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Species-specific probiotic *Lactobacillus johnsonii* CPN23 supplementation modulates blood biochemical profile and erythrocytic antioxidant indices in Labrador dogs

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ABSTRACT

The present investigation was conducted to study the blood biochemical profile and erythrocytic antioxidant indices in response to a canine-origin probiotic. Labrador adult female dogs (15) were distributed into 3 equal groups. Dogs in control group (CON) received a placebo MRS broth in the homemade basal diet as per the NRC requirements. In other 2 groups, dogs received either canine-origin probiotic (*Lactobacillus johnsonii* CPN23; cPRO) developed at our laboratory or dairy-origin probiotic (*Lactobacillus acidophilus* NCDC15; dPRO) in the same basal diet. The experimental duration was 13-weeks. The probiotics supplementation was discontinued after 9-weeks to study the withdrawal effects during the next 4-weeks. Blood was collected at 0, 30, 60 and 90 days of feeding and analyzed for various metabolites and antioxidants. The results indicated that the plasma glucose showed a reduction in both cPRO and dPRO groups vis-a-vis the CON group; however, total protein remained higher in both cPRO and dPRO as compared to CON. The total cholesterol was lower in cPRO and dPRO groups as compared to CON. The HDL/LDL ratio became wider in cPRO and dPRO groups in comparison to the CON. The erythrocytic lipid peroxidation and the levels of antioxidants including reduced glutathione, catalase and glutathione S-transferase remained unaltered by the probiotic use; however, the activity of superoxide dismutase and glutathione peroxidase was higher in cPRO. It is concluded that probiotic supplementation was effective besides having potential to augment the antioxidant status in dogs.

Key words: Antioxidant, Blood biochemistry, Canine-origin probiotic, *Lactobacillus johnsonii*

The use of probiotics, the live culture of host-friendly microbes which upon ingestion exert health benefits to the host, could be of great benefit to dogs who generally have a low microbial supply, concomitant low bacterial stimulation and interactions with the gut-associated lymphoid tissues together with incomplete digestion and altered barrier function allowing passage of large molecules and antigens (Guarner and Malagelada 2003, Strompfova *et al*. 2013). One of the most important criteria for the selection of probiotic is the host specificity (Ouwehand *et al.* 1999) because adhesion of probiotic is species-specific (Rinkinen *et al.* 2003). Native probiotic strains owing to well adaptation to the host ecological niche (O'Sullivan 2000) support the use of indigenous strains of probiotic for therapeutic strategies (Perelmuter *et al.* 2008).

Probiotics exhibit antioxidant activity in all major ways and also release and promote the production of major non-enzymatic antioxidant and free-radical scavenger glutathione (GSH). Moreover, they promote production of certain antioxidant biomolecules. Hence probiotics may have a potential therapeutic role in gastrointestinal disorders involving reactive oxygen species (Spyropoulos *et al.* 2011). However, there is a paucity of information, particularly of species-specific probiotics and antioxidant modulation in dogs. Keeping the above background in view, the present study was undertaken to ascertain the effects of the dietary supplementation of a probiotic of canine-origin on blood biochemical profile and antioxidant indices in Labrador dogs.

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MATERIALS AND METHODS

Animals, experimental design and diet: The study was approved by Institutional Animal Ethics Committee as well as CPCSEA. Adult healthy Labrador female dogs (15) were divided into 3 groups in randomized block design and fed on a pressure-cooked diet (Table 1) which was nutritionally adequate as per NRC (2006). The following treatments of the animals were arranged: (i) CON group received placebo (MRS broth, 0.1 mL/kg BW), (ii) cPRO groups received a overnight culture of canine-origin *Lactobacillus johnsonii CPN23* (0.1 mL/kg BW, 10^8 CFU/mL in MRS broth), (iii) dPRO groups received a overnight culture of dairy-origin

Lactobacillus acidophillus NCDC15 (0.1 mL/kg BW, 10^8 CFU/mL in MRS broth). Canine-origin probiotic was developed at our laboratory and identified as *Lactobacillus johnsonii* CPN23 (GenBank Accession Number - KP065494).

