad lib.* feed and water throughout the experiment of 90 days. The calves of Gr 1 (CON) fed on a basal diet (concentrate mixture and green fodder) alone while calves of Gr 2 (T1), Gr 3 (T2) and Gr 4 (T3) were supplemented with *L. acidophilus* in the form fermented milk @ 100 mL/calf/day having 10^8 CFU/mL, 200 mL/calf/day having 10^8 CFU/mL and 300 mL/calf/day having 10^8 CFU/mL, respectively.

The milk feeding was carried out two times in a day and whole milk fed to the calves @ 1/10th of BW up to 2nd week, 1/15th of BW in third to fourth week, 1/20th of BW in fifth and sixth week, 1/25th in the seventh to eighth week. Calf starter was offered from second week onwards (Table 1). The concentrate feed mixture contained maize, bajra, groundnut cake (GNC), soybean meal (SBM), mustard oil cake (MOC), wheat bran, rice polish and mineral mixture. Maize and sorghum were provided as green fodder.

Table 1. Feeding schedule of calves

Calves age (day)	Whole milk	Skim milk	Concentrate mixture	Fodder
1–15	1/10 of body wt	–	Introduced after 7 day	Introduce after 7 day
16–30	1/15 of body wt	–	<i>ad lib.</i>	<i>ad lib.</i>
31–45	1/20 of body wt	–	<i>ad lib.</i>	<i>ad lib.</i>
45–60	–	1/25 of body wt	<i>ad lib.</i>	<i>ad lib.</i>
61–90	–	–	<i>ad lib.</i>	<i>ad lib.</i>

All the buffalo calves were fed *ad lib.* concentrate and green (Ramaswami *et al.* 2005). Clean water was offered *ad lib.* to the calves.

Blood sampling and analyses: Blood samples were taken at monthly interval. The blood collection site was aseptically prepared by clipping the hairs from the collection site and using sterile gauze piece and spirit. Blood sample (6 mL) was collected from jugular vein aseptically using a disposable syringe (Dispovan HSMD) with 18 gauge hypodermic needle early in the morning at 7 AM. Blood (6 ml) after the collection was transferred into 2 (4 mL) EDTA coated tubes each and the blood samples were immediately transported to the laboratory in a box containing ice packs.

Plasma was extracted to study plasma total protein, plasma albumin, glucose, plasma cholesterol by using analytical kit (Recombigen Laboratories Pvt. Ltd., New Delhi, India). The globulin concentration was calculated by subtracting the albumin from total protein.

Total antioxidant was determined by enzymatic colorimetric method by using readymade diagnostic kit (Cayman chemicals, USA) according to Halliwell *et al.* (1996). Catalase was determined by enzymatic colorimetric method by using readymade kit (Cayman Chemicals, USA) according to Johansson and Borg (1988). SOD was determined by enzymatic colorimetric method by using readymade diagnostic kit (Cayman Chemicals, USA) according to Sandstrom *et al.* (1994). GPx was determined by enzymatic colorimetric method by using readymade diagnostic kit provided by (Cayman Chemicals, USA according to Forstrom *et al.* (1978).

Immunoglobulin G (IgG) was determined in plasma of calves by Bovine IgG ELISA kit (Bethyl Laboratories, Montgomery, USA). Total immunoglobulin was estimated in the plasma sample by Zn turbidity method reported by McEwan *et al.* (1970).

Statistical analysis: The data were analysed using the general linear model procedure of Statistical Package for the Social Sciences (SPSS for Windows, v21.0; SPSS Inc., Chicago, IL, USA). To estimate the effect of treatment and period, and their interaction, the following model was used:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ijk}$$

where Y_{ijk} , dependent variable; μ , overall mean of the

population; Ti, mean effect of the ith treatments; Dj, mean effect of day of sampling with day as a repeated factor ($j=0, 30, 60$ and 90 days of dietary treatment); (T×D)ij, effect of the interaction between effects of treatment and day of sampling; and eijk, unexplained residual element assumed to be independent and normally distributed. The individual animal was used as the experimental unit for all data. The pair-wise comparison of means was carried out using “Tukey’s honest significant difference (HSD) test”. Significance was determined at $P<0.05$.

