Lipid Metabolic Alterations and Satiety with a Pumpkin-Based supplement in Obese Dogs

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ABSTRACT

Objective−To evaluate the combined effects of a dietary fiber and carnitine supplement using a commercially available canned dog supplement on satiety, weight loss, and lipid metabolism.

Design−A randomized, crossover design (satiety study) and randomized complete block design (weight loss study)

Sample Population−12 (satiety study) and 7 (weight loss study) adult female overweight/obese Beagles.

Procedures−Two studies were conducted. In the satiety study, dogs were fed 1.2 times maintenance energy amounts of either high dietary fiber/high carnitine (HF/C) or low dietary fiber/low carnitine (LF/C) supplemented diet twice a day using a 3 hour interval and food intake was monitored. Blood samples were obtained at 0, 45, and 120 min postprandially for peptide YY determination. For the weight loss study, 60 % of maintenance energy amounts of either the HF/C or LF/C diet were fed for 42 days. Blood samples were collected at days 1, 28, and 42 to determine plasma lipid metabolites and peptide YY.

Results−The HF/C diet decreased both the amount of food and energy intakes at 3 hour post-feeding, suggesting improved 3 hour post-meal satiety. This combination supplement also increased postprandial plasma β-hydroxybutyrate at day 42 and was associated with greater body fat and weight loss without alteration of plasma peptide YY, triglyceride, total cholesterol, and lipoprotein-cholesterol concentrations.

Conclusions and Clinical Relevance– The combination of dietary fiber/carnitine from a commercially available canned supplement demonstrated the potential to decrease begging behavior between meals due to increased 3 hour post-meal satiety. This combination supplement also supported improved body weight reduction and increased fat utilization without altering plasma triglyceride, lipoprotein-cholesterol and cholesterol concentrations.

INTRODUCTION

Obesity is defined as abnormal or excessive fat accumulation that may impair health. It exists one's body weight exceeds 30% of a predefined optimal weight.[1] Obesity is a growing global problem in companion animals as well as humans, and it contributes to severe chronic diseases such as diabetes, hypertension, dyslipidemia, and cardiovascular disorders.[2] Thus, treatment of obesity has been intensively researched in both veterinary and human medical fields. One possible approach for weight management is to alter lipid metabolism to enhance fat mobilization and utilization, or to provide satiety using appropriate dietary nutrients. Both dietary fiber and carnitine may possess such beneficial functions on canine weight management.[3-11]

Dietary fibers are resistant to hydrolysis by digestive enzymes.[12] Because of their uniqueness, dietary fibers have many different effects and activities as they transit through the gastrointestinal tract, including energy intake management and, therefore, long-term weight loss.[13-15] In dogs, Jackson et al found a reduction of calorie intake by including 29% dietary fiber in a canine diet.[3] In support of this finding, Jewell et al reported that dogs consumed less metabolizable energy when fed diets containing from 12% to 21% of crude fiber on an as fed basis, compared to dogs fed a diet containing only 2 %crude fiber.[4,16] However, increased fiber may not necessary reduce energy intake depending on fiber type or food design. For example, when adult female English Pointers were fed 0 to 12.5% of beet pulp as dry matter in diets for 2 weeks, the incremental addition of beet pulp in the diets did not alter energy intake.[17]

Satiety is another area of interest regarding the effect of dietary fiber.[13-15] However, the current research findings have not fully elucidated the long term-satiety effects of increased dietary fiber.[6,7,17] Several studies have been conducted to evaluate dietary fiber effects on short term satiety (3-7hr intervals), but these data are inconsistent to date.[6,7,18,19]

Carnitine is an amino acid biosynthesized from lysine and methionine in the liver as well as kidney in the presence of ascorbic acid, iron, niacin, and pyridoxine.[8] The primary function of carnitine is the intracellular transport of long chain fatty acids from cytosol into the mitochondrial matrix where fatty acid β-oxidation occurs. Therefore, supplementation of dietary carnitine potentially enhances β-oxidation while conserving lean body mass during weight loss. Indeed, carnitine supplementation has been widely used in formulated dog foods for both weight reduction and maintenance purposes.

