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Effect of short-term probiotic *Enterococcus faecium* SF68 dietary supplementation in overweight and obese cats without comorbidities

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ABSTRACT

Obesity in cats is associated with metabolic abnormalities and increased susceptibility to diseases such as diabetes mellitus. Studies in mouse models and human beings have shown that probiotics can reduce food intake, promote weight loss and improve metabolic profile. Studies assessing the effects of probiotics on these same parameters are absent in cats. Therefore, the aim of this study was to determine if probiotic *Enterococcus faecium* strain SF68 dietary supplementation reduces food intake, promotes weight loss and improves metabolic profile in overweight and obese cats without comorbidities. Twenty overweight and obese specific pathogen-free cats without comorbidities were acclimatised to a dry diet for four weeks. After exclusion of four cats for unrelated reasons, eight cats received a daily oral probiotic for eight weeks and eight control cats received no probiotic. All cats were fed ad libitum with food intake measured daily and bodyweight weekly. Blood was collected at three time points: after four weeks of acclimatisation to the diet, after eight weeks of intervention and after six weeks of washout for measurement of glucose, triglyceride, cholesterol, fructosamine, insulin, leptin, total adiponectin and deuterium oxide for body composition. There were no differences in food intake, metabolic parameters and body composition between the probiotic and control groups after eight weeks of intervention and six weeks of washout ($P \geq 0.050$). Short-term use of *E faecium* SF68 dietary supplementation had no significant effect on food intake, bodyweight, body composition or metabolic parameters in overweight and obese specific pathogen-free cats without comorbidities.

INTRODUCTION

The incidence of obesity in cats varies from 17 per cent to 63 per cent in developed countries (Kronfeld and others 1994, Allan and others 2000, Cave and others 2012, Corbee 2014). Controlling calorie intake and adhering to a weight loss programme is difficult for many pet owners and prevention of weight gain and reversal of obesity in cats is often not successful. Excess bodyweight in cats is related to several diseases such as diabetes mellitus, hepatic lipidosis and osteoarthritis (Scarlett and Donoghue 1998, German 2006, German and others 2010). Therefore, it is important that overweight and obese cats achieve and maintain an ideal bodyweight, necessitating investigation of the efficacy of additional weight management strategies.

An imbalance in intestinal microbiota has been associated with metabolic diseases such as obesity, diabetes and atherosclerosis in human beings (Kelsen and Wu 2012, Moreno-Indias and others 2014, Nieuwdorp and others 2014). In knockout and diet-induced obese mice, obesity was associated with changes in the composition and metabolic function of the microbiome (Tilg and others 2009). Similarly, one study documented that overweight and obese cats had a significantly different gut microbiota compared with lean cats (Kieler and others 2012). Another study reported that human beings with low intestinal bacterial richness were characterised by more marked adiposity, insulin resistance and dyslipidaemia when compared with human beings with high intestinal bacterial richness (Le Chatelier and others 2013). Bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* have been reported to have many beneficial effects on metabolic syndrome, such as weight loss and improved glucose tolerance in human beings and mouse models (Cani and others 2008, F S Teixeira and others 2013). In addition, a study showed that faecal *Clostridium* cluster IV increased significantly and *Clostridium* cluster XIVa decreased after bodyweight reduction in human beings (Remely and others 2015).Therefore, promoting changes in the intestinal microbiota may play an important role in ensuring the efficacy and success of obesity treatments.

