Received: 3 May 2019 | Accepted: 4 September 2019

DOI: 10.1111/jvim.15625

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE

Effect of probiotic *Enterococcus faecium* SF68 on liver function in healthy dogs

Rosario Lucena | **Manuel Novales** | **Beatriz Blanco** | **Eduardo Hernández** | **Pedro José Ginel**

Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine of Córdoba, Campus of Rabanales, Córdoba, Spain

Correspondence
Rosario Lucena, Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine of Córdoba, Campus of Rabanales, Ctra Madrid-Cádiz, km 396, 14014 Córdoba, Spain.
Email: pv2lusor@uco.es

Abstract

Background: Probiotics are widely used in dogs but can be associated with alterations in some serum biochemistry test results.

Objective: To assess the effect of *Enterococcus faecium* SF68 administration for 14 days on serum alanine transferase (ALT) and alkaline phosphatase (ALP) activity and total cholesterol and triglyceride concentrations in healthy dogs.

Animals: Thirty-six healthy privately owned neutered dogs were randomly allocated, stratified by sex, to control or probiotic groups. Dogs were clinically healthy, with normal physical examination findings, blood, urine, and fecal analyses and ultrasonographic examinations.

Method: In this blinded, controlled study *E. faecium* SF68 was administered to the probiotic group for 14 days. Blood samples were taken from all dogs at days 0, 14, and 28. Serum ALT and ALP activity and total cholesterol and triglyceride concentrations were determined on these 3 days.

Results: The probiotic induced no significant changes in mean ALT and ALP activity. Mean cholesterol concentration did not change during probiotic administration but a significant decrease was seen on day 28 ($P < .01$). Mean triglyceride concentration increased progressively, becoming significant at day 28 ($P < .05$), with 1 dog developing hypertriglyceridemia.

Conclusions and Clinical Importance: *E. faecium* SF68 would not create confusion when monitoring dogs with hepatobiliary disease because ALT and ALP activity did not change significantly. A significant decrease in cholesterol and significant increase in triglyceride concentrations were seen at day 28 but were not clinically relevant, with 1 dog showing hypertriglyceridemia. A longer trial is warranted to assess if the probiotic effects could be clinically relevant and to assess its potential use in hypertriglyceridemic dogs.

KEYWORDS

dog, *enterococcus*, liver function, probiotic

List of Abbreviations: ALP, alkaline phosphatase; ALT, alanine transferase; AST, aspartate transferase; CIBDAI, canine inflammatory bowel disease activity index.

This article was published online on 2 October 2019. Errors were subsequently identified in Figures 1 and 2. This notice is included in the online version to indicate that has been corrected 11 October 2019.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2019 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

1 | INTRODUCTION

Probiotics are lactic acid bacteria such as *Enterococcus*, *Lactobacillus*, and *Bifidobacterium*,[1,2] that confer a health benefit on the host when administered in adequate amounts.[2] Probiotics may modulate the immune system, protect from enteropathogen infections, and enhance growth and development in dogs. They are advocated for treatment and prevention of gastrointestinal disorders and obesity.[2,3] Studies in mice, humans, and dogs have addressed the role of the gut microbiota in fat storage. Germ-free mice with no microbiota have 42% less body fat than do normal mice.[4] More *Firmicutes* and fewer *Bacteroidetes* have been found in obese mice and humans compared to their healthy counterparts.[5,6] In dogs, the phylum *Actinobacteria* and the genus *Roseburia* were more abundant in the intestinal microbiota of obese dogs than in lean dogs.[7] Obesity and hyperlipidemia are associated with hepatobiliary disease.[8] Thus, strategies to modify the gut microbiota would be indicated to decrease fat absorption and improve liver function in animals and humans. In human patients, probiotics represent a therapeutic alternative to improve liver function and serum cholesterol concentrations.[9] In obese dogs, probiotics seem to be able to restore the intestinal microbiota and modulate serum lipid concentrations,[2,10] improving the prognosis of patients with liver diseases.[3,11-14]

Hepatobiliary alterations can be assessed by determining serum hepatic enzyme activities. In dogs, serum alanine aminotransferase (ALT) is the most specific liver enzyme activities to detect hepatocyte membrane damage and necrosis, whereas serum alkaline phosphatase (ALP) increases with biliary stasis, corticosteroid administration, and bone lesions.[15,16] Cholesterol and triglycerides represent the main serum lipids in dogs.[15] Cholesterol participates in forming cell membranes and to bile acid and steroid hormone metabolism. Triglycerides constitute the most important energy reserve in fat tissue of animals. In dogs, serum total cholesterol and triglyceride concentrations depend on liver synthesis but also on regulatory hormones and dietary intake.[17]

Few studies have considered the effect of probiotics on liver function in dogs.[3,11-14,18] Reported results and conclusions can be controversial, because of the use of different species and strains that may exert variable biological effects, as reported in humans[10,19,20] and dogs.[2,11,21,22] Furthermore, these probiotics were neither standardized nor commercially available to other investigators.[3,11-14,18]

Enterococcus faecium SF68 is a commercially available probiotic product approved for use in dogs. It has been reported to induce variations in serum folate and cobalamin concentrations in healthy dogs,[23] but its effect on liver function tests has not been studied.