The pressure-cooked basal diet (containing rice 51%, Bengal gram 11%, soyben meal 32%, and soya oil 6%) was thoroughly mixed with mineral and vitamin premixes (each at 1g/head/day) (Kore *et al*. 2012) before feeding to individual dogs. All the dogs had 24 h access to *ad lib.* clean and freshwater. The diet of individual dog was divided in 2 equal portions and fed once in the morning (0900 h) and once in the evening (1700 h) while feeding of probiotics was done along with the morning feed. The experimental period was 13 weeks. However, probiotics supplementation was withdrawn after 9 weeks to ascertain the possible persistence of the probiotic-effects during the subsequent 4-weeks.

Blood biochemical indices: Blood samples were collected from the cephalic vein into anticoagulated vaccutainers from all the dogs at 0, 30, 60 and 90 days of the feeding trial to assess the blood biochemical profile and erythrocytic antioxidant indices.

Haemoglobin (Hb) in blood was determined by cyanmethemoglobin method (Dacie and Lewis 1968). Haematocrit of whole blood samples was estimated as per standard method using capillary centrifuge. Plasma concentrations of glucose, total protein, albumin, total cholesterol, triglycerides and high-density lipoprotein (HDL) were estimated by using diagnostic reagent kits in a UV-2601 double beam UV-vis spectrophotometer. The globulin value was calculated by subtracting the albumin from the total proteins. Very low-density lipoprotein (VLDL) was calculated as one-fifth of the triglycerides concentration. Low-density lipoprotein (LDL) was calculated as:

LDL= Total cholesterol – HDL – VLDL (Basoglu *et al*. 2002)

Erythrocytic antioxidant profile: Blood samples were collected in acid citrate dextrose buffer. The haemolysate and RBC suspension were prepared as per standard protocol (Choubey *et al*. 2015) and kept at –70°C and used for antioxidant assay. The concentration of reduced glutathione (GSH) in packed erythrocyte was estimated by 5,5-dithiobis-(2-nitro-benzoic acid; DTNB) method (Prins and Loos 1969). Catalase was assayed by the spectrophotometric method (Bergmeyer 1983). Superoxide dismutase (SOD) activity of the haemolysate samples was measured using nitro blue tetrazolium as a substrate after suitable dilution (Marklund and Marklund 1974) with certain modifications as suggested by Minami and Yoshikawa (1979). The lipid peroxides (malondialdehyde; MDA) was determined as per Placer *et al.* (1966). The glutathione S-transferase (GST) was estimated by monitoring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with GSH (Habig *et al*. 1974).

The experimental data generated were analyzed adopting repeated measures GLM procedure using the statistical software program SPSS.

RESULTS AND DISCUSSION

The basal diet contained 96.2% OM, 22.3% CP, 6.4% EE, 4.6% CF, 63.0% NFE, and 3.8% total ash, on DM basis.

Blood biochemical indices: Madubuike and Ekenyem

Table 1. Effect of source of probiotics on general blood metabolic profile of dogs

Attribute	Dietary groups†				Significance‡		
	Control	cPRO	dPRO	Period mean	T	P	T×P
Haemoglobin (g/dL)							
0d	13.97±0.17	14.02±0.23	14.16±0.98	14.05±0.31	0.032	0.200	0.810
30d	13.95±0.60	15.22±0.33	15.31±0.62	14.83±0.33			
60d	13.91±0.58	15.65±0.53	15.28±0.69	14.95±0.38			
90d	13.87±0.46	15.10±0.53	14.93±0.34	14.64±0.28			
Average	13.92^{a}±0.22	15.00^{b}±0.24	14.92^{b}±0.34				
Haematocrit (%)							
0d	52.20±2.03	51.60±1.29	50.80±2.54	51.53^{x}±1.09	0.441	<0.001	0.902
30d	54.80±2.08	57.80±1.77	53.80±1.98	55.47^{y}±1.14			
60d	56.60±2.11	57.40±1.60	53.80±0.97	55.93^{y}±0.96			
90d	58.00±1.95	59.60±3.52	58.60±0.93	58.73^{y}±1.29			
Average	55.40±1.06	56.60±1.23	54.25±1.03				
Glucose (mg/dL)							
0d	91.21±2.66	91.41±2.61	90.70±1.99	91.11^{z}±1.31	0.034	<0.001	0.248
30d	89.18±2.04	81.33±3.28	79.49±1.24	83.33^{x}±1.68			
60d	86.98±1.81	82.01±1.03	81.15±1.79	83.38^{x}±1.09			
90d	90.19±1.56	85.24±2.51	83.88±0.73	86.44^{y}±1.19			
Average	89.39^{b}±1.01	85.00^{a}±1.47	83.81^{a}±1.20				

