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Comparative effect of two probiotic lactic acid bacterial strains on nutrient digestibility and health status in geriatric dogs[#]

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Abstract

Similar to humans, the population of elderly companion animals is increasing. As dogs age, they experience diminished physical capabilities and metabolic changes, necessitating specialised senior care. Although the use of probiotics as functional food in animal feed is rising, research specifically focusing on geriatric dogs remains limited. This study evaluated the impact of two probiotic strains for their potential on nutrient utilisation and improving health among senior dogs. Fifteen neutered, medium-sized, non-descript geriatric dogs were randomly assigned to three equal groups and fed a basal pelleted diet (CP-25.5%, ME-3100 Kcal/kg) either without probiotics (control; T1) or supplemented with the probiotic strain Lactobacillus johnsonii (10^9 CFU)(T2) or Enterococcus faecium ($3x10^8$ CFU)(T3) of canine origin for 30 days. A three-day digestibility trial was conducted starting from the 28th day. Blood samples were collected on days 0 and 27 to assess haematological parameters. After the 30-day supplementation period, probiotics were discontinued and selected parameters were measured on day 40 to assess the persistence of the effect by the organisms. The study results indicated no significant variations ($p > 0.05$) in voluntary food intake between the groups. While the digestibility of most macronutrients remained consistent across groups, fibre digestibility improved significantly ($p=0.008$) in dogs receiving either of the probiotic strain. Additionally, both period-wise and treatment-wise values assessed on 0th, 27th and 40th days were similar across the group with respect to haematological parameters such as haemoglobin level, haematocrit values, differential leucocyte counts and total leucocyte counts. The supplementation L. johnsonii reduced serum glucose level but the persistence effect after the withdrawal of the probiotic was not evident. Overall, the Lactobacillus strain demonstrated superior effects compared to the control and enterococcus in geriatric dogs.

Keywords: *Geriatric dog, probiotics, Escherichia coli, Clostridium, Lactobacillus johnsonii, Enterococcus faecium*

The bond between humans and their animal companions dates back thousands of years, with evidence such as 12,000-year-old domesticated dog fossils found in Iraq and Northern Israel suggesting that this special relationship had ancient roots (Marples, 1981). As dogs age, they experience physical decline and metabolic changes, creating a need for specialised senior care, including tailored pet foods for prevention of age-related ailments (Fahey *et al*., 2008). Kuzmuk *et al.* (2005) observed reduced duodenal villous surface area and jejunal villi height in older dogs, potentially leading

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to decreased nutrient digestibility. Additionally, Bhatti *et al.* (2006) reported a reduced hormonal response to ghrelin, the appetite-stimulating peptide, in older dogs, contributing to a decline in appetite. The National Research Council (2006) noted that canines in domestic environments require approximately 95* $BW^{0.75}$ kilocalories of energy due to limited exercise and stimulation.

The health of companion animals, like their owners, is closely tied to their gut microbiota, which shares similarities with human microbiomes (Grześkowiak *et al*., 2015; Coelho *et al*., 2018). The gut microbiome is a key metabolic organ that transforms dietary substances and medications into bacterial metabolites, and its composition changes with age, notably with a decline in lactobacilli in older dogs (Mitsuoka, 2011; You and Kim, 2021). Benyacoub *et al.* (2003) stated that dietary modifications are a factor that affects the intestinal microbiota of dogs, and probiotics could offer benefits in such conditions. Yang and Wu (2023) highlighted that probiotics commonly employed in functional foods and dietary supplements belong to species and strains within Lactobacillus, Bifidobacterium, Enterococcus and Saccharomyces genera. While research on the impact of probiotics on dogs metabolic status is limited, this study aims at comparing the impact of supplementation of two probiotic strains *Lactobacillus johnsonii* (T2) and *Enterococcus faecium* (T3) on nutrient digestibility and haemato-biochemical parameters, in geriatric dogs.

Materials and methods

Fifteen healthy dogs aged from seven to ten years were selected from a reputed NGO, locally and housed at the Animal Production Shed of College of Veterinary and Animal Sciences, Mannuthy. All the animals were dewormed and vaccinated a week before introduction to kennel. The kennel was also disinfected and cleaned thoroughly to prevent the presence of ectoparasites.