RESULTS AND DISCUSSION

Effect of *Lactobacillus acidophilus* on plasma biochemical profile: The plasma level of glucose was statistically ($P<0.05$) higher in both T2 and T3 as compared to the CON group, with intermediate level in T1. Further, a significant reduction ($P<0.001$) in plasma glucose was also evident when period-wise comparison was made, with the value at 60 days being lower than that of the 0 day value. However, there was no significant difference between 0 day as compared to 90 days and 30 days as compared to 60

days (Table 2). The plasma glucose level indicates the physiological condition of the animals. Glucose is synthesized from carbohydrates, which are supplied to cell from body fluids. The serum glucose levels were within the normal range (Kaneko *et al.* 1997) though it was statistically ($P<0.05$) higher in both the treatment groups as compared to control. Hence, *S. cerevisiae* supplementation enhanced the serum glucose level in growing calves. Similarly, Abdalla *et al.* (2013) showed the significant effect on glucose level in the probiotic supplemented group as compared to control group. Hossain *et al.* (2012) and Hammon *et al.* (2002) also reported that due to supplementation of live yeast culture on calves, there was higher glucose concentration in supplemented group as compared to control group.

Supplementation of probiotic in calves did not effect ($P>0.05$) total protein (TP), plasma albumin (A), plasma globulin (G) and A : G (Table 2). Plasma total protein consists mainly of albumin and globulin. The plasma protein level at any given time interval is a function of nutritional status, hormonal balance, water balance and other factors

Table 2. Effect of dietary supplementation of *Lactobacillus acidophilus* on blood biochemical profile of Murrah buffalo calves

Attribute	Dietary groups [†]				Period Mean	Significance [§]		
	CON	T1	T2	T3		T	P	T×P
<i>Glucose (mg/dL)</i>								
0d	94.21±1.09	95.23±0.33	95.05±0.19	95.55±0.53	95.01 ^r ±0.31	0.013	<0.001	0.005
30d	86.45±1.09	87.79±2.06	87.94±2.92	86.75±2.28	87.23 ^{pq} ±1.01			
60d	82.36±1.99	83.55±3.64	84.00±2.84	82.64±4.18	83.14 ^p ±1.53			
90d	70.77 ^a ±3.13	94.79 ^b ±7.56	97.91 ^b ±5.98	99.62 ^b ±8.39	90.77 ^{qr} ±3.92			
Average	83.45 ^b ±1.99	90.34 ^{ab} ±2.26	91.23 ^a ±2.04	91.14 ^a ±2.66				
<i>Total protein (g/dL)</i>								
0d	7.58±0.23	7.67±0.25	7.61±0.36	7.68±0.19	7.64±0.13	0.985	0.315	0.998
30d	8.18±0.24	7.71±0.34	8.04±0.21	8.03±0.52	7.99±0.17			
60d	7.94±0.04	7.88±0.53	7.86±0.35	7.83±0.37	7.88±0.17			
90d	7.95±0.25	8.07±0.14	8.03±0.17	7.94±0.32	8.00±0.11			
Average	7.91±0.11	7.83±0.16	7.89±0.14	7.87±0.18				
<i>Albumin (g/dL)</i>								
0d	3.35±0.28	3.62±0.12	3.35±0.41	3.36±0.44	3.42 ^p ±0.16	0.962	0.016	1.000
30d	3.48±0.20	3.46±0.45	3.66±0.41	3.70±0.63	3.57 ^{pq} ±0.21			
60d	3.86±0.24	4.00±0.44	3.90±0.33	3.94±0.72	3.92 ^{pq} ±0.22			
90d	4.21±0.47	4.32±0.37	4.42±0.55	4.52±0.56	4.37 ^q ±0.23			
Average	3.72±0.16	3.85±0.19	3.83±0.22	3.88±0.29				
<i>Globulin (g/dL)</i>								
0d	4.23±0.47	4.45±0.15	4.26±0.44	4.58±0.36	4.38±0.18	0.955	0.065	0.997
30d	4.70±0.31	4.25±0.67	4.38±0.28	4.34±0.49	4.42±0.22			
60d	4.08±0.25	3.56±0.65	3.96±0.36	3.89±0.73	3.87±0.25			
90d	3.74±0.59	3.75±0.49	3.61±0.52	3.42±0.63	3.63±0.26			
Average	4.19±0.21	4.00±0.26	4.05±0.20	4.06±0.28				
<i>A:G</i>								
0d	0.87±0.15	0.82±0.05	0.87±0.20	0.80±0.19	0.84±0.07	0.636	0.051	0.987
30d	0.76±0.08	1.09±0.39	0.88±0.15	1.05±0.39	0.95±0.14			
60d	0.98±0.12	1.35±0.29	1.05±0.15	1.83±1.02	1.30±0.26			
90d	1.47±0.46	1.42±0.43	1.50±0.40	1.71±0.45	1.52±0.20			
Average	1.02±0.13	1.17±0.16	1.07±0.13	1.35±0.29				

