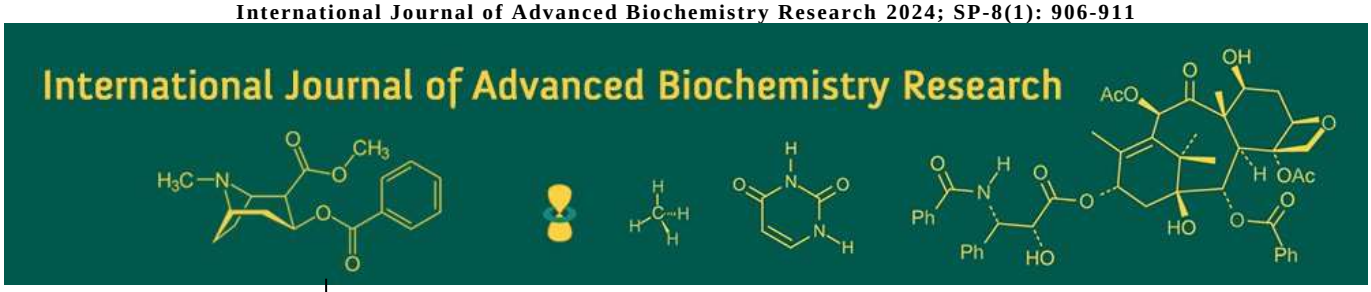

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(1): 906-911
www.biochemjournal.com
Received: 23-10-2023
Accepted: 26-12-2023

Kavipriya Jayswal
Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

LC Chaudhary
Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Anju Kala
Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Khushboo Panwar
Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Pooja
Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Corresponding Author:
Kavipriya Jayswal
Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Assessing the impact of gut-origin probiotic supplementation on haemato-biochemical and antioxidant profiles in neonatal murrah buffalo calves

Kavipriya Jayswal, LC Chaudhary, Anju Kala, Khushboo Panwar and Pooja

DOI: https://doi.org/10.33545/26174693.2024.v8.i1Sl.476

Abstract
This study explores the less investigated aspects of probiotics, focusing on their influence on haemato-biochemical and antioxidant profiles. These profiles encompass blood markers and biochemical parameters, offering insights into overall physiological health. The study specifically evaluates the impact of the gut-derived probiotic *Pediococcus pentosaceus* on haemato-biochemical and antioxidant profiles in neonatal Murrah buffalo calves over a four-month trial period.
The experiment involved two groups, control and treatment, with probiotic supplementation to the latter. Haematological and serum biochemical parameters were assessed at 0 and 4 months of feeding trial. The probiotic, *P. pentosaceus* RM119, was administered at 10^8 CFU/calf/day. Various haematological parameters, serum biochemical and erythrocytic antioxidant profile were investigated. Statistical analysis revealed no significant impact on haematological parameters. Serum biochemical profiles remained within normal ranges, indicating the overall well-being of experimental animals. Erythrocytic antioxidant glutathione showed an increase, suggesting a potential positive influence on antioxidant defenses. The study concludes that *P. pentosaceus* RM119 supplementation is safe and may offer health benefits in buffalo calves, warranting further exploration in animal health and welfare.

Keywords: Antioxidant profiles, buffalo calves, erythrocytic antioxidant, glutathione, haemato-biochemical, *Pediococcus pentosaceus*, probiotics

Introduction
In recent times, there has been an increasing focus on exploring the potential health advantages associated with probiotics. These are live microorganisms that provide beneficial effects to the host when administered in sufficient quantities. While much attention has been focused on their role in gut health and immune function, emerging research suggests that probiotics may also exert a notable influence on haemato-biochemical and antioxidant profiles within the body. Haemato-biochemical parameters encompass a range of blood and biochemical markers, providing valuable insights into the overall health and functioning of various physiological systems. The hematobiochemical profile encompasses parameters like the count of red blood cells, white blood cells, levels of hemoglobin, and various biochemical parameters such as glucose, cholesterol, and liver enzymes. Understanding how probiotics impact these parameters is crucial for unraveling their potential therapeutic applications beyond digestive health.
Moreover, the antioxidant profile, which involves the body's defense against oxidative stress, is gaining prominence due to its connection with numerous chronic diseases. Oxidative stress results from a disparity between the generation of reactive oxygen species and the body's capacity to counteract them, resulting in damage to cellular structures. Probiotics, with their ability to modulate the gut microbiota and produce bioactive compounds, are being investigated for their potential role in enhancing antioxidant defenses and mitigating oxidative stress.
This exploration into the effects of probiotics on haemato-biochemical and antioxidant profiles holds promise for advancing our understanding of the intricate interplay between the gut microbiota and systemic health. As we delve deeper into this emerging field of research, the implications for preventive and therapeutic interventions in various health conditions