Experimental Diets

A basal pelleted ration with 25 per cent crude protein and 3100 Kcal energy was prepared and fed to all the animals for a period of one week as adaptation. The probiotics *Lactobacillus johnsonii* ACC0035 (10^9 CFU/g) and *Enterococcus faecium* ACC0080 ($3x10^8$ CFU/g) of canine origin were procured from Anthem Biosciences Ltd., Bangalore and stored as prescribed. The dogs were divided into three groups of five animals randomly and the first group was fed on the basal ration (T1) and in the second group the basal diet was coated with one gram each of probiotic *Lactobacillus johnsonii* ACC0035 (10^9 CFU/g) daily to each dog (T2) and the third group was fed with the basal ration coated with 1 gram of *Enterococcus faecium* ACC0080 ($3x10^8$ CFU /g) daily to each dog (T3). The feeding trial was conducted for a period of 30 days. After 30 days of feeding the probiotic supplementation was discontinued and all the groups received the same basal diet for the next ten days to study the persistent effect of probiotics.

Table 1. Experimental diets of dogs during the first 30 days of the study

Treatment	Experimental diets
T1	Control
T2	Control + *Lactobacillus johnsonii* (10^9 CFU/g) (1g/day/dog)
T3	Control + *Enterococcus faecium* ($3x10^8$ CFU /g) (1g/day/dog)

FFeed was offered according to each dog's body weight to fulfill nutritional requirements as per NRC, 2006 (old dog with least activity) guidelines. All the dogs were fed in the evening (at 5 pm) and clean water was made available *ad libitum.* Any leftover feed was collected and weighed after each feeding, and its moisture content was analysed to calculate dry matter intake. Daily data on the quantity of feed consumed by each dog was recorded. The body weights of experimental animals were recorded at weekly intervals.

Digestion trial

A digestibility trial for three days duration was carried out towards the end of the feeding trial by total collection method (from day 28). The faeces voided by each animal were collected individually, uncontaminated with urine, feed residue, or dirt on a continuous 24-hour basis. The daily samples were kept in double-lined polyethylene bags and stored in deep freezer (-20ºC) for further analysis. During the digestion trial, quantities of daily feed offered, feed residue left and faeces voided per animal were recorded and their dry matter content was analysed. The proximate composition of feed and faeces was done as per the standard procedure (AOAC, 2016). The apparent digestibility coefficient of dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) were calculated based on the analytical data.

Blood analysis

Whole blood from all animals was collected on days 0, 27 and 40th day of the feeding trial. Six mL f blood was drawn under aseptic conditions and serum was separated for glucose analysis (GOD-PAP method) using diagnostic kits (Agappe Diagnostics Ltd. Kerala) Haematological analysis was conducted using whole blood.

Statistical analyses

All the data collected were analysed using a completely randomised design through ANOVA with the general linear model (GLM) procedures in SPSS for

Windows, version 24.0 (SPSS Inc., Chicago, IL, USA). The results from the digestion trials, body weight and average daily intake were evaluated using one-way ANOVA. The serum glucose was analysed using ANOVA for repeated measures, incorporating the variables of treatment (T), period (P) and T*P.

Results and discussion

The chemical composition of the basal diet showed on a dry matter basis 91.18% organic matter (OM), 25.5% crude protein (CP), 3.2% ether extract (EE), 4.54% crude fibre (CF), 57.94% nitrogen-free extract (NFE) and 8.82% total ash and the data is presented in Table 2. The dry matter content observed was 97.5% found to be similar as reported by Sachin (2014). The percentage composition of feed for adult dog in terms of crude fibre, nitrogen-free extract, total ash, calcium, and phosphorus were consistent with the findings of Akhil (2022), while the ether extract percentage aligned with the results from Abinaya (2019).

Table 2. Chemical composition (%) of basal diet based on dry matter.