Our aim was to assess the potential effect of *E. faecium* SF68 (Fortiflora; Proplan Purina, Spain) on serum activity of ALT and ALP and on serum total cholesterol and triglyceride concentrations when administered for 14 days to healthy dogs.

2 | MATERIALS AND METHODS

A randomized, blinded, controlled study was performed using serum samples obtained from dogs as a separate part of a previously published study about the effect of *E. faecium* SF68 on serum cobalamin and folate concentrations in healthy dogs.[23] Briefly, 36 healthy privately owned neutered dogs were enrolled in the study. Dogs were of different breeds of similar age (range, 2 to 5 years) that had not shown any clinical signs in the previous 12 months as reported by the owners. Physical examination findings, CBC, routine serum biochemistry profile, urinalysis, and 3 fecal examinations (direct smear observation and flotation techniques) at 48-hour intervals were normal. No abdominal abnormalities were detected by ultrasonographic examination in any of the dogs. For practical and ethical reasons, intestinal biopsy samples for cytological or histological examination were not taken.

Dogs were allocated, stratified by sex, to a control (18 dogs; 9 females, 9 males) or a probiotic group (18 dogs; 10 females, 8 males) using a computer random number generator set to generate numbers between 1 and 36. Randomization was stratified by sex to produce a balanced distribution of males and females between groups. When a group was completed (9 males and 9 females), the remaining unassigned animals were allocated to the other group.

A balanced diet for adult dogs (Brekkies, Affinity Petcare, SA, Spain)[23] was given to all dogs twice daily for 2 weeks before the study and during the study to eliminate the effect of diet on serum biochemical variables[8,24-26] and gut microbiota composition.[2] Owners having >1 dog living in the same house were instructed to feed the dogs separately. *E. faecium* SF68 NCIMB 10415 (5×10^8CFU/g; Fortiflora; Proplan Purina, Spain) was given PO to dogs in the probiotic group for 14 days. One sachet (1 g) of the probiotic was sprinkled daily over each dog's morning meal, as recommended by the manufacturer. No other PO supplement or treatment was allowed in the probiotic or control groups during the study.

After an 18-hour fast,[23] a blood sample was taken by venipuncture from each dog at 3 different time points: day 1 (when starting the probiotic administration), day 14 (when the probiotic administration ended), and day 28 (2 weeks later). All serum samples were harvested immediately and frozen at −20°C until analyzed.

Serum ALT and ALP activity and serum total cholesterol and triglyceride concentrations were determined at each time point using a COBAS 6000 analyzer and specific reagents (Hitachi-Roche Diagnostic). Serum ALT activity was determined by an absorbance technique. To determine hepatic ALP activity, serum of each dog was heated at 55°C as described,[15] and then ALP activity was measured using a colorimetric method. Serum total cholesterol and triglyceride concentrations were determined using a colorimetric method. All samples were run blindly and in duplicate using the same assay. The means of both results were calculated for each parameter.

Statistical analyses were performed using Prism 5.04 for Windows (GraphPad software, San Diego, California). Normal distribution of the data was assessed by the Kolmogorov-Smirnov test. A 2-way repeated measures (mixed model) analysis of variance (ANOVA) and Bonferroni multiple comparisons tests were performed to statistically compared serum ALT and ALP activity and serum total cholesterol and triglyceride concentrations within and between groups. The Grubbs' test for outliers was performed for all data sets (https://www.graphpad.com/quickcalcs/Grubbs1.cfm). A value of $P \leq .05$ was considered significant.

TABLE 1 Descriptive statistics for serum activity of ALT (IU/L) and ALP (IU/L) in the probiotic and control groups at days 1, 14, and 28 of the study

ALT						
	Probiotic group			Control group		
Days	1	14	28	1	14	28
Mean ± SD	33.67 ± 12.23	34.39 ± 14.40	34.50 ± 16.13	29.69 ± 4.30	30.22 ± 3.99	30.01 ± 2.80
Range	22.00 to 62.00	19.00 to 81.00	19.00 to 87.00	23.00 to 39.00	24.10 to 38.90	25.40 to 35.20
Out ref. range	0	dog #9	dog #5	0	0	0
ALP						
Mean ± SD	46.56 ± 23.33	49.28 ± 32.49	55.56 ± 33.25	47.33 ± 25.81	48.39 ± 20.94	45.06 ± 19.01
Range	17.00 to 108.0	8.00 to 154.0	14.00 to 129.0	23.00 to 137.0	26.00 to 115.0	23.00 to 109.0
Out ref. range	0	dog #2 (outlier)	0	0	0	0

Note: Dogs out of reference range and outliers have been included for each variable at the different time points. Range, minimum to maximum values; Out ref. range, dogs out of reference range.