The addition of prebiotics and probiotics to the diet of mice fed ad libitum is associated with decreased fat stores, reduction in adipocyte size, decreased food consumption and weight gain, modulation of microbiota, increased glucose tolerance, decreased total cholesterol and triglycerides, and reduction of low-grade inflammation (Aronsson and others 2010, Andersson and others 2010a, b, Takemura and others 2010, Everard and others 2011, Neyrinck and others 2012). *Lactobacillus rhamnosus* CGMCC1.3724 has been demonstrated to help obese women achieve sustainable weight loss, as well as reduce circulating leptin levels (Sanchez and others 2014). One study suggested that a weight loss diet and probiotic yogurt, enriched with *Lactobacillus acidophilus* La5, *Bifidobacterium* BB12 and *Lactobacillus casei* DN001 had synergistic effects in reducing body fat and weight among overweight and obese human beings (Zarrati and others 2014). In addition, consumption of probiotic yogurt has been associated with a reduced risk of weight gain and obesity as well as cardiovascular disease in an observational study in human beings (Astrup 2014). A study in healthy dogs reported that oral application of *Enterococcus faecium* strain EE3 decreased total blood lipid concentration and cholesterol concentrations were brought back within reference range (Marcináková and others 2006). Although studies have investigated the impact of probiotics on various parameters in cats with diarrhoea (Bybee and others 2011), herpesvirus (Lappin and others 2009) and chronic kidney disease (Rishniw and Wynn 2011); to the author's knowledge no studies have assessed probiotic effect on food intake, bodyweight and composition, and metabolic parameters in cats.

Therefore, the aims of this study were to evaluate the effects of probiotic *E faecium* SF68 dietary supplementation on daily food intake, bodyweight, body composition and concentrations of blood glucose, triglyceride, cholesterol, fructosamine, insulin, leptin, and total adiponectin concentrations in a population of overweight and obese cats without comorbidities. The probiotic *E faecium* SF68 dietary supplementation used in this study is the only probiotic that is licensed for use in cats and dogs in the European Union. Additionally, it has been studied most commonly in feline diseases (Lappin and others 2009, Bybee and others 2011), has quality assurance based on manufacturer's guarantee, and a previous study verified this product exceeded the claim for quantifiable viable organisms (Weese and Martin 2011). However, this probiotic is not of feline origin and is derived from healthy human infants. The results of this current study will help to determine if the probiotic *E faecium* SF68 might be a useful component of weight management plans for cats.

METHODS

Cats

Twenty specific pathogen-free, naturally occurring overweight and obese cats without comorbidities, based on absence of clinical signs and no abnormalities on physical examination, from a research colony at the University of California-Davis were included in this study. Cats were assigned a body condition score based on a 9-point scale (Laflamme 1997). Cats with a body condition score of 5.5/9 to 7/9 were considered overweight and cats with a score of 8/9 to 9/9 were considered obese. The facility maintains room temperatures between 18°C and 24°C, and has a 14-hour light/10-hour dark cycle. Cats were fed the same dry diet (Purina Cat Chow Complete Formula dry cat food; Nestle Purina PetCare Company, St Louis, Missouri, USA; Energy content (metabolisable energy; Maine, USA, calculated): 3873 kcal/kg, 926 kJ/kg, Caloric distribution: 34.3 per cent protein, 31.9 per cent fat and 33.8 per cent carbohydrate ME (based on conversion of guaranteed analysis using modified Atwater factors)) during the study that passed feeding trials in accordance with guidelines established by the Association of American Feed Control Officials for all life stages. Water was available at all times during the study with the exception of being withheld for two hours before body composition determination.

The cats were evenly allocated to two groups based on bodyweight and body condition score. A coin was flipped to randomly select which of the two groups would receive the probiotic *E faecium* SF68 supplementation versus control, which consisted of no supplementation. The probiotic group consisted of six male neutered cats and four female entire cats. The mean age of cats in the probiotic group was eight years and five months (range 4 years–12 years and 9 months) and the mean bodyweight and body condition score at inclusion were 6.5 kg and 7/9 (range 5.3–10.2 kg and 5.5/9–9/9, respectively). The control group consisted of two male neutered cats, five female entire cats and three female spayed cats. The mean age of cats in the control group was nine years and eight months (range 3 years and 6 months–12 years and 8 months) and the mean weight and body condition score were 6.8 kg and 7/9 (range 4.4–7.4 kg and 5.5/9–9/9, respectively).