Because pet foods contain a variety of nutrients, it is of interest to evaluate the synergistic effects of supplements containing more that one component. Thus, the combination of dietary fibers plus carnitine using a commercially available dietary supplement was evaluated in this study. In addition to a potential β-oxidation effect of carnitine and possible satiety effect of fiber, the presence of mixed dietary fiber types on canine cholesterol and lipid metabolism as has been reported in humans and rodents[14,15,20,21] was investigated. These aspects have not been fully explored in the canine. Thus, the objectives of the present study were to evaluate:

- Whether short-term satiety is observed with a dietary supplement containing mixed fiber types plus carnitine, and
- The extent to which dietary fibers plus carnitine supplementation affects canine weight management with respect to lipid metabolism including biomarkers of fat

mobilization and utilization, plasma cholesterol, and lipoprotein distributions.

MATERIALS AND METHODS

Animals

All Beagle dogs used in the studies were housed individually at the Laboratory Animal Research and Resources facility, Texas A&M University, according to the American Physiological Society Guidelines for Animal Research and guidelines set forth by Texas A&M University Care and Use Committee. Each dog kennel was 2.4 m (8 ft) long, 2.7 m (9 ft) high, and 1.2 m (4 ft) wide. Prior to the study, physical examinations, complete blood counts, and serum biochemistry profile tests were performed on all dogs to assure their normal clinical status.

Short-term Satiety Study

A commercially available dog food (Purina ONE®, healthy weigth formula, Nestle-Purina, St Louis, MO)and vegetable-based fiber supplement(Fruitables®, Pumpkin SuperBlend™, weight loss supplement, Vet Science LLC, Dallas, TX) were fed. The commercially available dog food was selected as a control diet containing lower fiber/low carnitine (LF/C). A mixture of this food plus the supplement, which contained higher fiber and carnitine, was prepared and designated as high fiber/high carnitine (HF/C). Both diets were nearly isocaloric (Table 1). The ratio of the dog food and supplement (HF/C diet) fed each day followed the feeding instructions provided on the supplement product label. For example, for each 8 oz measuring cup (ca. 96 g) of food fed to the LF/C group, 0.75 cup (ca. 72g) of food and 0.25 can (15 oz can) (ca. 106g) of supplement were mixed as the HF/C diet. Total dietary fiber (TDF) concentrations of the LF/C and HF/C diets were 35.0 and 45.3 g/1,000 kcal, respectively. In addition, 4.8 and 388.7 mg/1,000 kcal of carnitine was present in the LF/C and HF/C diets, respectively (Table 1). The HF/C diet contained 56% moisture, while that of the LF/C was 5%. Because moisture content can potentially alter satiety, the moisture content of each diet was equilibrated before feeding.

A 3-hr interval feeding period was chosen to evaluate a possible between meal-

Table 1. *Nutrient Compositions of Diets.*

Nutrient Compositions	Diet			
	LF/C	HF/C	LF/C	HF/C
	g/100g as is		g/1000 Kcal	
Protein-Combustion	12.7	12.3	78.2	78.8
Fiber, Crude	1.1	2.3	6.8	15.0
Total dietary fiber	5.7	7.0	35.0	45.3
Insoluble fiber	4.8	5.9	29.3	37.7
souluble fiber	0.9	1.2	5.7	7.6
Crude fat	4.7	4.3	29.0	27.6
Ash	3.0	3.0	18.4	19.5
Carbohydrates, Calculated	22.2	21.7	137.0	139.8
Moisture	56.3	56.3	347.1	362.0
Carnitine	0.8	60.5	4.8	388.7
	kcal/100g as is			
Metbolizable energy, Calculated	162.2	155.5		

satiety effect of the HF/C diet. Twelve adult female Beagles ranging in age between 4 to10 years were used. The average of their body weight was 15.2 ± 0.93 kg and body fat was 45.0 ± 1.46 %. Each dog was fasted overnight and fed at 8:00 am and 11:00 am using 1.2 times the amounts of calculated maintenance energy requirements for each dog (MER, kcal/d = 125 × (body weight) 0.75) for 2 consecutive days followed by a cross-over design with 7-day wash-out periods. Prior to the experiment, dogs were trained to consume foods within 15 min after offering their ration in order to properly evaluate satiety. Thus, any diet amounts left over 15 min after food presentation during the studies was considered as an index of satiety. Energy and gram food intake of each feeding period and daily total energy consumption were recorded. Dogs were allowed to freely exercise in their individual kennels during the study.