†Basal diet supplemented with no probiotics (CON), probiotics of canine-origin (cPRO), and dairy-origin (dPRO); $^{abc/xyz}$Means bearing different superscripts in a row (*abc*) or column (*xyz*) differ significantly (P<0.05); ‡Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

Table 2. Effect of source of probiotics on liver function indices of dogs

Attribute	Dietary groups[†]			Period mean	Significance[‡]		
	Control	cPRO	dPRO		T	P	T×P
Total protein (g/dL)							
0d	6.61±0.14	6.53±0.29	6.61±0.13	6.58^{x}±0.11	0.052	<0.001	0.006
30d	6.60^{a}±0.04	7.41^{b}±0.09	7.44^{b}±0.13	7.15^{y}±0.12			
60d	6.79^{a}±0.12	7.35^{b}±0.11	7.33^{b}±0.16	7.16^{y}±0.10			
90d	6.60±0.08	6.66±0.23	6.71±0.09	6.66^{x}±0.08			
Average	6.65^{a}±0.05	6.99^{b}±0.13	7.02^{b}±0.10				
Albumin (g/dL)							
0d	3.29±0.13	3.15±0.15	3.25±0.09	3.23^{x}±0.07	0.289	0.008	0.487
30d	3.44±0.06	3.61±0.07	3.41±0.08	3.49^{y}±0.04			
60d	3.27±0.08	3.43±0.08	3.20±0.09	3.30^{x}±0.05			
90d	3.22±0.06	3.36±0.11	3.25±0.05	3.28^{x}±0.04			
Average	3.31±0.04	3.39±0.06	3.28±0.04				
Globulin (g/dL)							
0d	3.32±0.24	3.37±0.26	3.36±0.20	3.35±0.12	0.063	<0.001	0.030
30d	3.15^{a}±0.06	3.80^{b}±0.15	4.03^{b}±0.11	3.66^{y}±0.12			
60d	3.52^{a}±0.09	3.92^{b}±0.11	4.13^{b}±0.15	3.86^{y}±0.09			
90d	3.38±0.10	3.30±0.20	3.46±0.07	3.38^{x}±0.08			
Average	3.34^{a}±0.07	3.60^{ab}±0.11	3.74^{b}±0.10				
A:G ratio							
0d	1.02±0.11	0.96±0.10	0.99±0.08	0.99^{y}±0.05	0.148	0.029	0.304
30d	1.10±0.04	0.96±0.05	0.85±0.03	0.97^{y}±0.04			
60d	0.93±0.04	0.88±0.04	0.78±0.04	0.86^{x}±0.03			
90d	0.96±0.04	1.04±0.07	0.94±0.02	0.98^{y}±0.03			
Average	1.00±0.03	0.96±0.04	0.89±0.03				

[†]Basal diet supplemented with no probiotics (CON), probiotics of canine-origin (cPRO), and dairy-origin (dPRO); [abc/xyz]Means bearing different superscripts in a row (*abc*) or column (*xyz*) differ significantly (P<0.05); [‡]Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

(2006) stated that haematology and serum biochemistry assay suggests the physiological disposition of the animals to their nutrition. Determination of the serum biochemistry reflects the physiological responsiveness of the animals to its internal and external environment (Kumar *et al.* 2012).

General metabolic profile: The supplementation of the basal diet with either of the probiotics boosted (P<0.05) Hb level of the dogs as compared to CON. There were no variations noticed upon period-wise comparison (Table 1). An opposite picture was obtained in haematocrit indicating no effects (P>0.05) of the supplementation; however, period-wise average values were significantly (P<0.05) higher at d30, d45, d60 and d90 when compared against the d0 value (Table 2). Baillon *et al.* (2004) found significant increases in both haematocrit and Hb concentration in healthy adult dogs supplemented with probiotic *L. acidophilus* strain DSM13241.