Study protocol

During the study, each cat was individually housed with a socialisation/enrichment period of four hours per day (08.00 till 12.00), during which time they were group-housed with free access to water only. During the four-week acclimatisation period they were placed back into individual housing after socialisation and fed ad libitum. During the following eight-week intervention period each cat in the probiotic group was given 1 g of probiotic *E faecium SF68* dietary supplement (Purina Veterinary Diets FortiFlora Feline Nutritional Supplement; Nestle Purina PetCare Company, St Louis, Missouri, USA; Energy content (metabolisable energy, calculated): 4 kcal/g.), based on manufacturers' recommendation (5×10^8 colony-forming units per gram, according to the manufacturers' guarantee) in 10 g of food with 1 ml of tap water to facilitate mixing daily when returned to their individual cages. During the

same time each control cat was given 10 g of food with 1 ml of tap water. Once the cats consumed their 10 g of food, they received additional food ad libitum. During the eight-week intervention period, all cats in the probiotic group were closely supervised to ensure they consumed all probiotic each day. Eight weeks was selected for the intervention period as studies in both mouse models and human beings have shown beneficial effects of probiotics on obesity and metabolic parameters following supplementation for this length of time (Agerholm-Larsen and others 2000, Everard and others 2011, Zarrati and others 2013, Wu and others 2015). During the following six-week washout period, the same protocol used for acclimatisation was applied. Six weeks were deemed adequate for the washout period, as studies have shown that probiotics do not persist in the faeces after a few weeks following their discontinuation (Goldin and others 1992, Spanhaak and others 1998, Jacobsen and others 1999). Food intake (grams/day) was measured daily and bodyweight with body condition score assessed weekly during the three time periods for all cats by the same investigator (AK). The study protocol was approved by the University of California-Davis Institutional Animal Care and Use Committee (Animal Welfare Assurance Number A3433-01) and complied with the recommendations of the National Research Council Guide for the Care and Use of Laboratory Animals.

Collection of blood samples

At the end of the acclimatisation, intervention and washout periods, food was withheld overnight for 12 hours. The following morning, each cat was weighed. Water was withheld from the cats and two hours later 6 ml of blood was collected by venepuncture. The cats were injected subcutaneously with their individually calculated dose of deuterium oxide (D20; tracer dose of 0.4 g/kg) and three hours later 2 ml of blood was collected by venepuncture.

Analysis of blood samples

Blood collected before administration of D20 was allowed to clot at room temperature for 20 minutes followed by centrifugation (room temperature, 2000 *g* for 10 minutes) and separation of serum into four microtubes (E&K Scientific, Santa Clara, California, USA); the first sample was submitted to the University of California-Davis Veterinary Medical Teaching Hospital Diagnostic Laboratory for measurement of glucose, triglyceride, cholesterol and fructosamine. An automated analyser (Cobas c501 analyzer, Hoffman-La Roche, Basel, Switzerland) was used to measure glucose, triglyceride and cholesterol; fructosamine was measured using a multispecies colorimetric assay incorporating nitroblue-tetrazolium reagent base with an immunoanalyser (Olympus AU5400 Chemistry Immuno Analyzer. Olympus America, Center Valley, Pennsylvania, USA). The second sample was submitted to the Diagnostic Center for Population and Animal Health at Michigan State University for measurement of insulin using a multispecies radioimmunoassay (RIA) kit (EMD Millipore, Billerica, Massachusetts, USA). The third sample was submitted to the University of California-Davis Department of Nutrition for measurement of leptin and total adiponectin concentrations. Total adiponectin was measured using a rat/mouse ELISA kit (B-Bridge, Cupertino, California, USA) and leptin was measured using a multispecies RIA kit (EMD Millipore, Billerica, Massachusetts, USA). Both assays have been previously validated for measuring total adiponectin and leptin in feline samples (Wei and others 2014). The fourth sample was sent to the University of Missouri for analysis of D20. Enrichment of D20 in serum was used to calculate total body water, lean body mass and fat mass using a previously described D20 isotopic dilution method (Backus and others 2010); water was distilled from serum (Backus and others 2000) and deuterium enrichment in water was determined using Fourier transform infrared spectrometry (Jennings and others 1999). Body fat mass was estimated as the difference between bodyweight and lean body mass, where the latter was estimated from body water mass indicated by deuterium dilution in serum water (lean body mass=body water mass/0.72 (Wolfe 1984).