Nutrient[1]	Percentage
Dry matter	97.50±0.07
Organic matter	91.18±0.09
Crude protein	25.50±0.04
Crude fibre	4.54±0.11
Ether extract	3.20±0.05
Total Ash	8.82±0.07
Nitrogen free extract	57.94±0.1

[1] Values expressed on a DM basis, an average of six values

Body weight

The weekly body weight recorded was not significant throughout the period of study and the data is presented in Table 3. The initial body weights of the dogs in all three groups were comparable, showing 16.46±1.17, 16.45±1.39 and 16.54±0.63 kg and the final weights were 16.52±1.15, 16.68±1.33 and 17.02±1.09 kg in control, Lactobacillus and Enterococci groups, respectively and all the animals maintained normal body weight throughout the study. Similar studies in healthy dogs documented similar bodyweights between the groups throughout the study (Baillon *et al.,* 2004; Sachin, 2014; Stefano *et al.,* 2020).

Average daily intake

The mean weekly dry matter intake for the three groups across the period of study is depicted in Table 4 and was statistically similar (p>0.05) between groups, aligned with the findings by Sachin (2014) and Kore *et al.* (2010), who observed no change in dry matter intake in young Labrador dogs provided with probiotics. Kore *et al.* (2012) also reported similar findings, where the intake of DM and various nutrients were comparable between dogs fed with or without probiotics.

Table 3. Weekly average body weights of dogs maintained on experimental diets, kg

Body weight (Kg)	Dietary groups			SEM	P- value
	T1	T2	T3		
Week 1	16.46±1.17	16.45±1.39	16.54±0.98	1.19	0.998^{ns}
Week 2	16.45±1.17	16.62±1.38	16.84±1.02	1.20	0.974^{ns}
Week 3	16.47±1.15	16.6±1.39	16.96±1.06	1.21	0.957^{ns}
Week 4	16.48±1.13	16.64±1.33	17.02±1.10	1.19	0.947^{ns}
Week 5	16.52±1.15	16.68±1.33	17.04±1.10	1.20	0.952^{ns}
Week 6	16.62±1.01	16.69±1.23	17.00±1.01	1.15	0.959^{ns}

ns- Non significant (P>0.05)

Table 4. Weekly average dry matter intake of dogs maintained on experimental diets

Parameters	Dietary groups			SEM	P- value
	T1	T2	T3		
Average dry matter intake (Grams)					
Week 1	209.63±10.46	206.7±12.86	206.7±12.86	12.11	0.981^{ns}
Week 2	206.70±10.86	208.46±12.80	226.2±14.59	12.84	0.513^{ns}
Week 3	209.82±10.55	216.45±11.29	236.73±12.17	11.36	0.257^{ns}
Week 4	210.01±10.12	217.21±11.04	236.73±12.17	11.12	0.251^{ns}
Week 5	213.73±9.14	219.32±9.69	236.73±12.17	10.53	0.293^{ns}
Week 6	216.45±9.07	222.3±9.45	236.73±12.17	10.32	0.389^{ns}

ns- Non-significant (P>0.05)

Table 5. Apparent digestibility coefficient of nutrients of the experimental rations, Per cent

Parameters	Dietary groups			p-value
	T1	T2	T3	
Dry matter				
Intake (g)	209.82±10.55	216.45±11.29	236.73±12.17	0.257^{ns}
Digested (g)	159.12±9.09	163.89±9.49	182.38±8.54	0.201^{ns}
Digestibility (%)	75.73±0.60	75.62±0.48	77.11±0.68	0.185^{ns}
Crude protein				
Intake (g)	53.48±2.69	55.17±2.88	60.34±3.1	0.257^{ns}
Digested (g)	43.62±2.65	45.04±2.65	49.2±2.84	0.352^{ns}
Digestibility (%)	81.38±0.96	81.55±0.89	81.43±0.57	0.989^{ns}
Crude fibre				
Intake (g)	9.53±0.48	9.84±0.51	10.76±0.55	0.257^{ns}
Digested (g)	3.72±0.33	4.44±0.32	4.71±0.29	0.109^{ns}
Digestibility (%)	38.74^{a}±1.61	45.02^{b}±0.97	43.7^{b}±0.97	0.008
Ether extract				
Intake (g)	6.71±0.34	6.93±0.36	7.58±0.39	0.257^{ns}
Digested (g)	5.77±0.34	5.93±0.37	6.48±0.32	0.344^{ns}
Digestibility (%)	85.77±0.84	85.48±0.9	85.55±0.91	0.97^{ns}
Nitrogen free extract				
Intake (g)	121.56±6.11	125.4±6.54	137.15±7.05	0.257^{ns}
Digested (g)	103.46±5.37	107.46±6.04	115.03±5.94	0.387^{ns}
Digestibility (%)	85.09±0.31	85.62±0.56	83.87±0.53	0.064^{ns}

ns- Non-significant (p>0.05), Mean of different treatments having a to c alphabets as superscripts within a row differ significantly