The plasma level of glucose (Table 1) showed a reduction (P<0.05) in both cPRO and dPRO groups as compared to the CON group, with no apparent variation between the probiotic groups. Further, a significant reduction (P<0.001) in plasma glucose was also evident when period-wise comparison was made; the values at d30, d60 and d90 were lower than that of the 0d value. However, the d90 glucose level was comparatively higher (P<0.001) than those of d30 and d60 which could be attributed to the effects of withdrawal of the probiotics. Similar to present findings, dogs fed with probiotics (*L. fermentum* AD1) showed significant reduction of glucose in serum (Strompfová *et al.* 2006). Altered gut microbiota in response to probiotics supplementation could bring about alterations in select gut hormone secretion (Firouzi *et al.* 2013). Gut hormones play important role in regulating glucose homeostasis by controlling beta-cell growth and survival. Further, probiotics may help in augmenting the antioxidant system of beta-cells and improve glucose homeostasis by slowing down the insulin reduction (Yadav *et al.* 2008).

Liver function indices: The serum levels of total protein (Table 2) increased with probiotic supplementation, and there was no variation evident between the 2 probiotics. A significant interaction between treatment and period was evident on the plasma total proteins, resulting in higher plasma total proteins in dogs under cPRO and dPRO on d30 and d60; but these values were reduced and became at par with the CON values at d90, following the withdrawal of probiotic supplementation beyond d60. The serum albumin levels remained similar (P>0.05) among the 3 groups, however, period-wise comparison indicated a significant (P<0.05) higher albumin levels on d30 as compared to all other days of measurement. The probiotic supplementation raised (P=0.003) the plasma levels of globulin over the corresponding CON values on d30 and

d60 of the study; but with the withdrawal of the probiotics post-d60, these values dropped to become comparable to the CON values. A significant period effect shown for the plasma globulin was essentially a reflection of the trend noticed for the treatment × period interaction. There was no variations observed for the A:G ratio among the treatments, except for a significant (P<0.05) period effect. Strompfová *et al.* (2006) reported that dogs fed with probiotics showed significant increase in serum total protein compared to control dogs.

Blood lipid profile of dogs: The plasma total cholesterol levels (Table 3) reduced (P<0.05) because of probiotic supplementation in cPRO and dPRO groups as compared to CON accompanying a significant treatment and period interaction. The plasma levels of triglycerides and VLDL-cholesterol were significantly (P<0.05) lower in cPRO as compared to CON while that of dPRO was comparable to both. These observations supported the hypothesis that a probiotic of canine-origin may serve better when supplemented in the diet of dogs. While no influence (P>0.05) of the dietary intervention was apparent on the plasma HDL-cholesterol, a significant (P=0.053) interaction between treatment and period implied that there was a reduction in the HDL-cholesterol in cPRO and dPRO groups at d30 and d60 in relation to the CON values. However, these differences cease to persist at d90 following 4-weeks of withdrawal of the probiotics. The HDL/LDL ratio became wider (P=0.005) in cPRO and dRO groups as compared to the CON. Moreover, a significant treatment × period interaction for the HDL/LDL was indicative of the persistently wider (P=0.017) ratio in both the probiotic groups (versus CON) starting with 30d of supplementation,