become increasingly apparent. Given this context, the current investigation sought to assess the impact of supplementing with a gut derived probiotic *P. pentosaceus* on haemato-biochemical and antioxidant profiles, shedding light on potential avenues for future research and clinical applications.

Material and Methods

Experimental Animal and Housing

The research was conducted on neonatal Murrah calves at the Cattle and Buffalo Farm of the LPM section, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India. Twelve unweaned Murrah buffalo calves were divided into two groups, consisting of six calves each- designated as the control (C) and treatment (T) groups. The grouping was done randomly, considering their body weight, within a completely randomized model. The trial spanned four months, during which all calves adhered to standardized, uniform routine practices. Throughout the experimental phase, the calves were housed individually in well-ventilated, clean, and dry concrete pens. During the trial period, the calves were nourished with whole milk. From the second week onwards, calf starter and roughage were made available ad-libitum. The treatment group received a freshly formulated probiotic supplement at a dose of 10^8 CFU/calf/d for the entire 4 months duration. It was ensured that the animals consumed the complete volume of the probiotic supplement.

Estimation of blood haemato-biochemical constituents

Blood samples, amounting to 6 ml per animal, were collected from the jugular vein at intervals of 0 and 4 months of feeding trial. A quantity of 2 ml from the sample was allocated for hematology, utilizing vials with sodium fluoride (NaF) at a rate of 0.1 ml per tube. An additional 2 ml from the sample was dedicated to serum biochemistry, collected in vials containing clot activator. To facilitate serum separation, the tubes were inclined for a duration of 4 to 5 hours, and the resultant clear serum was carefully extracted via pipette. The serum samples underwent comprehensive analysis for diverse biochemical constituents, encompassing total protein, albumin, SGPT, SGOT, and glucose. Diagnostic kits from CREST BIOSYSTEMS, a subdivision of Coral Clinical Systems in Goa, India, were employed for these analytical procedures. The remaining 2 ml of the blood sample was transferred to a microfuge tube along with 300 µl of ACD (acid citrate dextrose) for the evaluation of the erythrocytic antioxidant profile.

Haematocrit and Blood biochemical profile

Haematocrit profile was assessed using the Haematology Analyser by Clindiag device B.V.A (Cat.# HA-22/20/Vet) as per manufacturers' protocol. Blood for serum biochemistry was taken to the laboratory without disturbing the clots, and was centrifuged for 15 min at 3000 rpm to retrieve serum and preserved the serum for further estimations at -20 °C in sterilized microfuge tubes (2 ml). The serum samples were subjected to analysis of glucose, total protein, albumin, SGOT, and SGPT using the guidelines provided with diagnostic kits (Coral Clinical Systems) by spectrophotometric methods.

Serum glucose: The quantification of serum glucose employed the glucose oxidase (GOD) and peroxidase method (POD). In this process, glucose undergoes oxidation by the enzyme glucose oxidase, resulting in the formation of gluconic acid and hydrogen peroxide. The liberated oxygen is then detected by the chromogen system, leading to the production of a red-colored quinonimine compound through a subsequent peroxidase-catalyzed reaction. The intensity of the developed red color was measured at 505 nm, and the magnitude of the color is directly proportional to the concentration of glucose in the blood serum (expressed in mg/dl).

Total protein: The Biuret (Gornall *et al*., 1949) [4] procedure was used to measure serum total protein (TP). In an alkaline medium, proteins bind to the cupric ions present in the biuret reagent to form a complex of blue-violet colour. The strength of the colour produced is directly correlated to the quantity of protein in the sample.