Statistical analysis

Analyses were performed using a computer software package (Stata IC/V.13.1, StataCorp LP, College Station, Texas, USA). Data were assessed for normality using the Shapiro-Wilk test. Data were analysed using mixed-effects analysis of variance to account for the repeated measures. Effects of group were evaluated at individual time points or periods (time point 1: after a four-week period of acclimatisation, time point 2: after an eight-week period of intervention and time point 3: after a six-week period of washout); effects of time were evaluated separately by group; this was done by creating separate models evaluating the main effect of group at each time point, and by creating separate models evaluating the main effect of time for each group separately. The repeated measures were handled by designating the individual as the random effect. Post hoc contrasts were adjusted for multiple comparisons using a Bonferroni correction. Pearson correlation between continuous variables was estimated using linear regression with robust variance estimation to account for replicate values within individuals. Differences in age and bodyweight between the two groups, at inclusion and after exclusion of necessary cats was analysed using the Student's *t* test. Differences in body condition score between the two groups, at inclusion and after exclusion of necessary cats was analysed using the Mann–Whitney U test. Differences in sex and neuter status between the two groups, at inclusion and after exclusion of necessary cats was analysed using the χ^2 test. Statistical significance was defined as $P \leq 0.050$ in all analyses.

Data are presented as mean with se of the mean in Figs 1 and 2 and mean with sd in Table 1.

RESULTS

No abnormalities were noted on the basis of prestudy physical examinations before the four-week acclimatisation period or laboratory findings after the four weeks of acclimatisation. Two cats were eliminated from the probiotic group as one became febrile from suspected cutaneous abscess on day 1 of the acclimatisation period and the second cat developed diabetes mellitus on day 28 of the intervention period. Two cats were eliminated from the control group; one became acutely anorexic on day 24 of the acclimatisation period and was subsequently diagnosed with metastatic ovarian carcinoma. The second cat became too fractious for blood collection and was also eliminated. Data from these four cats were excluded from all analyses. The final probiotic group consisted of eight cats (six male neutered and two female entire). The mean age of cats in the probiotic group was seven years and seven months (range 4 years–12 years and 3 months) and the mean bodyweight and body condition score at inclusion was 6.7 kg and 7/9 (range 5.3–10.2 kg and 6/9–9/9, respectively). The final control group consisted of eight cats (two male neutered, three female entire and three female neutered). The mean age of cats in the control group was 10 years and 2 months (range 5 years and 8 months–12 years and 8 months) and the mean weight and body condition score was 5.7 kg and 6.5/9 (range 4.4–6.5 kg and 6/9–8/9, respectively). There were no significant differences in age, bodyweight or body condition score between the two groups at inclusion (P>0.157), or after exclusion of the four cats (P>0.155). All cats in the probiotic group accepted and consumed all their daily amount of probiotic *E faecium* SF68 dietary supplementation during the eight-week period.

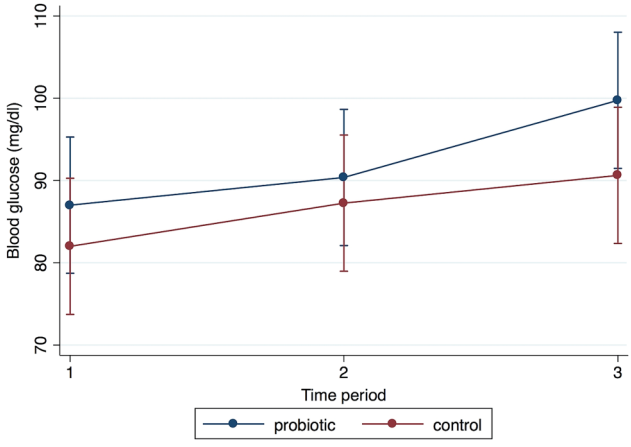

FIG 1: Comparison of blood glucose within and between probiotic and control groups at the three time points. Line graph of average blood glucose (mg/dl) for cats in the probiotic *Enterococcus faecium* strain SF68 supplementation group (n=8) and control (unsupplemented) group (n=8) at the three time points (1, after four weeks of acclimatisation; 2, after eight weeks of intervention; 3, after six weeks of washout). Dots represent mean with lines representing se of the mean. There was a trend (P=0.081) towards a significant increase in blood glucose at time point 3 when compared with time point 1 within the probiotic group