Table 3. Effect of source of probiotics on blood lipid profile of dogs

Attribute	Dietary groups†			Period mean	Significance‡		
	Control	cPRO	dPRO		T	P	T×P
Total cholesterol (mg/dL)							
0d	222.28±4.00	225.84±4.01	225.56±3.80	224.56^{y}±2.15	0.002	<0.001	<0.001
30d	217.76^{b}±5.36	196.14^{a}±1.67	211.96^{b}±4.67	208.62^{y}±3.32			
60d	212.76^{b}±4.96	186.80^{a}±1.38	194.24^{a}±3.75	197.93^{x}±3.52			
90d	213.20^{b}±3.75	184.46^{a}±3.67	182.54^{a}±4.06	193.40^{x}±4.27			
Average	216.50^{b}±2.27	198.31^{a}±4.01	203.58^{a}±4.22				
Triglycerides (mg/dL)							
0d	40.82±0.50	39.79±2.23	43.28±1.09	41.30^{y}±0.88	0.036	<0.001	0.074
30d	40.95±2.38	32.13±1.17	34.04±1.93	35.71^{x}±1.43			
60d	38.38±1.48	32.88±1.48	38.22±2.22	36.49^{x}±1.17			
90d	37.12±2.91	35.04±1.80	33.28±1.28	35.15^{x}±1.20			
Average	39.32^{b}±1.01	34.96^{a}±1.04	37.21^{ab}±1.20				
HDL-cholesterol (mg/dL)							
0d	57.26±2.59	55.04±3.31	55.78±2.51	56.02^{x}±1.53	0.154	<0.001	0.053
30d	57.42^{a}±2.26	68.04^{b}±3.81	70.14^{b}±3.64	65.20^{y}±2.31			
60d	58.60^{a}±1.09	67.43^{b}±2.02	65.93^{b}±3.29	63.99^{y}±1.61			
90d	60.71±1.55	65.51±2.37	63.03±4.44	63.08^{y}±1.71			
Average	58.50±0.96	64.00±1.82	63.72±2.02				
VLDL- cholesterol (mg/dL)							
0d	8.16±0.10	7.96±0.45	8.66±0.22	8.26^{y}±0.18	0.035	<0.001	0.074
30d	8.19±0.48	6.43±0.23	6.81±0.39	7.14^{x}±0.29			
60d	7.68±0.30	6.57±0.30	7.64±0.44	7.30^{x}±0.23			
90d	7.42±0.58	7.01±0.36	6.66±0.26	7.03^{x}±0.24			
Average	7.86^{b}±0.20	6.99^{a}±0.21	7.44^{ab}±0.24				
LDL-cholesterol (mg/dL)							
0d	156.86±2.31	162.82±4.79	161.14±5.31	160.27^{y}±2.41	<0.001	<0.001	<0.001
30d	152.16^{b}±6.26	121.66^{a}±3.89	135.06^{ab}±7.48	136.29^{y}±4.65			
60d	146.48^{b}±5.40	112.78^{a}±3.12	120.66^{a}±4.41	126.64^{x}±4.51			
90d	145.06^{b}±3.30	111.96^{a}±5.81	112.88^{a}±2.47	123.30^{x}±4.66			
Average	150.14^{b}±2.37	127.31^{a}±5.21	132.44^{a}±4.85				
HDL:LDL							
0d	0.36±0.02	0.34±0.03	0.35±0.02	0.35^{x}±0.01	0.005	<0.001	0.017
30d	0.38^{a}±0.03	0.56^{b}±0.05	0.53^{b}±0.05	0.49^{y}±0.03			
60d	0.40^{a}±0.02	0.60^{b}±0.03	0.55^{b}±0.04	0.52^{y}±0.03			
90d	0.42^{a}±0.01	0.59^{b}±0.05	0.56^{b}±0.05	0.52^{y}±0.03			
Average	0.39^{a}±0.01	0.52^{b}±0.03	0.50^{b}±0.03				

†Basal diet supplemented with no probiotics (CON), probiotics of canine-origin (cPRO), and dairy-origin (dPRO); $^{abc/xyz}$Means bearing different superscripts in a row (*abc*) or column (*xyz*) differ significantly (P<0.05); ‡Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

Table 4. Effect of source of probiotics on erythrocytic non-enzymatic antioxidant profile of dogs

Attribute	Dietary groups†			Period mean	Significance‡		
	CON	cPRO	dPRO		T	P	T×P
Lipid peroxidation (nmol MDA/mg Hb)							
0d	4.46±0.30	4.62±0.30	4.30±0.23	4.46^{y}±0.15	0.347	<0.001	0.238
30d	3.78±0.12	3.46±0.02	3.30±0.23	3.51^{x}±0.10			
60d	4.69±0.28	4.51±0.09	4.52±0.32	4.57^{y}±0.14			
90d	4.78±0.19	4.10±0.23	4.94±0.27	4.61^{y}±0.16			
Average	4.43±0.14	4.17±0.14	4.27±0.18				
Reduced glutathione (nmol/mg Hb)							
0d	5.37±0.35	5.52±0.32	5.25±0.27	5.38^{z}±0.17	0.334	<0.001	0.978
30d	3.64±0.19	3.75±0.17	3.64±0.08	3.68^{x}±0.08			
60d	4.15±0.32	4.38±0.20	3.93±0.22	4.15^{y}±0.14			
90d	3.64±0.38	3.97±0.14	3.62±0.15	3.74^{xy}±0.14			
Average	4.20±0.22	4.40±0.18	4.11±0.18				