Table 6. Effect of experimental diet on general metabolic profile

Parameters	Dietary groups			Period mean	Significance		
	T1	T2	T3		T	P	T*P
Haemoglobin (g/dL)							
0 d	14.50±0.26	14.48±0.37	14.58±0.11	14.52±0.16	0.058	0.059	0.301^{ns}
27 d	14.66±0.29	15.24±0.12	15.86±0.33	15.25±0.15			
40 d	14.64±0.27	15.18±0.08	15.74±0.30	15.19±0.14			
Average	14.60±0.30	14.97±0.30	15.39±0.30				
Haematocrit (%)							
0 d	43.56±0.82	43.34±1.13	44.26±1.11	43.72±0.87	0.069	0.061	0.184^{ns}
27 d	43.86±0.81	45.78±0.35	47.56±1.04	45.73±0.86			
40 d	43.40±0.81	45.56±0.42	47.20±1.11	45.39±0.88			
Average	43.61±0.92	44.89±0.92	46.34±0.92				
WBC ($x10^3$ cells per µL)							
0 d	9.10±0.95	10.10±1.00	9.06±0.78	9.42±0.83	0.576	0.052	0.160^{ns}
27 d	9.65±0.70	11.34±1.16	10.36±0.91	10.45±0.74			
40 d	9.61±0.65	10.94±1.14	10.26±0.85	10.27±0.82			
Average	9.45±0.89	10.79±0.89	9.89±0.89				
Glucose (mg/dL)							
0 d	103.14±2.90	102.05^{B}±7.11	102.49±0.91	102.56^{B}±2.58	0.263	<0.001	<0.001
27 d	101.32^{b}±2.26	89.36^{aA}±2.42	101.16^{b}±1.02	97.28^{A}±1.16			
40 d	100.42±2.67	96.99^{B}±1.00	100.41±0.34	99.27^{B}±0.96			
Average	101.63±2.53	96.13±2.53	101.36±2.53				

ns- Non-significant (p>0.05), Means of different treatments having a to c alphabets as superscripts within a row differ significantly, Means of different periods having A to C alphabets as superscripts within a column differ significantly