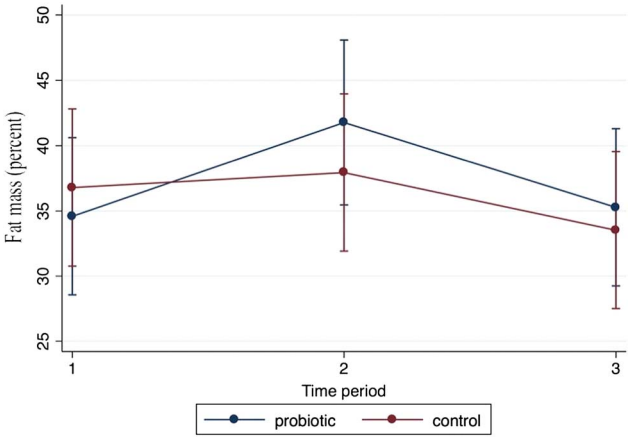

FIG 2: Comparison of body fat mass within and between probiotic and control groups at the three time points. Line graph of average body fat mass for cats in the probiotic *Enterococcus faecium* strain SF68 supplementation group (n=8) and control (unsupplemented) group (n=8) at the three time points (1, after four weeks of acclimatisation; 2, after eight weeks of intervention; 3, after six weeks of washout). Dots represent mean with lines representing se of the mean. There was a trend (P=0.089) towards an increase in per cent body fat mass at time point 2 compared with time point 1 within the probiotic group

Comparisons between the control and probiotic groups

There was an almost significantly higher insulin concentration in the probiotic group (80.8±17.50) compared with the control group (65.8±15.14) at the end of the four-week acclimatisation period (time point 1, Table 1, P=0.050, Table 2). There was a trend (P<0.1) towards a significant difference in body weight at time points 1 and 2, with the cats in the probiotic group being heavier. Additionally, there was a trend towards a significantly greater lean body mass of the cats in the probiotic group at time point 1. There were no statistically significant differences in the remaining measured parameters (daily food intake, body condition score, fat mass per cent, blood glucose, triglyceride, cholesterol, fructosamine, leptin and total adiponectin concentrations) between the probiotic and control groups at any of the three time points (P> 0.10, Table 2).

Comparisons among the three time points within each group

There was a decrease in average daily food intake during time points 2 and 3, compared with time point 1, within each group of cats (P<0.001, mean daily food intake (grams/day): probiotic group; time point 1: 91.4, time point 2: 83.3, time point 3: 76.9, control group; time

TABLE 1: Descriptive results showing the means and sds of various parameters for the probiotic *Enterococcus faecium* strain SF68 supplementation group (eight cats) and control group consisting of no supplementation (eight cats)

	Probiotic group			Control group		
	Time point 1* mean (SD)	Time point 2† mean (SD)	Time point 3‡ mean (SD)	Time point 1 mean (SD)	Time point 2 mean (SD)	Time point 3 mean (SD)
Daily food intake (g/day)	91.4 (19.31)	83.3 (15.82)	76.9 (15.60)	83.9 (25.00)	70.9 (21.72)	74.2 (20.50)
Blood glucose (mg/dl)	87.0 (8.83)	90.4 (9.21)	99.8 (17.12)	82 (10.41)	87.3 (13.12)	90.6 (15.67)
Triglyceride (mg/dl)	69.5 (43.73)	77.0 (38.14)	77.3 (43.40)	79.4 (49.66)	72 (38.14)	90.5 (44.96)
Cholesterol (mg/dl)	149 .0 (38.60)	147.4 (38.31)	146.4 (42.30)	151.8 (28.01)	140.3 (18.22)	145.8 (20.72)
Fructosamine (umol/l)	208.8 (17.24)	201.8 (16.62)	209.8 (21.55)	214.0 (25.25)	200.1 (14.25)	197.3 (17.62)
Insulin (pmol/l)	80.8 (17.50)	96.0 (23.30)	88.6 (20.04)	65.8 (15.14)	79.5 (20.05)	79.4 (20.91)
Leptin (ng/ml)	10.5 (4.76)	12.5 (5.62)	13.6 (6.44)	9.3 (3.33)	10.4 (5.02)	10.3 (4.16)
Adiponectin (μg/ml)	1.7 (1.85)	1.4 (1.50)	1.5 (1.68)	2.1 (0.96)	1.9 (1.25)	1.7 (1.03)
Bodyweight (kg)	6.9 (1.58)	7.1 (1.63)	7.1 (1.65)	5.8 (0.88)	5.9 (1.20)	6.1 (1.33)
Body condition score	7.5 (0.98)	7.8 (1.04)	7.8 (1.04)	7.1 (0.58)	7.2 (1.41)	7.1 (1.55)
Lean body mass (kg)	4.5 (0.91)	4.1 (1.02)	4.6 (1.29)	3.7 (0.96)	3.7 (0.94)	4.0 (0.76)
Fat mass %	34.6 (7.80)	42.1 (7.09)	35.3 (6.47)	36.8 (13.51)	37.9 (11.73)	33.5 (6.64)