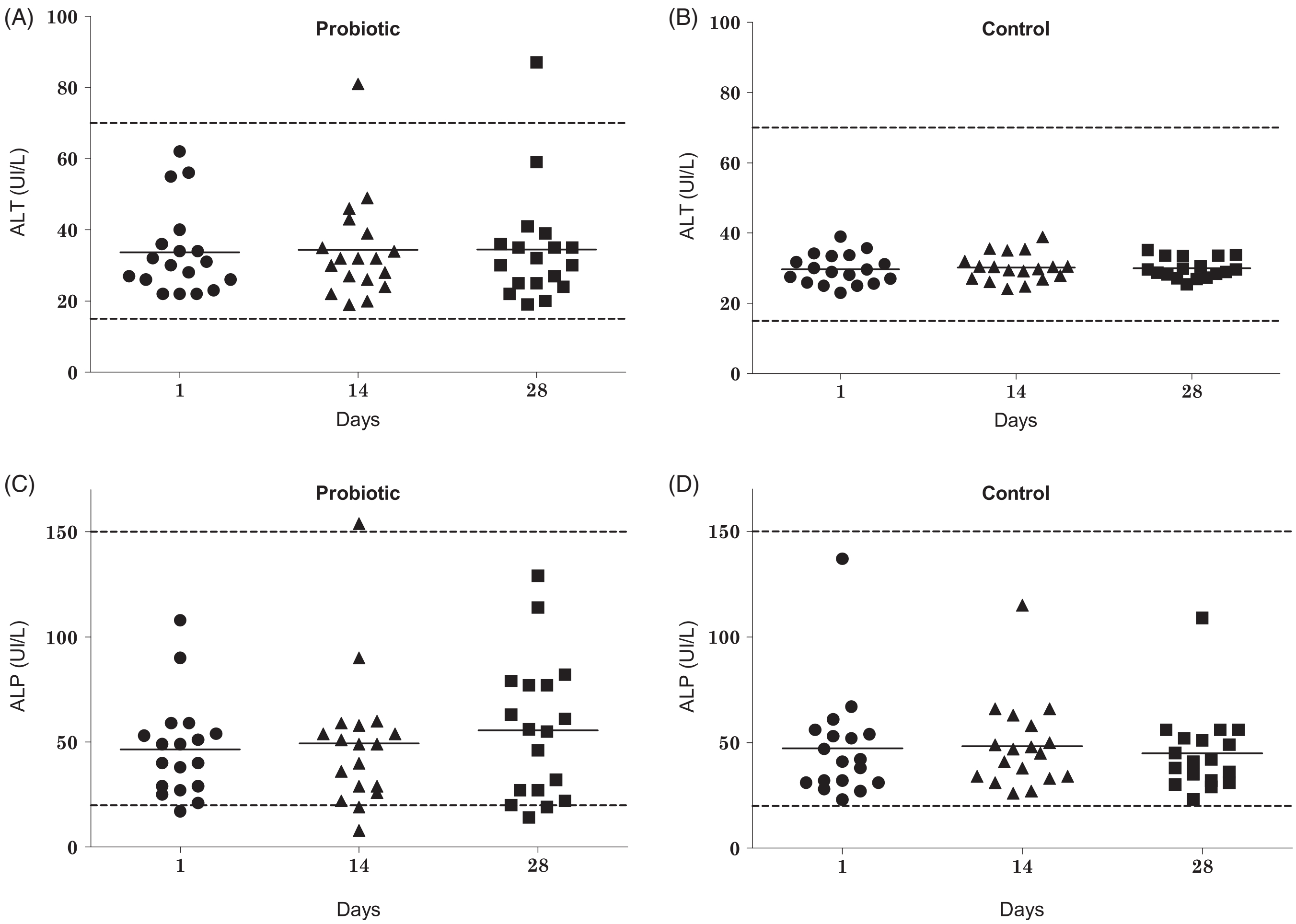

FIGURE 1 Scatter plot of serum ALT (IU/L) and ALP (IU/L) in the probiotic, A,C, and control, B,D, groups of dogs at days 1, 14, and 28. Solid lines: mean serum concentrations. Dotted lines: reference range of each variable (ALT: 15-70 IU/L; ALP: 20-150 IU/L). (GraphPad software, San Diego, California)

In all dogs at the 3 time points, ultrasonographic examination was performed to evaluate abdominal organs. The canine inflammatory bowel disease activity index (CIBDAI)[27] also was scored to document any clinical sign or adverse effect at these 3 time points.