†Basal diet supplemented with no probiotics (CON), probiotics of canine-origin (cPRO), and dairy-origin (dPRO); $^{abc/xyz}$Means bearing different superscripts in a row (*abc*) or column (*xyz*) differ significantly (P<0.05); ‡Significant effects of dietary treatment (T), period (P) or their interaction (T×P).

which persisted till the end of the withdrawal period, i.e., at d90. Products of bacterial fermentation, specifically SCFAs, may inhibit cholesterol synthesis in the liver and/or cause the mobilization of plasma cholesterol to the liver (Pereira and Gibson 2002). Some gastrointestinal bacteria may also prevent cholesterol absorption by deconjugating bile salts that then affect cholesterol metabolism. However, some lactobacillus has direct effect on cholesterol through its assimilation and removal from the growth medium (Fuller 1989).

Erythrocytic antioxidant indices

Non-enzymatic indices: The data on the non-enzymatic antioxidants in the erythrocytes of the experimental dogs is presented in Table 4. There was no effect of the probiotic supplementation observed on the MDA levels (indicative of the extent of lipid peroxidation) and GSH concentrations. However, period-wise comparison showed a significant (P<0.001) effect. The findings are supported by Aluwong *et al.* (2013) who observed no significant (P>0.05) difference in MDA levels in broiler chickens fed diet supplemented with yeast probiotic. Castex *et al.* (2009) reported no significant influence of probiotic supplementation on the GSH contents.

Enzymatic indices: The activity of SOD was higher (P<0.05) in cPRO compared to dPRO, and the level observed in CON was intermediate and comparable to both, indicative of the superiority of the canine-origin *L. johnsonni* CPN23 over the dairy origin probiotic (Fig. 1). The effect of period was also found to be significant (P<0.05) for SOD, with the values recorded on d30 and d60 being higher (P<0.05) than the corresponding values on d0 and d90. This, in turn, indicates that the positive stimulus of the probiotics on the SOD activity diminished following 4-weeks of withdrawal. There was also a significant (P=0.002) treatment × period interaction observed for SOD. The activity of GPx (Fig. 2) exhibited a significantly (P<0.05) higher value in cPRO as compared

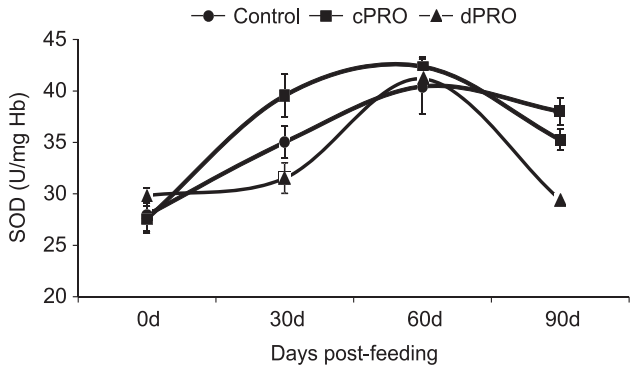

Fig. 1. Periodic changes in erythrocytic superoxide dismutase activity of dogs supplemented with probiotics of canine-origin (cPRO), and dairy-origin (dPRO) as compared to the non-supplemented control (CON). Significance: T, 0.037; P<0.001; T×P, 0.002.