Peptide YY (PYY), a satiety hormone that is secreted by the gastrointestinal tract, was determined as an endogenous satiety marker. Its peak level has been reported to appear in the circulation 1-2 hr after food, fat, and fermentable fiber ingestion.22 Therefore, blood samples were collected 0, 45, and 120 min after the foods were offered at 8:00 am from a cephalic vein into heparin containing tubes. Three hundred and sixty KIU/mL whole blood of aprotenin (protease inhibitor) was added into the tubes immediately before the blood collection. Plasma was separated by slow speed centrifugation and stored at -80 °C until analyses.

Plasma PYY determination

Plasma PYY was determined by an enzyme immunoassay kit (Phoenix Pharmaceuticals Inc, Burlingame, CA) after protein extraction according to the manufacturer's protocol. Dilution parallelism showed a linear curve fit with $\gamma 2=0.999$. The spike recovery was in range between 95.5 and 112.9 %. The typical detection limit of this assay was 0.07 ng/mL with an interassay CV of 9.3 %.

Six-Wweek Weight Loss Study

Seven intact adult female obese Beagles ranging in age between 4 and 10 years were used employing a completely randomized design. The recommended amount of food restriction without losing excessive lean body mass loss is generally considered to be 60 % MER.23 Therefore, each dog was fed either the LF/C (n=3) or HF/C (n=4) diet at 60 % MER once daily in the morning for 42 days. The initial body weight in each group was 14.6 ± 1.3 kg in LF/C and 15.8 ± 1.6 kg in HF/C, respectively. Body weights were monitored weekly. Percentage body fat was initially 44.3 ± 2.0 % in LF/C and 45.8 ± 2.5 % and was measured using a body fat analyzer employing bioelectrical impedance (Kao Corporation, Tokyo, Japan) at days 1, 28, and 42. On days 1 and 28, 60

Table 2. *Food Intake and Energy Intake of 3 hr Feeding Interval Trial.*

	3 h interval		
Food intake (g/d)	LF/C	HF/C	*P*-value
1st fed	490.3 ± 49.1	420.9 ± 45.0	ns
2nd fed	281.4 ± 61.1*	149.8 ± 36.7*	0.040
total	771.6 ± 93.1	570.7 ± 66.6	0.014
Energy intake (kcal/d)			
1st fed	744.5 ± 82.5	692.0 ± 74.6	ns
2nd fed	445.7 ± 96.8*	246.7 ± 60.5*	0.049
total	1190.2 ± 151.8	938.7 ± 110.0	0.039

Values are mean ± SEM. P-values are for Paired t-test between diets: ns denotes non significance. Asterisks denote significant difference from the 1st fed period within diet. P < 0.05 is considered significant.

Table 3.*Postprandial Plasma Peptide YY Concentrations After Food Was Consumed at the First Feeding Period During 3hr Feeding Interval Trial.*

Postprandial time	Diet		*P*-Value
	LF/C	HF/C	
Plasma PYY (pg/mL)			
0 min	119.9 + 25.5	76.8 + 15.9	ns
45 min	122.1 + 22.1	79.7 + 13.4	ns
120 min	204.5 + 112.8	101.1 + 19.6	ns

Values are mean ± SEM. P-value is for Paired t test; ns represents non significant. P < 0.05 is considered significant.

min postprandial blood was collected. On the final day of the study (day 42), 0 and 60 min postprandial blood was also collected. Plasma was separated by low speed centrifugation and stored at -80 C until analysis except that lipoprotein electrophoresis was performed on freshly separated samples.

Plasma Lipid Determination

Plasma triglyceride (TG) concentrations were measured by an enzymatic colorimetric assay using a commercially available reagent (Triacylglycerol GPO reagent, Bayer HealthCare AG, Leverkusen, Germany) Total cholesterol (TC) was measured using an enzymatic colorimetric assay described by Warnick.24 Lipoprotein (LP) fractions (β, pre-β, α1, and α2 -LPs) were separated by electrophoresis using 1 % agarose gel (TITAN GEL Lipoprotein Electrophoresis System, Helena Laboratories, Beaumont, TX).