Albumin: Albumin was estimated by the BCG dye-binding method. Albumin binds with the dye bromocresol green in a buffered medium to form a green coloured complex. The strength of the colour produced is directly correlated to the quantity of albumin in the sample.

Globulin: The quantification of globulin involved determining the difference between the total protein and albumin concentrations in the serum. The results were expressed in grams per deciliter (g/dl).
Albumin to globulin ratio (A:G) ratio: A:G ratio was determined by dividing the amount of albumin in the serum by the globulin.

Aspartate aminotransferase (AST)/SGOT: Utilizing diagnostic kits supplied by Span Diagnostics, Surat, India, the determination of Aspartate Aminotransferase (AST) in the serum was conducted. AST facilitates the transfer of the amino group from L-aspartate to α-ketoglutarate, resulting in the synthesis of oxaloacetate and glutamate. The oxaloacetate formed reacts with NADH in the presence of Malate Dehydrogenase, generating NAD. The rate of oxidation of NADH to NAD is quantified as a reduction in absorbance, measured at 505 nm, and is directly proportional to the concentration of SGOT/AST in the sample. Alanine aminotransferase (ALT): The quantification of Alanine Aminotransferase (ALT) utilized a diagnostic kit manufactured by Span Diagnostics, Surat, India. ALT catalyzes the transfer of the L-alanine amino group to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The generated pyruvate reacts with 2,4-dinitrophenyl hydrazine to produce a brown-colored complex in an alkaline medium. The measurement of absorbance for the derivative of hydrazone, recorded at 505 nm, is indicative of ALT activity. This is determined by constructing a pyruvate calibration curve as a standard reference.

Erythrocytic antioxidant profile

Preparation of hemolysate: Blood samples collected in ACD buffer for oxidative stress index assays were subjected to centrifugation for 20 minutes at 2000 rpm in refrigerated conditions. The resulting packed erythrocytes were isolated, and the buffy coat (supernatant) was discarded. The packed erythrocytes underwent three washes with ice-cold phosphate-buffered saline (PBS) solution. These packed erythrocytes were employed for estimating reduced

glutathione (GSH). An erythrocytic suspension was prepared by mixing equal volumes of erythrocytes and normal saline solution (NSS). This suspension was utilized for assessing hemoglobin. For the estimation of reduced glutathione (GSH), 4.5 mL of stabilizing solution [comprising 0.5025 g EDTA (2.7 mM, pH-7.0) and β-Mercapto-ethanol (0.7 mM) with 24.55 µL in 500 mL distilled water] was combined with 0.5 mL of the erythrocytic suspension to form hemolysate. The resulting hemolysate and red blood cell (RBC) suspension were preserved at -70 °C for subsequent use in antioxidant assays. Erythrocytic haemoglobin concentration: Hemoglobin concentration in the erythrocyte suspension was determined spectrophotometrically using the Richterich (1969) [11] procedure.

Estimation of antioxidant indices

Reduced glutathione (GSH): Following the protocol outlined by Prins and Looss (1969) [10], the concentration of reduced glutathione (GSH) in packed erythrocytes was determined utilizing the DTNB method.

Catalase (CAT): Catalase activity in erythrocytes was assessed using the spectrophotometric method outlined by Bergmeyer (1983) [1].

Superoxide dismutase (SOD): The activity of superoxide dismutase (SOD) in RBC hemolysate samples was determined following the procedure described by Marklund and Marklund (1974) [6], utilizing Nitro blue tetrazolium (NBT) as a substrate. The method was adjusted with specific modifications recommended by Minami and Yoshikawa (1979) [7] after appropriate dilution.

Lipid peroxidation (LPO): The quantity of lipid peroxides in RBC hemolysate was determined using the method outlined by Placer *et al*., (1966) [9].

Statistical Analysis

The experimental data were subjected to analysis employing independent t-tests and two-way analysis of variance (ANOVA) using the statistical software IBM SPSS Statistics 20, following established statistical methodologies (Snedecor and Cochran, 1994) [13]. Treatment means were compared utilizing Tukey's test, with significance considered at the 5% level ($p<0.05$).