Time point 1 (after a four-week period of acclimatisation), time point 2 (after an eight-week period of intervention) and time point 3 (after a six-week period of washout)
*Time point 1: after a four-week period of acclimatisation
†Time point 2: after an eight-week period of intervention
‡Time point 3: after a six-week period of washout

TABLE 2: P values obtained from the mixed-effects ANOVA* analysis for parameters compared among and between probiotic (*Enterococcus faecium* strain SF68) supplemented group (n=8 cats) and control group consisting of no supplementation (n=8 cats)

	Probiotic v control Mixed-effects ANOVA			Probiotic group			Control group		
	Time point 1†	Time point 2‡	Time point 3§	Time point 2 v 1	Time point 3 v 1	Time point 3 v 2	Time point 2 v 1	Time point 3 v 1	Time point 3 v 2
Daily food intake	0.365	0.158	0.771	0.00	0.00	0.00	0.00	0.00	0.0
Blood glucose	0.268	0.556	0.233	1.0	0.081	0.312	1.0	0.487	1.0
Triglyceride	0.652	0.779	0.521	1.0	0.781	0.611	1.0	1.0	1.0
Cholesterol	0.862	0.612	0.968	1.0	1.0	1.0	0.836	1.0	1.0
Fructosamine	0.559	0.304	0.179	1.0	1.0	1.0	0.390	0.203	1.0
Insulin	0.050	0.105	0.334	1.0	1.0	1.0	0.214	0.214	1.0
Leptin	0.511	0.397	0.192	1.0	0.746	1.0	1.0	1.0	1.0
Adiponectin	0.575	0.374	0.759	1.0	1.0	1.0	1.0	1.0	1.0
Bodyweight	0.060	0.072	0.143	1.0	1.0	1.0	1.0	1.0	1.0
Body condition score	0.246	0.331	0.311	1.0	1.0	1.0	1.0	1.0	1.0
Lean body mass	0.055	0.292	0.226	1.0	1.0	1.0	1.0	1.0	1.0
Fat mass %	0.670	0.383	0.568	0.089	1.0	0.145	1.0	1.0	1.0

*ANOVA: analysis of variance with post hoc pairwise comparisons using a Bonferroni correction
†Time point 1: after a four-week period of acclimatisation
‡Time point 2: after an eight-week period of intervention
§Time point 3: after a six-week period of washout

point 1: 83.9, time point 2: 70.9, time point 3: 74.2; Tables 1 and 2). Within the probiotic group there was a tend (P=0.081) towards a significant increase in blood glucose at time point 3 when compared with time point 1 (Table 2 and Fig 1). Additionally, there was a trend (P=0.089) towards an increase in per cent body fat mass at time point 2 when compared with time point 1 within the probiotic group (Table 2 and Fig 2). There were no significant differences in the remaining measured parameters (bodyweight, body condition score, lean body mass, triglyceride, cholesterol, fructosamine, insulin, leptin and total adiponectin concentrations) for any of the three time point comparisons within the probiotic or control groups (P>0.145, Tables 1 and 2).