β-hydroxybutyrate (BHB) is one of the end products of fatty acid oxidation in the liver. Because BHB is not utilized in the liver, it is mobilized to peripheral tissues via the circulation and used as one form of energy. Therefore, plasma BHB was measured as a marker of fat oxidation in vivo using a two point kinetic method.25

STASTICAL ANALYSES

Data were expressed as means ± SEM. All data were analyzed by SPSS 15.0 for Windows. Repeated measures ANOVA using a general linear model was performed using all data. Diet and time (feeding time periods or postprandial times) were included as a within-subject factor for the satiety study. For the data obtained from the weight loss study, diet was considered as a between-subject factor and week as a within-subject factor. Follow up tests using pairwise comparisons were performed where statistical significance was found with Bonferroni adjustment. Student's t-test was assessed to check for a diet effect in the satiety studies between each feeding time period. For the weight loss study, in order to assess the independent effects of diet on d 42 plasma lipids independent of body weight and fat loss, an analysis of covariance model was used controlling for body weight and fat change from baseline (day 1). Normality and homogeneity of variances of all data were analyzed before all tests were conducted. Non-normally distributed data were assessed by appropriate non parametric tests (ie, Mann-Whitney U test). Where variances were not homogeneous, data were transformed as log10. Differences were considered significant at $P < 0.05$.

RESULTS

Food Intake during the Satiety Studies

The first objective of this study was to measure the short-term satiety effect of the dietary fiber/carnitine supplement combination. To do this we conducted the 3 hr interval feeding trials and monitored food intake and measured plasma PYY. Although dogs fed both diets consumed less food and energy at the 2nd feeding period, the degree of suppression of intake was higher with the HF/C diet. This lower intake at the 2nd feed-

ing period with the HF/C diet consequently resulted in a decreased total daily weight of food and energy intake (Table 2, $P < 0.05$).

Plasma PYY Concentrations in the Satiety Studies

PYY concentrations in the LF/C diet were numerically greater at each postprandial time including 0 min (Table 3). Therefore, a significant main diet effect was observed in postprandial plasma PYY with the greater PYY concentrations in the LF/C diet ($P = 0.039$). However, when PYY concentrations were converted to relative change based on time 0, the main diet effect was eliminated (data not shown). Moreover, no statistically significant diet effect and PYY concentration were observed at any postprandial sample period (Table 3).

Body Weight and Fat Loss in the Weight Loss Study

All dogs lost body weight ($P = 0.003$), however, repeated measures revealed that only the HF/C diet group had a significant decrease in body weight at day 42 compared with days 1 and 28. This same effect was observed for body fat content. While all dogs lost some body fat ($P = 0.030$), the HF/C diet resulted in a statistically significant decrease in body fat at day 42 compared with days 1 and 28 (Table 4).

Plasma Lipid profiles in the Weight Loss Study

The HF/C diet fed dogs showed significantly elevated BHB concentrations at day 42 postprandially compared with the LF/C diet ($P = 0.049$, Table 5), whereas the BHB concentrations at 0 min on day 42 were the same independent of diet.

Neither time nor diet effects were observed for plasma TG, TC, and PYY (Table 5). In addition, these findings were unchanged when loss of body weight and body fat were included as a covariate at d 42.

A pre-β LP fraction was not clearly observed after electrophoresis of the plasma samples. Therefore, the pre-β fraction was combined with the β fraction and data were presented as pre- β + β LP-cholesterol. Statistical analyses did not show any significant time or/and diet effects in any of LP cholesterol fractions including pre- β + β LP, α2, and α1 LP-cholesterols (Table 5). Moreover, the loss of body weight and body fat included as covariates at d 42 of LP-cholesterol fractions also did not show any significant diet effect.

DISCUSSION

The present study aimed to evaluate the effect of carnitine and dietary fiber supplementation on satiety as well as weight loss using a commercially available dog food and supplement in order to address a practical question in obesity management. The supplement selected was enriched in dietary

Table 4. *Body Weight and Body Fat During Weight Loss Study.*

		Day			P- value		
		d 1	d 28	d 42	time	diet	time x diet
Body weight (kg)							
	LF/C	14.6 + 1.3	13.5 + 1.3	13.2 + 1.3	0.003	ns	ns
	HF/C	15.8 + 1.6a	14.3 + 1.4[a]	13.8 + 1.4[b]			
Body fat (%)							
	LF/C	44.3 + 2.0	36.6 + 6.6	24.6 + 4.1	0.030	ns	ns
	HF/C	45.8 + 2.5[a]	38. + 3.8[a]	25.6 + 3.8[b]			

Values are mean ± SEM. Letters not in common denote significant differences among days within the diet, P<0.05. P-value is for repeated measures ANOVA using GLM model; ns represents non significant.