Results and Discussion

The impact of administering the gut-derived probiotic *Pediococcus pentosaceus* RM119 to pre-ruminant buffalo calves was assessed in terms haemato-biochemical and antioxidant parameters. For this, twelve buffalo calves (15 d old) were selected from LPM buffalo calf section, ICAR-IVRI and divided into two groups of six calves each. The calves of control group (C) were fed with milk plus basal diet without probiotic while, calves of the treatment (T) group were fed with milk plus basal diet along with probiotic @ 10^8 CFU/calf/d for 4 months of duration. The basal diet comprised of calf starter I with berseem fodder and calf starter II with maize fodder to make the diets isonitrogenous and isocaloric. The two fodders were used depending on the availability. The comprehensive results of the investigation are outlined herein.

Haematological parameters

Haematological parameters of experimental buffalo-calves are presented in Table 1. The mean Hb concentration was 12.86 and 12.64 in C, and T groups. Hb concentration did not vary ($p>0.05$) between the groups, whereas, the significant ($p<0.001$) effect of period was observed in both the groups. The mean PCV values (%) were 34.4 and 34.72 in C, and T groups with no variation ($p>0.05$). Although, the PCV (%) demonstrated significantly higher ($p<0.001$) values with the time progression. The mean values of RBC (millions/µl) were 8.60, and 8.80 in C, and T groups with no significant ($p>0.05$) interaction between the treatments and period. The mean values of TLC (1000/µl) were 13.32, and 13.53 in C, and T groups and were statistically similar ($p>0.05$) between the groups.

The lymphocyte count and monocyte count were found to be comparable ($p>0.05$) between C, and T groups. The granulocytes values were 4.41 and 4.91 in C and T groups depicting no difference between the two groups. The eosinophils count was found statistically lower ($p<0.05$) in T group (0.24) compared to C group (0.54). Further, in both the groups, the count was significantly higher ($p<0.05$) at 4 months of the study with respect to 0 day. The mean platelet count was 219.1, and 219.0 for C, and T group, and was statistically comparable ($p>0.05$). Platelet count was also similar ($p>0.05$) in respect of duration. Overall, the blood haematology observations indicated no impact of probiotic feeding and the calves of both the groups were clinically normal. Roodposhti and Dabiri (2012) [12] observed no notable variance in leukocyte count among Holstein calves administered commercially available probiotics.

Serum Biochemical Parameters

The serum biochemical profile provides valuable insights into health conditions, deficiency statuses, treatment evaluations, and dietary imbalances. The concentration of plasma proteins at any given time reflects hormonal equilibrium, nutritional status, water balance, and other health-related factors. The serum concentrations of total proteins, albumin, globulin, and the albumin-globulin ratio (A:G ratio) are presented in Table 2. The mean values of total proteins (g/dl) were 7.68 and 7.74 for C and T, with no statistically significant differences ($p>0.05$) between the groups. Total protein values exhibited significant variation ($p<0.05$) over the duration, with mean values of 7.66 and 7.96 at 0 day and 4 months. The mean serum albumin concentrations (g/dl) were 3.52 and 3.38 in C and T, with no statistical differences ($p>0.05$) between the groups and over time. The mean values of serum globulin (g/dl) were 4.15 and 4.35 for C and T, showing no significant difference ($p>0.05$) between the groups. However, serum globulin values exhibited significant variation ($p<0.05$) over the duration. The average A:G ratio was statistically similar between the groups but showed significant variation ($p<0.05$) with duration, having mean values of 1.31 and 3.15 at 0 day and 4 months. In both the C and T groups, total serum protein, albumin, globulin, A:G ratio, and glucose levels were within the physiological range. These findings align with Chaudhary *et al*., (2008) [2] and Ojha *et al*., (2020) [8], who reported comparable concentrations in control animals and those supplemented with probiotics. Frizzo *et al*., (2008) [3] observed similar results after supplementing probiotics in Holstein calves, where globulin significantly increased, indicating enhanced immunity. The mean glucose concentrations (mg/dl) exhibited no statistical differences ($p>0.05$) between the groups but showed significant changes ($p<0.05$) over time, with mean values of 101.75 and 109.44 at 0 day and 4 months, respectively.

Plasma glucose levels reflect the physiological state of the animals, and variations may be linked to carbohydrate metabolism.