3 | RESULTS

Serum baseline ALT and ALP activity and serum total cholesterol and triglyceride concentrations were within the reference range in all dogs in the probiotic and control groups. In dogs of both the probiotic and control groups, ultrasonographic examination of the abdomen was normal at the 3 time points. Similarly, all dogs had a null CIBDAI score from the beginning to the end of the study. None of the dogs showed alterations in general attitude, appetite, weight, consistency or frequency of feces, and no adverse effects were observed.

The Grubbs' test showed that, in dog 2 in the probiotic group, results for ALP activity and triglyceride concentration at time points 14 and 28, respectively, were outliers. These values were not omitted because they were not caused by incorrect measurements and may have represented an idiosyncratic response in this dog. Moreover, the results of the statistical analysis did not change when the results of dog 2 were excluded.

The repeated measures mixed-model ANOVA found no significant interaction between time and probiotic administration levels; differences were significant ($P < .05$) at time level (within group comparison) and not significant at probiotic administration level (between group comparison).

In the probiotic group, mean serum ALT activities were not significantly different when comparing day 1 with day 14 and 28 ($P > .05$; Table 1). Dog 9 had ALT activity higher than the reference range at day 14 (81 IU/L) but activity returned to normal at day 28 (35 IU/L; Figure 1A). In the control group, mean ALT activities were not significantly different at the 3 time points of the study ($P > .05$; Table 1). In all control dogs, ALT activity remained within the reference range (15-70 IU/L; Figure 1B).

TABLE 2 Descriptive statistics for serum concentrations of total cholesterol (mg/dL) and triglycerides (mg/dL) in the probiotic and control groups at days 1, 14, and 28 of the study

Total cholesterol	Probiotic group			Control group		
Days	1	14	28	1	14	28
Mean ± SD	191.8 ± 36.00	198.2 ± 33.92	185.4 ± 29.64	192.1 ± 27.03	195.0 ± 32.63	194.1 ± 34.76
Range	143.0 to 294.0	129.0 to 263.0	135.0 to 245.0	156.0 to 259.0	156.0 to 301.0	149.0 to 298.0
Out ref. range	0	0	0	0	0	0
Triglycerides						
Mean ± SD	59.39 ± 14.65	61.17 ± 11.03	69.00 ± 20.96	56.39 ± 9.61	53.67 ± 12.26	57.56 ± 13.22
Range	38.00 to 85.00	46.00 to 89.00	51.00 to 145.00	45.00 to 79.00	32.00 to 82.00	35.00 to 80.00
Out ref. range	0	0	Dog #2 (outlier)	0	0	0

Note: Dogs out of reference range and outliers have been included for each variable at the different time points. Range, minimum to maximum values; out ref. range, dogs out of reference range.

TABLE 3 Experimental procedures and conclusions of the studies previously reported about the effect of different probiotics on serum hepatic variables in dogs

Probiotic	Control group	Probiotic group	Probiotic administration time	Effect*	Authors
Enterococcus faecium EE3	No	11 healthy dogs	7 days	Cholesterol brought to physiological levels Decreased total lipids	Marciňáková et al[11]
Lactobacillus fermentum AD1	No	15 healthy dogs	7 days	Increased total lipids Unaltered ALT and cholesterol	Strompfová et al[3]
Bilavet	7 healthy German Shepherd dogs	7 healthy German Shepherd dogs	30 days	Decreased ALT and cholesterol Increased AST	Sengaut et al[12]
Bifidobacterium animalis B/12	10 healthy dogs	10 healthy dogs	14 days	Increased ALT and ALP Unaltered AST and cholesterol Decreased triglycerides	Strompfová et al[13]
L. fermentum VET9A *L. rhamnosus* VET16A *L. plantarum* VET14A	19 dogs with diarrhea	25 dogs with diarrhea	7 days	Unaltered ALT and ALP	Gómez-Gallego et al[18]
Lactobacillus acidophilus *Lactobacillus johnsonii*	5 healthy Labrador Retriever dogs	10 healthy Labrador Retriever dogs	9 weeks	Decreased cholesterol Increased HDL/LDL ratio	Kumar et al[14]

Note: All these studies were carried out using commercially unavailable probiotics. *Effect of the probiotic on the hepatic variables compared to the control group.

Abbreviations: ALP, alkaline phosphatase; ALT, alkaline phosphatase; AST, aspartate transferase.