There were small significant positive correlations between the following parameters when all cats were analysed at all three time points; body fat mass and blood glucose (P=0.043, r=0.179), body fat mass and fructosamine (P=0.025, r=0.276) and body fat mass and body condition score (P=0.002, r=0.400). There was a significant negative correlation between body fat mass and total adiponectin concentration (P=0.002, r=0.400).

DISCUSSION

The high incidence of obesity in pet cats is a growing concern, and this problem is associated with serious diseases. Studies in mice models and human beings have shown that probiotics can reduce food intake, promote weight loss and improve metabolic profile (Aronsson and others 2010, Andersson and others 2010a, b, Takemura and others 2010, Everard and others 2011, Neyrinck and others 2012, Sanchez and others 2014, Zarrati and others 2014); however, similar studies are absent in cats. The study reported here did not identify any significant change within or between the control and probiotic *E faecium* SF68 supplementation group with respect to bodyweight, body condition score, lean body mass, fat mass per cent, blood glucose, cholesterol, triglyceride, insulin, fructosamine, leptin and total adiponectin concentrations after eight weeks of intervention and six weeks after discontinuation. Although daily food intake was significantly decreased after eight weeks of intervention and six weeks after discontinuation compared with the end of the four-week acclimatisation period in both groups, there were no significant differences between groups at any of the three time points. This effect within both groups was likely due to wide variation in daily food intake or self-regulation of food intake rather than probiotic supplementation.

This study showed a trend towards a significant increase in fat mass and blood glucose in the probiotic group after eight weeks of probiotic supplementation and six weeks after discontinuation compared with the end of the four-week acclimatisation period. Both parameters did not reach significance; this could have been due to a small sample size resulting in an underpowered study and therefore, the potential impact of probiotic supplementation on body fat mass and blood glucose cannot be definitively determined. Further studies are needed to ascertain if probiotic supplementation in overweight and obese cats without comorbidities results in increased body fat mass and blood glucose.

In contrast to the findings of the present study, the addition of prebiotics and probiotics to the diet of mice fed ad libitum has been shown to be associated with decreased fat stores, reduction in adipocyte size, decreased food consumption and weight gain, modulation of microbiota, increased glucose tolerance, decreased total cholesterol and triglycerides and reduction of low-grade inflammation (Aronsson and others 2010, Andersson and others 2010a, b, Takemura and others 2010, Everard and others 2011, Neyrinck and others 2012). Probiotics belonging to *Lactobacillus* and *Bifidobacterium* species have been studied most commonly in obese human beings and have been reported to reduce bodyweight (Ilmonen and others 2011, Mikirova and others 2011, Kadooka and others 2013, Sanchez and others 2014, Zarrati and others 2014, Minami and others 2015). Although the exact underlying mechanism remains unclear, one study showed a moderate increase in plasma lipopolysaccharide coupled with a decreased number of intestinal *Bifidobacteria* in mice fed a fat-enriched diet, resulting in the onset of metabolic diseases such as obesity and diabetes (Cani and others 2007a). One study adduced that the effect of *Bifidobacteria* on energy and fat metabolism is strain dependent (Yin and others 2010). Differences in probiotic strain may explain why the results of this study may be in contrast to a study in dogs, which reported that *E faecium* strain EE3 decreased total blood lipid concentration and influenced cholesterol levels (Marcináková and others 2006). Therefore, further research to determine the therapeutic response of each bacterial type and strain to treat or prevent obesity is needed and may explain why results from this current study differ from what has been shown in mice and human beings.