Regarding SGOT and SGPT, probiotic supplementation had no significant effect. SGOT values were significantly lower at 4 months (105.1) compared to 0 day (145.2). SGPT values were higher at 0 d (59.91) and lower at 4 months (47.04).

Erythrocytic Antioxidant Profile

The results pertaining to the impact of probiotic supplementation on erythrocytic antioxidant indices in buffalo-calves are outlined in Table 3. The concentration of reduced glutathione (GSH) (mmol/mg Hb) was notably higher ($p<0.05$) in the treatment group (1.56) compared to the control (1.37). Additionally, the influence of the time period exhibited significantly higher ($p<0.01$) values at 4 months (1.76) in comparison to the baseline at 0 days (1.23). The interaction pattern revealed that the significant difference between the groups was notable only at the 4 months of the experiment. Higher activity of GSH in the *P. pentosaceus* supplemented group, is suggestive of prolonged GSH activity by a dietary probiotic of host origin. The super oxide dismutase (SOD) activity exhibited no significant variation ($p>0.05$) in control and treatment groups. Lipid peroxidase (LPO) and catalase (CAT) activities also followed the same trend with no ($p>0.05$) effect of probiotic supplementation also, the values were similar at different time periods. The probiotic exhibits defense mechanisms against the detrimental effects of reactive oxygen species (ROS), involving both enzymatic (SOD and catalase) and non-enzymatic components. Probiotics mitigate ROS activity by producing SOD, which transforms superoxide radicals into oxygen and hydrogen peroxide. Kutluyer *et al*., (2017) found that the level of SOD was not influenced by potassium humate and probiotic supplementation, while the level of GPx ($p>0.05$) was reduced by supplementation in brown Swiss calves. In a study by Ojha *et al*., (2020) [8], an increase in total antioxidant (TA) activity was observed in T_2 (*L. acidophilus* fermented milk at 200 mL/calf/day with 10^8 CFU/ml) and T_3 (*L. acidophilus* fermented milk at 300 ml/calf/day with 10^8 CFU/mL) compared to the control, with an intermediate level in T_1 (*L. acidophilus* fermented milk at 100 mL/calf/day with 10^8 CFU/mL). The activity of SOD was significantly higher in the T_1, T_2, and T_3 groups compared to the control, while the activities of catalase and glutathione peroxidase (GPx) remained consistent across all groups during the experimental period.

Table 1: Effect of dietary supplementation of probiotic on haematological profile of calves

Duration	Dietary groups		Period mean	Significance		
	Control	treatment		T	T	T*P
Haemoglobin (g/dL)						
0 day	10.22±0.22	10.48±0.50	10.36^{a}±0.28	0.913	<0.001	0.791
4 months	13.82±0.68	13.32±0.29	13.57^{b}±0.35			
Average	12.86±0.67	12.64±0.55				
Paked cell volume (%)						
0 day	34.64±1.37	34.30±1.47	34.45^{a}±0.97	0.7	0.001	0.839
4 months	32.00±1.06	32.36±1.35	32.18^{a}±0.81			
Average	34.40±0.84	34.72±0.94				
Red blood cells (millions/µl)						
0 day	8.65±0.28	8.41±0.37	8.52±0.23	0.905	0.687	0.918
4 months	8.86±0.62	8.89±0.63	8.87±0.43			
Average	8.60±0.24	8.80±0.27				
White blood cells(1000/µl)						
0 day	13.30±2.16	16.53±1.39	15.06^{b}±1.28	0.883	0.009	0.58
4 months	14.92±1.23	13.86±0.69	14.39^{b}±0.69			
Average	13.32±1.04	13.53±1.04				
Platelets(1000/µl)						
0 day	195.2±52.32	152.3±55.57	171.8±37.22	0.932	0.176	0.508
4 months	231.2±43.62	297.0±44.28	264.1±31.28			
Average	219.1±25.01	219.0±31.82				
Lymphocytes (1000/µl)						
0 day	7.94±1.06	8.98±1.19	8.51±2.62	0.506	0.171	0.343
4 months	12.84±3.30	9.16±0.46	11.00±5.33			
Average	9.70±1.37	8.73±0.49				
Monocytes (1000/µl)						
0 day	0.020±0.02	0	0.009^{a}±0.03	0.669	<0.001	0.158
4 months	0.300±0.07	0.200±0.12	0.250^{b}±0.14			
Average	0.121±0.04	0.093±0.11				
Eosinophils (1000/µl)						
0 day	0.24±0.11	0.25±0.13	0.25^{a}±0.08	0.013	0.002	0.008
4 months	1.06±0.20	0.28±0.08	0.67^{b}±0.16			
Average	0.54^{B}±0.13	0.24^{A}±0.03				
Granulocytes (1000/µl)						
0 day	5.10±1.31	7.30±0.83	6.30^{c}±0.79	0.711	0.001	0.112
4 months	4.72±0.47	4.18±0.47	4.45^{b}±0.33			
Average	4.41±0.53	4.91±0.66				