Table 5. *Postprandial Plasma Lipid, Lipoproteins, and Peptide YY Concentrations*

			Diet		P-value	
		Postprandial time	LF/C	HF/C	Repeated measures ANOVA	Student's test
BHB (mg/dL)						
	d1	60 min	1.68 + 0.32	2.10 + 0.58	ns	ns
	d28	60 min	1.67 + 0.22	1.99 + 0.11	ns	ns
	d42	0 min	2.02 + 0.34	2.20 + 0.24	ns	ns
		60 min	1.15 + 0.19	1.85 + 0.32	ns	0.049
TG (mg/dL)						
	d1	60 min	50.14 + 4.76	71.91 + 12.59	ns	ns
	d28	60 min	28.08 + 8.16	46.14 + 13.63	ns	ns
	d42	0 min	36.12 + 0.34	42.05 8.21	ns	ns
		60 min	34.42 + 2.73	42.18 + 8.39	ns	ns
TC (mg/dL)						
	d1	60 min	244.17 + 38.37	226.75 + 22.42	ns	ns
	d28	60 min	232.30 + 35.70	234.30 + 36.32	ns	ns
	d42	0 min	255.10 + 42.01	211.98 + 55.45	ns	ns
		60 min	237.63 + 32.27	208.58 + 49.13	ns	ns
Pre-β + β LP-cholesterol (mg/dL)						
	d1	60 min	48.07 + 7.62	68.75 + 1.29	ns	ns
	d28	60 min	69.02 + 14.96	73.44 + 14.06	ns	ns
	d42	0 min	56.16 + 7.03	54.16 + 15.15	ns	ns
		60 min	51.99 + 4.27	51.27 + 12.39	ns	ns
α2 LP-cholesterol (mg/dL)						
	d1	60 min	76.76 + 15.25	57.35 + 11.06	ns	ns
	d28	60 min	57.54 + 12.25	53.25 + 9.26	ns	ns
	d42	0 min	75.82 + 24.01	54.88 + 19.85	ns	ns
		60 min	79.57 + 24.19	49.32 + 14.03	ns	ns
α1 LP-cholesterol (mg/dL)						
	d1	60 min	113.24 + 16.15	95.64 + 10.51	ns	ns
	d28	60 min	104.26 + 9.86	102.87 + 16.16	ns	ns
	d42	0 min	123.12 + 16.90	102.52 + 22.23	ns	ns
		60 min	11.08 + 12.09	102.33 + 24.86	ns	ns
PYY (pg/mL)						
	d1	60 min	35.161 + 4.146	97.859 + 39.277	ns	ns
	d28	60 min	62.656 + 21.795	94.110 + 44.347	ns	ns
	d42	0 min	30.995 + 19.095	66.739 + 46.963	ns	ns
		60 min	51.325 + 8.515	79.238 + 35.090	ns	ns

Values are mean ± SEM. D 1, 28, and 42 indicate sample taking in days.
P-value of repeated measures includes diet, time, and diet × time effect. No such significances were observed in plasma lipid profiles. P-value of Student's t-test indicates the difference between diet at each day or each postprandial period; ns represents non significant. $P < 0.05$ is considered significant.

fiber including both soluble and insoluble fiber types and carnitine. In order to provide similar nutrient compositions and calories, a commercially available, dry-extruded, complete and balanced food for weight management was selected. The combination of this diet plus supplement provided 30 % more dietary fiber with the same ratio of soluble and insoluble fiber, and 82 times more carnitine compared to the extruded product alone.