Apart from an almost significant increase in blood insulin in the probiotic group at the end of the four-week acclimatisation period there were no significant differences in the remaining parameters between the probiotic and control groups at any time point. Similarly, a recent meta-analysis of four randomised clinical trials suggested that probiotics given over a three to eight week period might not affect bodyweight, body mass index or visceral fat of obese human patients (Park and Bae 2015). As there are multiple mechanisms potentially involved in changing microbiota that influence adiposity, a longer intervention period may be needed to see an effect in both cats and human beings (Agerholm-Larsen and others 2000, Sharafedtinov and others 2013, Zarrati and others 2013, Lee and others 2014). To that effect, collection of faecal samples before and after probiotic intervention to investigate the effects of living probiotics and to confirm changes in microbiota were not performed in either of the four human studies or in this

current study. While the intent of this preliminary study was to simply determine if probiotic supplementation would affect change in bodyweight, body condition score or biochemical parameters, investigating changes in the microbiota following probiotic intervention in this current study may have been beneficial. A prior study performed in kittens indicated that blood triglyceride concentration was negatively correlated with faecal *Veillonellaceae* and *Bifidobacteriaceae,* while faecal *Lactobacillaceae* had a negative association with bodyweight and a positive association with blood leptin concentration (Hooda and others 2013). In addition the lack of significance in this current study and in the meta-analysis may have been due to the absence of concurrent restrictive control of food intake, which may be especially important as one study showed synergism between probiotic and food restriction on weight loss in human beings (Zarrati and others 2014).

Other factors may also confound outcomes in studies of probiotics and their impact on bodyweight and metabolic parameters. For example, activity level may influence body composition but this was not investigated in the studies used in the meta-analysis or in this current study; therefore, future studies using activity collars on cats to track and determine if activity could be a confounding factor should be considered. In addition, two separate studies have shown that probiotics specifically cause weight loss in women (Kadooka and others 2013, Sanchez and others 2014); unfortunately the sample size of the present study was too small to specifically look at the effects of sex. If the effects of probiotic are specific to female or male cats, this may explain the lack of significance in this current study.

An important consideration for selection of a probiotic is host species specification, as this has been shown to be important for manifestation of beneficial effects of the probiotic (Dunne and others 1999, Ouwehand and others 2002). However, the current study utilised probiotics that originated from healthy human being infants instead of healthy cats. Therefore, the lack of a significant finding in the present study may have been because a probiotic derived specifically from healthy cats was not used. This may also explain why results from this current study may differ from what has been shown in mouse models and human beings, where species-specific probiotics were commonly used. Another possible limitation of the current study is that it was performed in specific pathogen-free cats. Although these cats were naturally overweight or obese, they may have a different microbiota compared with conventionally raised cats (Ritchie and others 2010). Therefore, findings from the current study may not be applicable to client-owned cats. However, the previous study only reported findings from one specific pathogen-free cat, so it is difficult to know how applicable the findings are to the present study or to such populations in general.

Blood leptin is known to correlate positively with body fat mass in cats (Appleton and others 2000, Backus and others 2000, Martin and others 2006, Hoenig and others 2007, 2013); however, this current study did not find an association between leptin and body fat mass. This may have been due to other confounding factors such as sex or age of the cats, which have been shown to also effect leptin concentration (Appleton and others 2000, Backus and others 2007). Furthermore the small sample size of the current study may have precluded any finding of a significant positive association. Similarly, although previous studies have shown a positive correlation between blood insulin and body fat mass in cats (Hoenig and others 2013, Bjornvad and others 2014), this current study did not show such an association. This again may have been due to confounding factors of age and sex (Hoenig and others 2011, Bjornvad and others 2014) or due to the small sample size used in the current study. The findings of a positive association between fat mass and fructosamine in this study has been previously described (Gilor and others 2010). In this current study, blood total adiponectin concentration was negatively associated with body fat mass in all cats similar to other reports (Hoenig and others 2007, 2013, Ishioka and others 2009, Takashima and others 2016). However, recent studies have reported that the high molecular weight form in particular is associated with adiposity in cats and human beings (Hara and others 2006, Sinha and others 2007, Kaser and others 2008, Bjornvad and others 2014, Witzel and others 2015), and is thus functionally more important; unfortunately this was not assessed in this study.

In conclusion, short-term use of probiotic *E faecium* SF68 dietary supplementation had no effect on food intake, bodyweight, body composition or metabolic parameters in overweight or obese specific pathogen-free cats without comorbidities. However further studies are needed to assess the effect of a longer intervention period, larger sample size, different types and strains of probiotics, and concurrent calorie restriction on these parameters. In addition, any significant findings need to be confirmed in client-owned cats.

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