C: Basal diet devoid of probiotic supplementation; T: Basal diet enriched with probiotic @ 10^8 CFU/calf/d

AB…Means marked with distinct superscripts within a column exhibit significant differences ($p<0.05$)

abc…Means marked with different superscripts indicate significant differences ($p<0.05$)

Table 2: Effect of dietary supplementation of probiotic on serum biochemical profile of calves

Duration	Dietary groups		Period mean	Significance		
	C	P		P	D	P*D
Total protein (g/dL)						
0 day	7.52±0.17	7.80±0.26	7.66^{ab}±0.16	0.598	0.015	0.436
4 months	7.97±0.08	7.95±0.13	7.96^{b}±0.07			
Average	7.68±0.08	7.74±0.10				
Albumin (g/dL)						
0 day	4.40^{q}±0.16	4.02^{q}±0.16	4.20^{b}±0.12	0.157	0.277	<0.01
4 months	3.06^{p}±0.25	3.25^{p}±0.12	3.15^{a}±0.14			
Average	3.52±0.16	3.38±0.12				
Globulin (g/dL)						
0 day	3.12±0.25	3.79±0.30	3.45^{a}±0.20	0.263	<0.001	0.158
4 months	4.91±0.29	4.70±0.29	4.81^{b}±0.17			
Average	4.15±0.20	4.35±0.20				
A:G ratio						
0 day	1.51^{q}±0.19	1.12^{pq}±0.11	1.31^{b}±0.12	0.517	<0.001	0.044
4 months	4.15±0.20	4.35±0.20	0.68^{a}±0.05			
Average	1.20±0.11	1.14±0.09				
Glucose (mg/dL)						
0 day	102.54±4.45	100.95±5.17	101.75^{ab}±3.30	0.732	0.008	0.986
4 months	110.55±4.87	108.34±2.32	109.44^{b}±2.62			
Average	101.93±3.72	100.49±2.51				
SGOT (U/L)						
0 day	148.8±3.70	142.2±1.09	145.2^{b}±1.97	0.255	<0.001	0.004
4 months	108.2±3.12	102.0±4.24	105.1^{a}±2.66			
Average	110.1±5.86	118.7±5.11				
SGPT (U/L)						
0 day	65.79±1.25	55.02±2.0	59.91^{c}±2.06	0.918	<0.001	0.01
4 months	47.81±2.18	46.28±1.34	47.04^{b}±1.25			
Average	45.70±4.10	47.93±1.22				

C: Basal diet devoid of probiotic supplementation; T: Basal diet enriched with probiotic @ 10^8 CFU/calf/d
abc…Means marked with different superscripts indicate significant differences (p<0.05)

Table 3: Effect of dietary supplementation of probiotic on the erythrocytic antioxidant profile in buffalo-calves