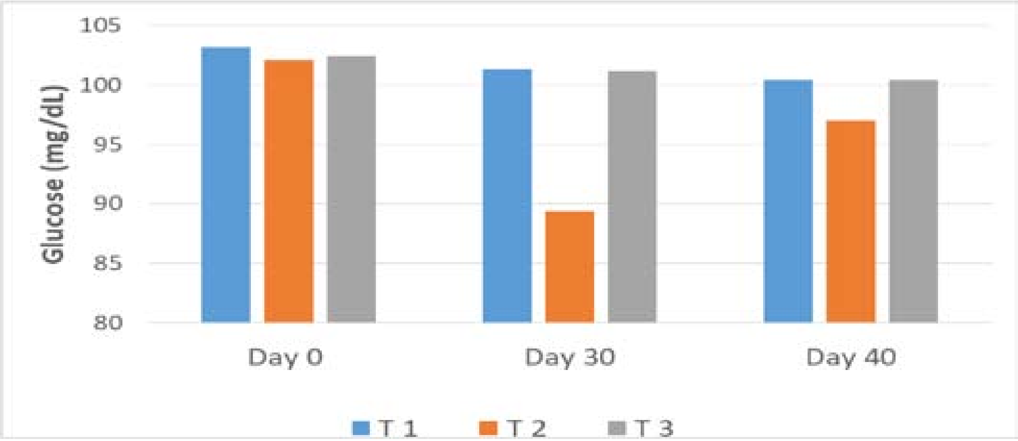

Fig 1. Effect of experimental diet on serum glucose

Digestibility coefficients

The average nutrient intake, nutrient digested and the digestibility coefficients of nutrients (Table 5) showed a significant difference in crude fibre digestibility ($p<0.05$) while other components were non-significant among the three groups. Swanson *et al*. (2002) observed improved DM and OM digestibility in dogs supplemented with *Lactobacillus acidophilus* compared to the control diet. Maxwell *et al.* (1983) and Hong *et al*. (2002) noted positive effects on nutrient digestibility with probiotics containing lactobacillus species. However, Gonzalez-Ortiz *et al.* (2013) investigated the effects of supplementing *Bacillus amyloliquefaciens* CECT 5940 and *Enterococcus faecium* CECT 4515 in the diet of healthy dogs and observed that the supplementation did not result in any significant change in the digestibility coefficients of nutrients. Crude fibre digestibility was significantly higher ($p<0.05$) in the T2 group (45.02±0.97 %) and T3 group (43.7±0.97 %) compared to the T1 group (38.74±1.61 %). The observed improvement in crude fibre digestibility in this study might be attributed to the increased fermentability of undigested fibre, with the probiotics in the hindgut. Supporting these findings, El-Gaafary *et al.* (1992), Kore *et al.* (2010) and Kumar *et al.* (2017) reported similar positive effects of probiotics on crude fibre digestibility.

Haematobiochemical parameters

The haemoglobin, haematocrit, total WBC counts and differential leucocyte count of dogs were estimated in the blood collected on days 0, 27 and 40 (Table 6) and there was no impact on hematological parameters by the probiotic supplementation. However, the average serum glucose value on the 27th day was significantly reduced ($p<0.01$) in T2 (89.36±2.42 mg/dL) compared to T3 (101.16±1.02 mg/dL) and T1 (101.32±2.26 mg/dL) groups, respectively. Additionally, in the period-wise comparison, a significant decrease ($p<0.01$) in serum glucose level was observed on day 27 compared with values on day 0 and day 40 in the T2 group.

Similar to the result above, Allenspach *et al*. (2023) also observed that administering probiotics did not significantly impact the CBC values. Conversely, Kumar *et al.* (2016) noted supplementation of probiotics *L. johnsonii* resulted in elevated levels of haemoglobin. Baillon *et al.* (2004) had also observed a notable rise in both haematocrit and hemoglobin levels in healthy adult dogs given the probiotic *L. acidophilus* strain DSM13241. The inclusion of *L. johnsonii* in the diet resulted in a significant reduction ($p<0.05$) in serum glucose levels in the present study and is aligning with the previous findings where dogs fed with the probiotic *L. johnsonii* showed a decrease in serum glucose (Strompfová *et al.,* 2006; Kumar *et al.,* 2016). Probiotics have been linked to improved glucose metabolism. Specific probiotics enhanced glucose and insulin tolerance, potentially through modifications in gut microbiota and energy metabolism (Kang *et al.,* 2024). The results of this study clearly showed that probiotic supplementation can effectively lower glucose levels.

Overall, the results showed significant improvement in crude fibre digestibility in dogs receiving either probiotic strain compared to the control. Additionally, *Lactobacillus johnsonii* reduced serum glucose levels, although this effect did not persist after discontinuation of the probiotics. Haematological parameters, such as haemoglobin and leucocyte counts, remained consistent across all groups. Overall, *Lactobacillus johnsonii* demonstrated a superior effect in improving nutrient utilisation and regulating glucose levels compared to *Enterococcus faecium.*

Conclusion

Fifteen, seven to ten year old dogs were divided into three groups where the first group (T1) was fed with the basal diet alone, the second group (T2) was supplemented with *Lactobacillus johnsonii* (10^9 CFU) and the third group (T3) was supplemented with *Enterococcus faecium* ($3x10^8$ CFU)to the basal diet. Analysis of the experimental data showed that weekly average body weight and weekly average daily DM intake were similar (p>0.05) among the treatment groups. The digestibility coefficient of crude fibre significantly improved in the treatment groups whereas the digestibility of DM, NFE, CP and EE were comparable between treatment groups. Haematological values such as haemoglobin, DLC, haematocrit and total counts were also similar across the groups. Dietary inclusion of lactobacillus significantly decreased the glucose levels on day 27 of the feeding trial. It is concluded that the probiotics improved the fibre digestibility without affecting the intake or body weight of the animals.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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