[†]Basal diet with no supplementation (CON) or supplemented as *Lactobacillus acidophilus* T1 (100 g/calf/d), T2 (200 g/calf/d), T3(300 g/calf/d). ^{a,b/p,q,r}Means bearing different superscripts in a row (a,b) or column (p,q,r) differ significantly ($P<0.05$). [§]Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

affecting the state of health. Noori *et al.* (2016) also observed no effect on TP levels. Al-Saidy (2010) demonstrated that serum TP, A and G levels were comparable between probiotic supplemented groups as compared to control group. Lesmeister *et al.* (2004) and Chaudhary *et al.* (2008) also reported similar total protein, albumin and globulin concentration in control animals and the animals given probiotics. However, Hossain *et al.* (2012) reported that serum profiles of total protein and globulin significantly ($P<0.05$) higher in probiotic supplemented group as compared to T1 group.

There was no influence ($P>0.05$) of the dietary intervention on the plasma total cholesterol and plasma HDL cholesterol levels among the 4 groups (Table 3). Singh *et al.* (2016) reported that there were significantly ($P<0.05$) lower cholesterol concentration in the probiotic fed group compared to control. The lower level of cholesterol may be attributed to stimulation of bacterial lipid synthesis or due to antichol estroleamic effect of yeast culture treatment (Fuller 1989). Al-Saiady (2010) reported that probiotic bacteria (*L. acidophilus* and *L. plantarum*) fed to Holstein Friesian calves (3–4 day old) and serum cholesterol level found significantly reduced. Noori *et al.* (2016) reported that supplementation of probiotic improved serum triglyceride ($P<0.01$) and cholesterol ($P<0.05$) levels.

Effect of *Lactobacillus acidophilus* on erythrocytic antioxidant enzyme activity: The level of total antioxidant (TA) was higher ($P=0.007$) in T2 and T3 as compared to CON, whereas T1 level was intermediate. Due to supplementation of probiotic the activity of Super oxide dismutase (SOD) was significantly increased ($P<0.05$) in T1, T2 and T3 groups as compared to CON. While no effect ($P>0.05$) of probiotic supplementation was evident on the Glutathione peroxidase (GPx) and catalase (CAT) activity (Table 4). The probiotic have defence mechanisms against the damaging effects of reactive oxygen species (ROS) by involving both in enzymatic (SOD and catalase) and non-

enzymatic components. The lactic acid bacteria decreased the activity of ROS through the production of SOD that converts superoxide radicals to oxygen and hydrogen peroxide. Tunç *et al.* (2017) reported that the level of SOD were not affected from humate and probiotic additives whereas the additives decreased the GPx ($P>0.05$) level in brown swiss calves. Seifzadeh *et al.* (2016) reported that supplementation of medical plant mixture and a probiotic to calves enhanced the plasma anti-oxidant activity ($P<0.05$).

Effect of *Lactobacillus acidophilus* on plasma immunoglobulin level: The total plasma immunoglobulin (mg/mL) remained uninfluenced in all the groups. However, period-wise comparison indicated a significant ($P<0.05$) higher level on 90 days as compared to 30 days and in case of 0 day and 60 days no difference was seen as compared to 30 days and 90 days. Likewise, the plasma IgG levels remained similar ($P>0.05$) among the 4 groups. However, period-wise comparison indicated a significant ($P<0.05$) higher level on 0 day and 90 days as compared to 30 days and 60 days (Table 5). According to Besser and Gay (1994) at the time of birth, neonatal calves do not have immunoglobulin like IgG and IgA in their blood streams and maternal colostrum is important for their immunity and thus passive immunity is important for disease control of new born calves before the weaning periods. Galvão *et al.* (2005) reported that calves were characterized as having inadequate passive transfer of immunity because the concentrations of IgG in serum were below the recommended levels for adequate passive transfer of immunity (>1.0 g/dL).