Days	Dietary groups		Period mean	Significance		
	C	P		P	D	P*D
Reduced glutathione (mMol/mgHb)						
0	1.24^{p}±0.05	1.22^{p}±0.02	1.23^{a}±0.02	0.012	<0.001	0.014
4 months	1.52^{p}±0.13	2.01^{q}±0.05	1.76^{b}±0.10			
Average	1.37^{A}±0.06	1.56^{B}±0.10				
Superoxide dismutase (unit/mgHb)						
0	71.00±6.07	70.00±4.49	70.50±3.56	0.30	0.179	0.543
4 months	78.40±13.40	97.47±8.55	87.93±8.14			
Average	74.92±5.26	83.21±5.73				
Lipid peroxidase (nmol MDA/mgHb)						
0	2.22±0.09	2.26±0.20	2.24^{a}±0.10	0.137	0.045	0.629
4 months	2.55±0.14	2.96±0.28	2.75^{b}±0.16			
Average	2.39±0.09	2.64±0.14				
Catalase (unit/mgHb)						
0	65.24±0.58	62.96±1.87	64.10^{c}±0.99	0.144	<0.001	0.9
4 months	51.24±2.44	48.96±2.20	50.10^{a}±1.59			
Average	58.53±2.03	55.68±2.10				

C: Basal diet devoid of probiotic supplementation; T: Basal diet enriched with probiotic @ 10^8 CFU/calf/d
AB…Means marked with distinct superscripts within a column exhibit significant differences (p<0.05)
abc…Means marked with different superscripts indicate significant differences (p<0.05)

Conclusions

In conclusion, our study demonstrated that supplementing pre-ruminant buffalo calves with *Pediococcus pentosaceus* RM119 probiotic had no adverse effects on haemato-biochemical parameters. The haematological and serum biochemical profiles remained within normal ranges, reflecting the clinical well-being of the experimental animals. Notably, an increase in erythrocytic antioxidant glutathione suggests a potential positive impact on antioxidant defenses. These findings underscore the safety and potential health benefits of probiotic supplementation in buffalo calves, paving the way for further exploration in animal health and welfare.

References

1. Bergmeyer HU. Methods of Enzymatic Analysis. 1983;2:165-166.
2. Chaudhary LC, Sahoo A, Agarwal N, Kamra DN, Pathak N. Effect of direct fed microbials on nutrient utilization, rumen fermentation, immune and growth

response in crossbred cattle calves. Indian Journal of Animal Sciences. 2008;78:515-521.
3. Frizzo LS, Soto LP, Bertozzi E, Zbrun MV, Sequeira GJ, Santina DR, *et al*. The effect of supplementation with three lactic acid bacteria from bovine origin on growth performance and health status of young calves. Journal name not provided; c2008.
4. Gornall AG, Bardawill CJ, David MM. Biuret method of protein determination. Journal of Biochemical and Biophysical Methods (Poland). 1949;52:665-671.
5. Kutluyer F, Sirkeciodlu AN, Aksakal E, Aksakal FY, Tunc A, Gunaydin E. Effect of dietary fish oil replacement with plant oils on growth performance and gene expression in juvenile rainbow trout (*Oncorhynchus mykiss*). Annals of Animal Science. 2017;17:1135-1153.
6. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 1974;47:469-474.
7. Minami M, Yoshikawa H. Simplified assay method of SOD activity for clinical use. Clinica Chimica Acta. 1979;92:337-342.
8. Ojha L, Kumar S, Kewalramani N, Sarkar S, Singh AK, Tyagi AK. Effect of dietary supplementation of *Lactobacillus acidophilus* on blood biochemical profile, antioxidant activity and plasma immunoglobulin level in neonatal Murrah buffalo calves. Indian Journal of Animal Sciences. 2020;90:48-54.
9. Placer ZA, Cushman LL, Johnson BC. Estimation of production of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical Biochemistry. 1966;16:359-367.
10. Prins HK, Loos JA. Biochemical methods in red cell genetics. Academic Press NY; c1969. p. 115-137.
11. Richterich R. Clinical chemistry: Theory and practice. Karger, Basel (Switzerland) Academic Press. 1969:336-337.
12. Roodposhti PM, Dabiri N. Effects of probiotic and prebiotic on average daily gain, faecal shedding of *Escherichia coli*, and immune system status in newborn female calves. Asian-Australasian Journal of Animal Sciences. 2012;25(9):1255-1261.
13. Snedecor GW, Cochran WB. Statistical Methods. 8th Edn. Lowa State University Press, Iowa; c1994.
14. Sreedhar S, Sreenivas D. A study on calf mortality and managemental practices in commercial dairy farms. Livestock Research International. 2015;3:94-98.