In the probiotic group, no significant differences were found when comparing mean serum ALP activities at day 1, day 14, and day 28 ($P > .05$; Table 1). In dog 2, ALP activity was higher than the reference range (154 IU/L) at day 14, returning to normal at the end of the study (82 IU/L; Figure 1C). In the control group, mean ALP activity did not change significantly at the 3 time points ($P > .05$; Table 1). The ALP activity was within the reference range (20-150 IU/L) in all control dogs (Figure 1D).

Mean serum cholesterol concentration in the probiotic group was not significantly different at day 1 and day 14 ($P > .05$; Table 2) but at day 28 mean serum cholesterol concentration decreased significantly compared to day 14 ($P < .01$; Table 3; Figure 2A). In the control group, mean serum cholesterol concentrations did not change significantly during the study ($P > .05$; Table 2; Figure 2B). Cholesterol concentrations were within the reference range (108-310 mg/dL) in all dogs of the probiotic and control groups.

Mean serum triglyceride concentrations in the probiotic group were significantly higher ($P < .05$) at day 28 compared to day 1, but no differences were found when comparing each with day 14 (Table 2). Dog 2 had a higher serum triglyceride concentration (145 mg/dL) at day 28 than the reference range (Figure 2C). In the control group, mean serum triglyceride concentrations were not significantly different during the study ($P > .05$; Table 2). In all control dogs, serum triglyceride concentrations were within the reference range (20-112 mg/dL; Figure 2D).

4 | DISCUSSION

One of our inclusion criteria for dogs was baseline serum activities of ALT and ALP and serum total cholesterol and triglyceride concentrations within the reference range. As expected, no significant changes were found in the control group, but administration of *E. faecium*