Similar to the present study, Bakhshi *et al.* (2006) reported that due to probiotic supplementation, blood plasma immunoglobulin were not affected. Similarly, Brakefield *et al.* (2010) also reported that supplementation of probiotic had no effect on serum IgG level on calves. Kim *et al.* (2011) reported that after supplementation of

Table 3. Effect of dietary supplementation of *Lactobacillus acidophilus* on blood lipid profile of Murrah buffalo calves

Attribute	Dietary groups [†]				Period Mean	Significance [§]		
	CON	T1	T2	T3		T	P	T×P
<i>Total cholesterol (mg/mL)</i>								
0d	142.1±3.14	142.88±3.76	142.5±3.59	142.9±0.59	142.59±1.42	0.151	0.748	0.984
30d	141.7±4.04	144.38±2.66	145.1±2.26	145.1±1.77	144.05±1.34			
60d	140.7±6.08	145.80±2.85	146.7±1.87	145.8±1.24	144.74±1.72			
90d	138.7±5.94	146.10±1.84	147.4±2.04	147.2±2.44	144.83±1.79			
Average	140.8±2.33	144.79±1.36	145.4±1.24	145.2±0.84				
<i>HDL (mg/dL)</i>								
0d	83.42±5.59	84.32±2.30	85.4±1.99	84.7±2.73	84.44±1.62	0.991	0.739	0.966
30d	83.85±6.51	86.53±3.34	87.1±4.99	85.8±4.29	85.83±2.31			
60d	84.24±6.55	87.56±2.65	87.6±3.98	87.2±4.28	86.66±2.16			
90d	93.38±7.69	86.18±3.56	86.7±2.14	85.0±2.51	87.84±2.23			
Average	86.22±3.21	86.15±1.42	86.7±1.65	85.7±1.67				

[†]Basal diet with no supplementation (CON) or supplemented as *Lactobacillus acidophilus* T1 (100 g/calf/d), T2(200 g/calf/d), T3 (300 g/calf/d). ^{P,q}Means bearing different superscripts in a column (p,q) differ significantly ($P<0.05$). [§]Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

Table 4. Effect of dietary supplementation of *Lactobacillus acidophilus* on antioxidant activity of Murrah buffalo calves

Attribute	Dietary groups [†]				Period Mean	Significance [§]		
	Con	T1	T2	T3		T	P	T×P
<i>Total antioxidant (mmol/l)</i>								
0d	0.48±0.06	0.50±0.06	0.50±0.05	0.48±0.06	0.49 ^P ±0.03	0.007	0.007	0.849
30d	0.42±0.04	0.52±0.02	0.53±0.02	0.50±0.04	0.49 ^P ±0.02			
60d	0.50±0.07	0.55±0.02	0.60±0.03	0.60±0.04	0.56 ^{Pq} ±0.02			
90d	0.47±0.04	0.58±0.02	0.63±0.02	0.60±0.03	0.57 ^q ±0.02			
Average	0.47 ^a ±0.03	0.54 ^{ab} ±0.02	0.57 ^b ±0.02	0.55 ^b ±0.02				
<i>Super oxide dismutase (U/ml)</i>								
0d	5.35±0.40	5.37±0.46	5.35±0.37	5.37±0.52	5.36 ^P ±0.21	0.002	<0.001	0.435
30d	5.95±0.89	6.35±0.24	6.53±0.22	6.65±0.26	6.37 ^q ±0.24			
60d	6.74±0.35	7.84±0.21	7.99±0.37	8.26±0.22	7.71 ^r ±0.18			
90d	6.95±0.20	8.48±0.03	8.64±0.04	8.19±0.16	8.07 ^r ±0.15			
Average	6.25 ^a ±0.28	7.01 ^b ±0.29	7.13 ^b ±0.30	7.12 ^b ±0.29				
<i>Glutathione peroxidase (nmol/min/ml)</i>								
0d	5.01±0.18	5.27±0.47	5.25±0.53	5.28±0.33	5.20±0.19	0.071	1.650	0.213
30d	4.85±0.25	4.88±0.48	4.96±0.32	4.94±0.17	4.91±0.15			
60d	4.91±0.35	4.73±0.46	4.79±0.22	4.72±0.16	4.79±0.15			
90d	5.08±0.35	5.23±0.25	5.24±0.17	5.26±0.14	5.20±0.11			
Average	4.96±0.14	5.03±0.20	5.06±0.16	5.05±0.11				
<i>Catalase (nmol/min/ml)</i>								
0d	20.48±1.42	20.66±0.99	20.58±0.65	20.70±1.04	20.60 ^P ±0.50	0.909	<0.001	0.992
30d	19.12±1.16	19.53±1.61	20.68±0.60	20.46±0.67	19.95 ^P ±0.53			
60d	20.81±1.77	20.67±0.75	20.19±0.92	20.32±0.68	20.50 ^P ±0.52			
90d	22.17±0.57	23.43±1.47	23.16±0.92	22.76±0.92	22.88 ^q ±0.49			
Average	20.65±0.65	21.07±0.66	21.15±0.44	21.06±0.44				