For the 3 hr interval satiety study, an equivalent amount of moisture was added to the LF/C diet as was present in the HF/C diet to control for any palatability and satiety differences due to moisture content.[26,27] The results indicated that the HF/C diet provided better satiety by decreasing both food (gram basis) and energy intake at the 3 hr post food administration period. Weber et al also reported lower food consumption at the 2nd feeding period after a 3 hr interval when dogs were fed a high fiber/high protein containing diet (97.9 g/1,000 kcal total dietary fiber) compared with a high protein diet only (55.9 g/1,000 kcal total dietary fiber). Jackson et al also found a satiety effect on dietary fiber in dogs.[3] Therefore, the satiety effect observed in this 3 hr interval study may be the result of higher dietary fiber contents in the HF/C diet compared to the LF/C diet. Whether carnitine also provides an additive effect on satiety is unknown at this time. Several researchers have reported that fat oxidation is an important regulatory stimulus for the regulation of food intake.[28-30] Because carnitine is considered to support increased β-oxidation postprandially, the possibility exists that carnitine may also affect food intake. However, food intake regulation by fat oxidation may more likely occur due to subsequent high fat intake.[28-30] If so, in the present study, because the fat content in both diets was similar, the different carnitine contents in the diets probably would not have contributed to food intake regulation.

In the present study, PYY was selected as an endogenous satiety marker and was measured. The reason for this is because rat studies consistently showed the increased PYY secretion in the large intestine as well as blood circulation by fermentable fiber administration. [32-33] In the present study, although the extent of fiber fermentation resulting from the diets was specifically unknown, soluble fiber is generally considered to largely reflect fermentable fiber.[34] Because the HF/C diet contained 30 % more soluble fiber than the LF/C diet, it was expected to increase plasma PYY postprandially in the HF/C diet vs. the LF/C diet. Nevertheless, plasma PYY concentrations were not different between diets and interestingly, this finding is consistent with several canine and human reports. For example, Bosch et al measured the alteration of canine plasma PYY concentrations during 495 min post-feeding of high or low fermentable fiber to healthy mixed gender Beagle dog groups, yet they did not find any differences in plasma PYY due to diet.19 Human studies measuring plasma PYY after fermentable fiber ingestion found blunted PYY concentrations compared to a rat study with a similar design.[34,35] Furthermore, it has been reported that insoluble fiber intake eliminated postprandial PYY elevation in adult women.36 Taken together, these reports indicate that PYY in dogs and humans may not be as sensitive to fermentable fiber as rats. In addition, higher insoluble fiber contents in the HF/C diet may attenuate the possible fermentable fiber effect on plasma PYY concentrations. Another factor to also consider is that dogs used in the present study were either overweight or obese. Obese subjects usually show a delayed onset of satiety after consuming a meal.[37] Therefore, the overweight/obese condition of dogs in the present study may also have resulted in less sensitivity to 2-hr postprandial PYY secretion. In order to clarify these speculations, further investigations will be needed.

During the 6-week weight loss study, all dogs consumed all foods that were offered. The amount of food offered aimed to achieve a 1.0-2.0 % rate of weight loss per week. Therefore, as expected, all dogs lost weight. However, only the HF/C diet group

significantly decreased their body weight at day 42 compared with days 1 and 28. The rate of weight loss was 2.1 % for the HF/C diet, which is close to the range of recommended safe weight loss.23 On day 42, a significant difference in postprandial BHB concentrations was observed with the HF/C diet vs the LF/C diet. Consequently, our finding of less of a decrease in BHB concentration with the HF/C diet suggested greater β-oxidation at day 42. This finding is consistent with significant body fat loss with the HF/C diet on this same day and is reflected by greater β-oxidation. Therefore, the HF/C diet appeared to preferentially reduce body weight in these dogs at 42 days of feeding with more body fat loss due to β-oxidation. Although soluble fibers have been reported to have a hypocholesterolemic effect,38 the 7.6 g/1000 kcal amount of soluble fiber used in this study did not influence the plasma lipid profiles including cholesterol, TG, and LPs during the 6 week feeding period.

CONCLUSION

In conclusion, the combination of dietary fiber and carnitine supplementation using a commercially available dog food and supplement improved satiety at 3 hr postprandially while increasing fat oxidation and was successful in decreasing a greater degree of body fat loss associated with body weight loss. Plasma lipids including triglyceride, total cholesterol, and lipoprotein fractions were unchanged with this supplement combination during a 42 day feeding period. These findings may help provide better choices for both owners and professionals to achieve healthy weight loss for their pets.

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