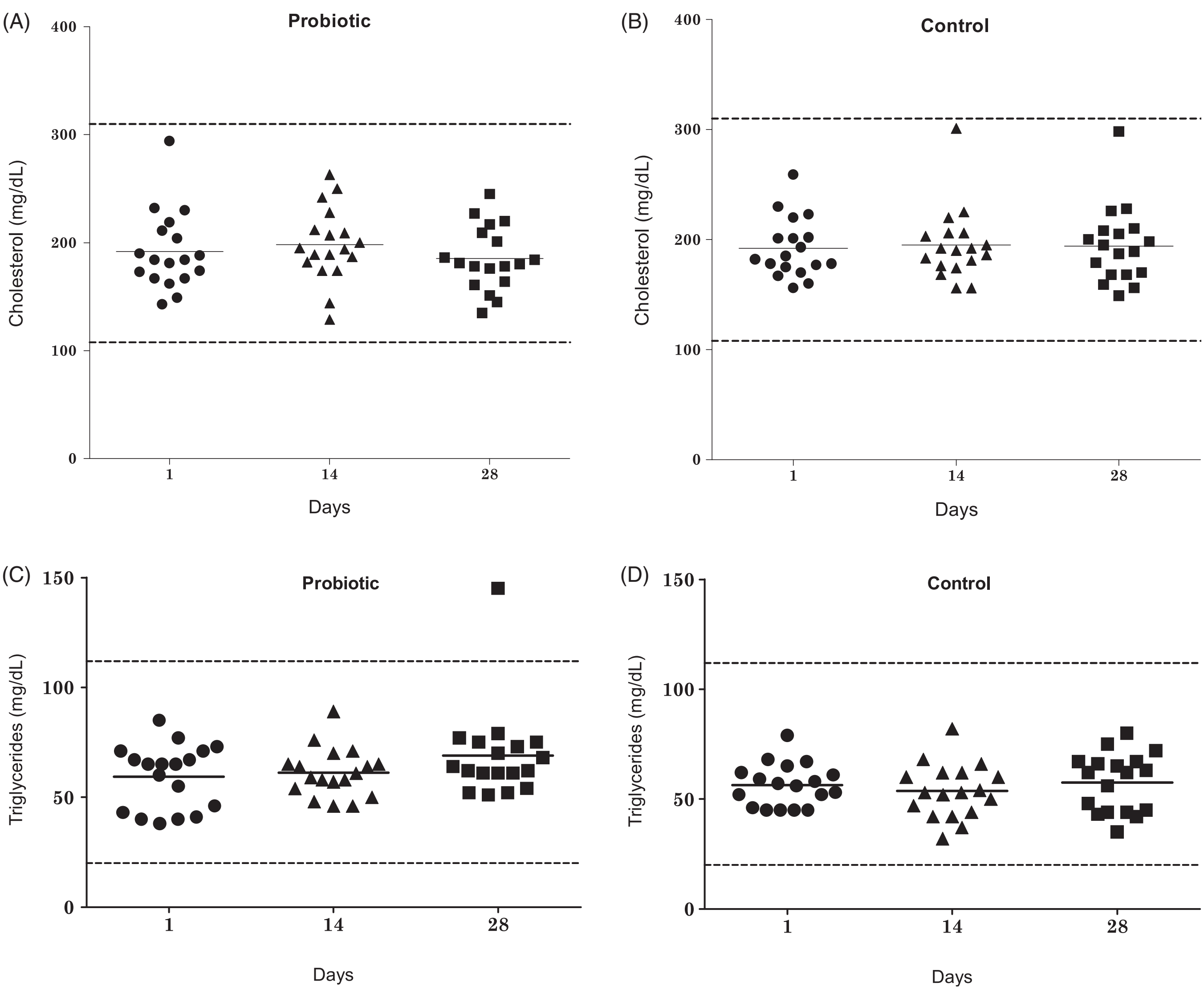

FIGURE 2 Scatter plot of serum total cholesterol (mg/dL) and triglycerides (mg/dL) in the probiotic, A,C, and control, B,D, groups of dogs at days 1, 14, and 28. Solid lines: mean serum concentrations. Dotted lines: reference range of each variable (total cholesterol: 108-310 mg/dL; triglyceride: 20-112 mg/dL). (GraphPad software, San Diego, California)

SF68 was associated with a significant serum total cholesterol concentration decrease and a significant increase in serum triglyceride concentration, both 14 days after ending probiotic administration. Any potential effect of diet[8] on these results was eliminated by feeding all dogs with the same balanced commercial diet.

E. faecium SF68 administration did not induce any significant change in mean serum ALT and ALP activities during the study. Only 2 dogs (5.5%, each variable) showed concentrations higher than the reference range at day 14, but in both cases the dogs remained clinically normal and enzyme activities returned to normal after discontinuing probiotic administration.

E. faecium SF68 is a probiotic product approved for use in dogs and cats but, surprisingly, few previous reports assess its potential effect on the canine liver[2,10,28] in contrast to reports on commercially unavailable probiotics. The variables determined in our study to assess hepatobiliary alterations and lipid status in dogs[15-17] were selected because they had been included in most related reports investigating the potential effect of probiotics on the canine liver.[3,11-14,18] Nevertheless, a direct comparison of results among these studies was precluded by differences in the numbers of dogs that received the probiotic (range, 7-25 dogs), the clinical status of dogs (healthy dogs and dogs with diarrhea) and the probiotic administration period (range, 1-9 weeks; Table 3). *Lactobacillus* spp. did not induce any change in ALT activity in healthy dogs[3] and a mixture of 3 different *Lactobacillus spp*. did not alter ALT and ALP activity in dogs with diarrhea.[18] On the other hand, *Bifidobacterium animalis* B/12 administered to healthy dogs for 14 days significantly increased ALT and ALP activity,[13] and the probiotic *Bilavet* slightly decreased ALT activity in German Shepherd dogs.[12] It therefore can be concluded that probiotics may have variable biological properties and different effects on these laboratory tests depending on the species and strain. This fact must be taken into account when prescribing any specific probiotic. *E. faecium* SF68, by itself or through changes promoted in the intestinal microbiota, did not induce alterations in ALT or ALP activity in healthy dogs. The ALT and ALP activities were slightly increased above the reference range in only 2 dogs (5.5%). In any case, ALT and ALP activity returned to normal when the probiotic was discontinued. In contrast to other probiotics capable of increasing ALT and ALP activity, *E. faecium* SF68 may be the probiotic of choice in dogs with hepatobiliary alterations in which interference in analytical monitoring is undesirable. This lack of effect of *E. faecium* SF68 on serum ALT and ALP activities should be confirmed in further studies including longer administration periods and dogs with hepatobiliary disease.