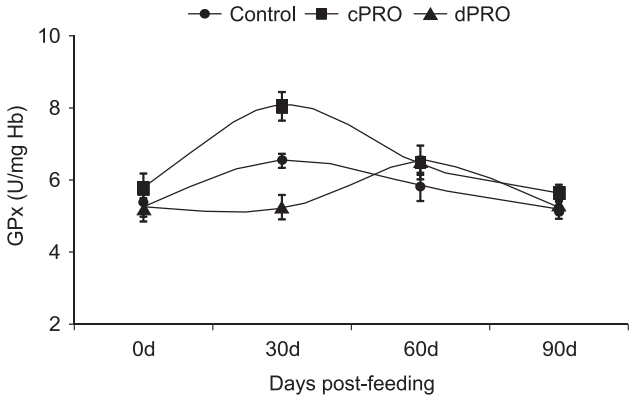

Fig. 2. Periodic changes in erythrocytic glutathione peroxidase activity of dogs supplemented with probiotics of canine-origin (cPRO), and dairy-origin (dPRO) as compared to the non-supplemented control (CON). Significance: T, 0.001; P<0.001; T×P, 0.006.

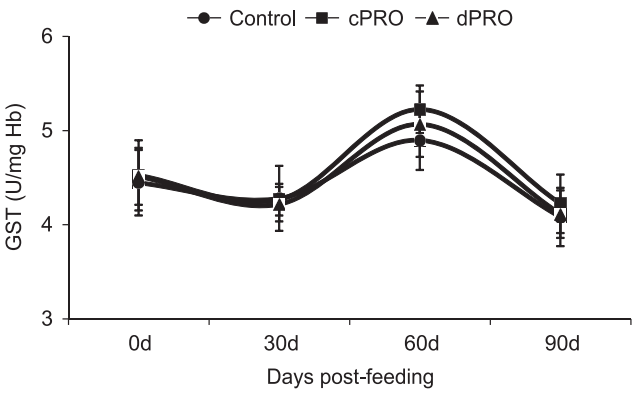

Fig. 3. Periodic changes in erythrocytic glutathione S-transferase activity of dogs supplemented with probiotics of canine-origin (cPRO), and dairy-origin (dPRO) as compared to the non-supplemented control (CON) [Significance: T=0.001; P<0.001 and T×P=0.006].

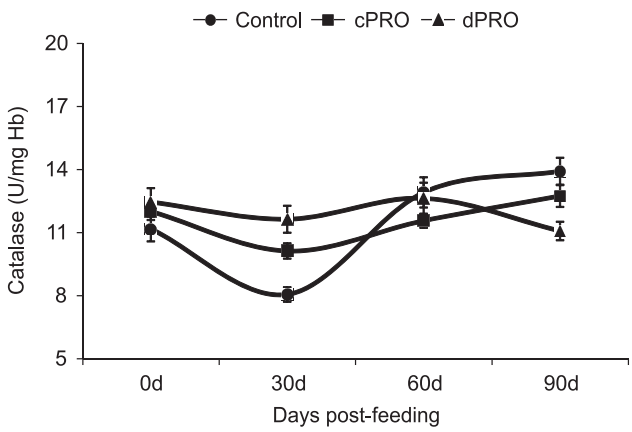

Fig. 4. Periodic changes in erythrocytic catalase activity of dogs supplemented with probiotics of canine-origin (cPRO), and dairy-origin (dPRO) as compared to the non-supplemented control (CON) [Significance: T=0.554; P<0.001 and T×P<0.001].

to dPRO as well as CON. Further, there was a significant (P=0.006) interaction between treatment and period evident for the GPx activity. These present findings are in agreement with Ghoneim and Moselhy (2013) who had observed elevated SOD and GPx activities in probiotics-administrated rabbits. There was no effect of probiotic supplementation noticed on the activity of GST (Fig 3). However, a period-wise comparison indicated a higher (P<0.05) value on d60 compared to all other days of assessment. While no effect (P>0.05) of probiotic supplementation was evident on the erythrocytic catalase activity (Fig. 4), significant (P<0.001) interaction between treatment and period was observed. Similar to present findings, Capcarova *et al.* (2010) while evaluating the effects of two probiotic strains *Lactobacillus fermentum* CCM 7158 and *Enterococcus faecium* M 74 in broiler chickens observed that the antioxidant status in both probiotic groups was significantly increased.

These results indicated that supplementation of the probiotics was effective in raising the antioxidant status and metabolic profile of the dogs. Additionally, the present results are also indicative of the superiority of canine-origin probiotic (cPRO) over the dairy-origin (dPRO) one, as far as its application for canines is concerned.

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