[†]Basal diet with no supplementation (CON) or supplemented as *Lactobacillus acidophilus* T1 (100 g/calf/d), T2 (200 g/calf/d), T3 (300 g/calf/d). ^{P,q}Means bearing different superscripts in a column (p,q) differ significantly (P<0.05). [§]Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

Table 5. Effect of dietary supplementation of *Lactobacillus acidophilus* on immunity indices in Murrah buffalo calves

Attribute	Dietary groups [†]				Period Mean	Significance [§]		
	Con	T1	T2	T3		T	P	T×P
<i>Total immunoglobulin (mg/ml)</i>								
0d	61.66±25.17	62.89±25.67	62.66±25.58	62.79±25.63	62.50 ^{Pq} ±12.76	0.944	0.006	0.889
30d	62.23±25.41	60.53±24.71	61.34±25.04	62.79±25.63	61.72 ^P ±12.60			
60d	63.44±25.90	64.31±26.25	63.70±26.01	63.14±25.77	63.65 ^{Pq} ±12.99			
90d	63.68±26.00	64.40±26.29	64.41±26.29	64.09±26.16	64.14 ^q ±13.09			
Average	62.75±12.81	63.03±12.87	63.03±12.87	63.20±12.90				
<i>Plasma IgG (mg/mL)</i>								
0d	6.50±0.08	6.51±0.02	6.56±0.08	6.51±0.07	6.52 ^q ±0.03	0.826	<0.001	0.991
30d	5.67±0.05	5.68±0.06	5.71±0.05	5.70±0.03	5.69 ^P ±0.02			
60d	5.76±0.17	5.72±0.07	5.72±0.07	5.73±0.03	5.73 ^P ±0.05			
90d	6.55±0.05	6.67±0.06	6.68±0.05	6.68±0.10	6.65 ^q ±0.03			
Average	6.12±0.10	6.15±0.10	6.16±0.10	6.16±0.10				

[†]Basal diet with no supplementation (CON) or supplemented as *Lactobacillus acidophilus* T1 (100 g/calf/d), T2 (200g/calf/d), T3 (300 g/calf/d). ^{P,q}Means bearing different superscripts in a column (p,q) differ significantly (P<0.05). [§]Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

probiotic in calves, the total serum IgG and IgA decreased up to 20d and thereafter increased in the calves of both control as well as supplemented group. Supplementation of probiotic in calves significantly improved serum IgG concentration in probiotic treated groups as compared to control group (Al-Saiady 2010). Hong *et al.* (2005)

hypothesized that addition of probiotic, prebiotic and synbiotic to milk would stimulate an increase in plasma IgG concentration as an anti-spore immune response in animal. However, Morill *et al.* (1995) showed that supplementation of probiotic in calves had no effect on IgG levels. Riddell *et al.* (2010) observed that supplementation

of probiotic had no significant effect on plasma IgG1 concentration of pre ruminant calves. Roodposhti and Dabiri (2012) reported that plasma IgG1 concentration was higher in synbiotic and prebiotic treatments but the difference was not significant.

The results of the present study showed a positive effect on energy metabolism reflected in the higher plasma glucose level due to the feeding of the *L. acidophilus* as fermented milk. The antioxidant capacity of the animals improved as the total antioxidant level and the super oxide dismutase level increased. Therefore, the use *L. acidophilus* is effective for maintaining the health status and performance of the neonatal Murrah buffalo calves.

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