Mean serum cholesterol concentrations did not change significantly and remained within the reference range during probiotic administration. However, at day 28 after ending the administration, mean serum total cholesterol concentration was significantly decreased compared to day 14. Alternatively, mean serum triglyceride concentration progressively increased with probiotic administration and became significant 14 days after discontinuing its administration. At the end of the study, 1 dog (5.5%) had serum triglyceride concentrations higher than the reference range but remained clinically normal. Similar to the ALP and ALT activity, the effect of *E. faecium* SF68 on serum cholesterol or triglyceride concentrations in dogs has not been reported previously but has been assessed when evaluating other probiotics, with different study designs and conflicting results. Regarding cholesterol, when *E. faecium* EE3 was administered to asymptomatic dogs, high cholesterol concentrations were brought into the reference range,[11] whereas *Lactobacillus fermentum* AD1[3] and *Bifidobacterium animalis* B/12[13] did not modify serum cholesterol concentrations in healthy dogs. On the contrary, dogs receiving *Lactobacillus johnsonii* and *Lactobacillus acidophilus* experienced a decrease in serum cholesterol concentrations[14] as did German Shepherd dogs treated with the probiotic *Bilavet*[12] and dogs given *Lactobacillus rhamnosus*.[29] With regard to triglycerides, *Bifidobacterium animalis* B/12 significantly decreased serum triglyceride concentrations in healthy dogs,[13] but an increase in serum triglyceride concentrations was seen in healthy dogs given *L. rhamnosus*.[29] The effect of a probiotic on specific serum lipids seems to be variable. This variability also was reported in a previous study on the effect of *E. faecium* SF68 on serum vitamin concentrations in healthy dogs.[23] In our study, *E. faecium* SF68 administration was associated with a slight but significant decrease in serum total cholesterol concentration, but a slight significant increase in serum triglyceride concentrations occurred in healthy dogs, once the probiotic was discontinued. *E. faecium* EE3 can survive gastrointestinal transit and persist in canine feces for 3 months after its administration has been discontinued.[11] Although the viability of *E. faecium* SF68 in the canine gut has not been assessed, the prolonged effect on serum cholesterol and triglyceride concentrations could be a result of persistence of this bacterial strain within the canine gut after probiotic discontinuation. Moreover, the effects on serum cholesterol and triglyceride concentrations could be proportional to the duration of probiotic administration. It would be interesting to assess if these effects associated with *E. faecium* SF68 would reach statistical significance and be clinically relevant with longer administration, because a clear relationship has been described between hyperlipidemia and liver and gallbladder diseases in dogs[30,31] and humans.[9,32]

In conclusion, *E. faecium* SF68 administered to 18 healthy dogs for 14 days induced no significant variation in serum ALT or ALP activity during the study. In dogs with hepatobiliary alterations, this probiotic could be administered to monitor the treatment response of dogs. In contrast, both serum total cholesterol and triglyceride concentrations were significantly decreased or increased, respectively, after discontinuing the probiotic, with 1 dog experiencing hypertriglyceridemia although these changes were not clinically relevant. A longer trial will be necessary to assess if the probiotic effects could be clinically relevant and to assess its potential use in dogs with hypertriglyceridemia.

ACKNOWLEDGMENT

The authors thank Laboratorios Garfia SL for their technical assistance performing all the serum biochemical analyses.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study protocol was revised by the University of Córdoba Animal Welfare Ethics Committee and oversight by one of the authors (Pedro J Ginel) certified to conduct and design study procedures with animals as legally required.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Rosario Lucena https://orcid.org/0000-0002-6181-9349

REFERENCES

1. Sauter SN, Benyacoub J, Allenspach K, et al. Effects of probiotic bacteria in dogs with food responsive diarrhea treated with an elimination diet. *J Anim Physiol Anim Nutr*. 2006;90:269-277.
2. Grzeskowiak L, Endo A, Beasley S, Salminen S. Microbiota and probiotics in canine and feline welfare. *Anaerobe*. 2015;34:14-23.
3. Strompfova V, Marciňáková M, Simonova M, et al. Application of potential probiotic *Lactobacillus fermentum* AD1 strain in healthy dogs. *Anaerobe*. 2006;12:75-79.
4. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A*. 2004;101:15718-15723.
5. Weiss G, Christensen HR, Zeuthen LH, Vogensen FK, Jakobsen M, Frøkiær H. *Lactobacilli* and *Bifidobacteria* induce differential interferon-beta profiles in dendritic cells. *Cytokine*. 2011;56:520-530.
6. Xu RY, Wan YP, Fang QY, Lu W, Cai W. Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat non alcoholic fatty liver disease model. *J Clin Biochem Nutr*. 2012;50:72-77.
7. Handl S, German AJ, Holden SL, et al. Faecal microbiota in lean and obese dogs. *FEMS Microbiol Ecol*. 2013;84:332-343.
8. Xenoulis P, Steiner J. Canine hyperlipidaemia. *J Small Anim Pract*. 2015;56:595-605.
9. Altamirano-Barrera A, Uribe M, Chávez-Tapia NC, Nuño-Lambarri N. The role of the gut microbiota in the pathology and prevention of liver disease. *J Nutr Biochem*. 2018;60:1-8.
10. LeBlanc JG, Laiño JE, Juarez del Valle M, et al. B-group vitamin production by lactic acid bacteria-current knowledge and potential applications. *J Appl Microbiol*. 2011;111:1297-1309.
11. Marciňáková M, Simonova M, Strompfova V, Laukova A. Oral application of *Enterococcus faecium* strain EE3 in healthy dogs. *Folia Microbiol*. 2006;51:239-242.
12. Sengaut J, Januskevicius A, Januskeviciene G, Gabinaitis P. The influence of probiotic bilavet on morphological blood parameters, digestibility and chemical composition of faeces in German shepherd dogs. *Vet Zootech*. 2011;55:72-78.
13. Strompfova V, Simonova MP, Gancarcikova S, et al. Effect of *Bifidobacterium animalis* B/12 administration in healthy dogs. *Anaerobe*. 2014;28:37-43.
14. Kumar S, Pattanaik AK, Sharma S, Jadhav SE. Species-specific probiotic *Lactobacillus johnsonii* CPN23 supplementation modulates blood biochemical profile and erythrocytic antioxidant indices in Labrador dogs. *Indian J Anim Sci*. 2016;86:918-924.
15. Willard MD, Tvedten H. Gastrointestinal, pancreatic, and hepatic disorders. In: Willard MD, Tvedten H, eds. *Small Animal Clinical Diagnosis by Laboratorial Methods*. 4th ed. St. Louis, MO: Saunders; 2012:208-246.
16. Lawrence YA, Steiner JM. Laboratory evaluation of the liver. *Vet Clin Small Anim*. 2017;47:539-553.
17. Nelson RW, Turnwald GH, Willard MD. Endocrine, metabolic and lipid disorders. In: Willard MD, Tvedten H, eds. *Small Animal Clinical Diagnosis by Laboratorial Methods*. 4th ed. St. Louis, MO: Saunders; 2004:165-207.
18. Gómez- Gallego C, Junnila J, Mannikko S, et al. A canine-specific probiotic product in treating acute or intermittent diarrhea in dogs: a double blind placebo controlled efficacy study. *Vet Microbiol*. 2016;197:122-128.
19. Pompei A, Cordisco L, Amaretti A, Zanoni S, Matteuzzi D, Rossi M. Folate production by *Bifidobacteria* as a potential probiotic property. *Appl Environ Microbiol*. 2007;73:179-185.
20. Rossi M, Amaretti A, Raimondi S. Folate production by probiotic bacteria. *Nutrients*. 2011;3:118-134.
21. Vahjen W, Männer K. The effect of a probiotic *Enterococcus faecium* in diets of healthy dogs on bacteriological counts of *Salmonella spp*., *Campylobacter spp*. and *Clostridium spp*. in faeces. *Arch Tierernahr*. 2003;57:229-233.
22. Baillon ML, Marshall-Jones ZV, Butterwick RF. Effects of probiotic *Lactobacillus acidophilus* strain DSM13241 in healthy adult dogs. *Am J Vet Res*. 2004;65:338-343.
23. Lucena R, Olmedilla AB, Blanco B, Novales M, Ginel PJ. Effect of *Enterococcus faecium* SF68 on serum cobalamin and folate concentrations in healthy dogs. *J Small Anim Pract*. 2018;59:438-443.
24. Davenport DJ, Ching RJW, Hunt JH, Bruyette DS, Gross KL. The effect of dietary levels of folate and cobalamin on the serum concentration of folate and cobalamin in the dog. *J Nutr*. 1994;124:2559S-2562S.
25. Dossin O. Laboratory tests for diagnosis of gastrointestinal and pancreatic diseases. *Topics Comp Anim Med*. 2011;26:86-97.
26. Kook PH, Lutz S, Sewell AC, Bigler B, Reusch CE. Evaluation of serum cobalamin concentration in cats with clinical signs of gastrointestinal disease. *Schw Arch Tierheilkd*. 2012;154:479-486.
27. Jergens AE, Schreiner CA, Frank DE, et al. A scoring index for disease activity in canine inflammatory bowel disease. *J Vet Intern Med*. 2003; 17:291-297.
28. Chávez-Tapia NC, González-Rodríguez L, Jeong M, et al. Current evidence of the use of probiotics in liver diseases. *J Funct Foods*. 2015;17:137-151.
29. Hasiri MA, Gheisari HR, Khadernolhosseini AA. *Lactobacillus rhamnosus* as a probiotic for the health of adult dogs. *Int J Probiot Prebiot*. 2015;10:69-75.
30. Lee S, Kweon OK, Kim WH. Associations between serum leptin levels, hyperlipidemia and cholelithiasis in dogs. *PLos One*. 2017;31 (12):e0187315.
31. Kutsunai M, Kanemoto H, Fukushima K, Fujino Y, Ohno K, Tsujimoto H. The association between gallbladder mucoceles and hyperlipidaemia in dogs: a retrospective case control study. *Vet J*. 2014;199:76-79.
32. Mokhtari Z, Gibson DL, Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome and diet. *Adv Nutr*. 2017;8:240-252.

How to cite this article: Lucena R, Novales M, Blanco B, Hernández E, Ginel PJ. Effect of probiotic *Enterococcus faecium* SF68 on liver function in healthy dogs. *J Vet Intern Med*. 2019;33:2628–2634. https://doi.org/10.1111/jvim.15625