

Effect of short-chain fructooligosaccharide-enriched energy-restricted diet on weight loss and serum haptoglobin concentration in Beagle dogs

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Abstract

The effects of the dietary inclusion of two levels of short-chain fructooligosaccharides (sc-FOS) on weight loss, biochemical parameters and serum haptoglobin concentration were investigated in twelve experimental obese Beagle dogs. Dogs were randomised into two groups and submitted to a weight loss program (WLP): the control group (C) received a commercial energy-restricted high-protein diet containing 1% DM sc-FOS, whereas the test group (T) received the same diet enriched with sc-FOS to attain a 3% DM content. Body weight (BW) and body condition score were weekly assessed in each dog and blood was collected before and after WLP to measure total plasma cholesterol (CHOL), TAG, NEFA, glucose (GLUC), insulin, serum leptin and haptoglobin. Groups showed similar BW and blood parameters before treatment. When values before and after treatment of the dogs were compared, significant reductions were observed for all parameters, with the exception of NEFA and GLUC. However, when these reductions were compared between C and T groups, significant differences were detected only for haptoglobin (T before *v.* T after: 1545 *v.* 605 mg/l, *P*=0.03; C before *v.* C after: 1635 *v.* 1400 mg/l, *P*=NS). Positive correlations between haptoglobin and CHOL and between haptoglobin and TAG were observed before but not after WLP. In conclusion, feeding obese dogs with the energy-restricted diet caused significant weight loss and reduction of blood parameters, irrespective of the sc-FOS content included. However, serum haptoglobin level, and the subclinical inflammatory condition associated with it, was significantly lowered in the T but not in the C group.

Key words: Canine obesity; Short-chain fructooligosaccharides; Weight loss; Haptoglobin

Recent studies have underscored the increased efficiency of obese mice's microbiota in harvesting energy from the diet^(1,2), suggesting that the gut microbial population acts as an environmental factor influencing fat storage and obesity development⁽³⁾. On the basis of these findings, the increased attention recently addressed to the role of dietary fiber on the lipid profile and glucose (GLUC) metabolism of obese dogs seems appropriate, as it has the potential to modulate gut microbiota⁽⁴⁾. Short-chain fructooligosaccharides (sc-FOS) are non-viscous β -fructan fibers that enhance a beneficial microbial fermentation in the colon by producing SCFA, mainly acetate and propionate. A series of studies carried out on rodents demonstrated that inulin-type fructans (e.g. inulin and oligofructosaccharides) affect lipid metabolism by lowering serum TAG concentration⁽⁵⁾ and, even if to a lesser degree, decreasing serum cholesterol (CHOL)⁽⁶⁾. The suggested mechanisms to explain these effects are related

mainly to the decrease of the activity of lipogenic hepatic enzymes and to the production of butyrate, a SCFA that inhibits liver CHOL synthesis^(7,8). In human subjects, however, results on the effect of inulin-type fructans on lipid metabolism are conflicting. Most of the studies failed to demonstrate a beneficial effect in reducing plasma lipid concentrations in healthy volunteers, whereas hypotriglyceridaemic and/or hypocholesterolaemic effects were achieved in type-2 diabetic and hyperlipidaemic patients; in all studies, the dietary supplementation of either inulin or oligofructosaccharides ranged from 7 to 20 g/d and the time of administration was between 2 and 8 weeks⁽⁶⁾. The variability of the basal diet and the difficulties in controlling the nutrient intake in human subjects may explain, at least in part, the lack of consistent results achieved in human studies⁽⁹⁾. Our hypothesis is that sc-FOS dietary inclusion may influence the metabolism of obese dogs and reduce the inflammatory state due to the

Abbreviations: BW, body weight; C, control group; CHOL, cholesterol; GLUC, glucose; sc-FOS, short-chain fructooligosaccharides; T, test group; WLP, weight loss program.

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chronic overweight condition. To date, no indications are available on the optimal sc-FOS level in the diet to enhance beneficial effects on the metabolism of obese dogs.

In the present study, we therefore investigated the effects of two levels of sc-FOS (1 *v.* 3% DM) on weight loss, biochemical parameters and serum haptoglobin in obese dogs. With this aim, a commercial dry energy-restricted diet containing 1% DM sc-FOS was used as control and supplementary sc-FOS were added to the diet to reach 3% DM of dietary content.

Experimental methods

The present study was carried out using twelve chronically obese Beagle dogs (six neutered males, three entire females and three neutered females, aged between 3 and 9 years; mean body weight (BW) 21.9 (SEM 2.7) kg) with a body condition score of 7 or 8 on a nine-point scale⁽¹⁰⁾. Dogs were randomised into two groups of six individuals and submitted to a weekly 1–2% weight loss program (WLP) until optimal body condition score was obtained. The experimental protocol was approved by the Ethical Committee of the University of Liege, Belgium, before experimentation.

The WLP was implemented using a commercial energy-restricted high-protein extruded diet (as fed: 34% crude protein, 9.5% fat and 12 kJ Metabolizable Energy (ME)/g, Obesity Veterinary Diet, Royal Canin, Aimargues, France), which contains 1% DM sc-FOS, as included by the manufacturer. The control group (C) received the commercial diet only, whereas the test group (T) received the same diet enriched with a sc-FOS supplement (Beghin-Meiji Industrie, Marckolsheim, France) in order to attain a 3% DM sc-FOS dietary content.

BW and body condition score were weekly assessed for each dog and an individual blood collection was carried out before and after WLP to quantify plasma CHOL, TAG, NEFA, GLUC, insulin, leptin and haptoglobin. Briefly, blood samples were collected in tubes containing K₃EDTA as anticoagulant and kept frozen at –20°C until assayed. Plasma CHOL, TAG and GLUC were analysed by Technicon autoanalyser RA-1000 (Bayer Diagnostics, Holliston, MA, USA) using reagents from Bayer (Leverkusen, Germany), whereas NEFA concentrations were assayed by means of an enzymatic method (NEFA, Half microtest; Roche Diagnostics GmbH, Penzberg, Germany). Insulin plasma concentrations were

analysed using a commercial RIA kit (INS-IRMA kit; Biosource Europe, Nivelles, Belgium) and leptin levels were measured using a canine-specific ELISA method validated by Iwase *et al.*⁽¹¹⁾. An assay was developed at the laboratory of Biochemistry Unit of the Faculty of Veterinary Medicine in Liege in order to measure plasma canine haptoglobin concentration. This is a photometric method that measures the peroxidase activity of the haptoglobin–cyanmethaemoglobin complexes, as described elsewhere⁽¹²⁾.

Data were analyzed using PROC GLM of SAS (Statistical Analysis Systems statistical software package version 6.11; SAS Institute, Cary, NC, USA) to initially detect the effect of group (C *v.* T) and sex (females *v.* neutered females *v.* neutered males) on BW and blood parameters measured before WLP. A SPLIT PLOT model using PROC GLM of SAS (SAS Institute) was then adopted to detect the effects of dietary treatment, time (before *v.* after WLP) and the interaction between dietary treatment and time on BW and all blood parameters of dogs being treated. *P* values for the multiple comparisons of the interaction effect have been adjusted using the Bonferroni method. Pearson correlation analyses were performed on BW and blood parameters using data measured before and after WLP, with *P* values <0.05 considered significant.

Results

All dogs reached body condition score of 5–9 between 21 and 32 weeks (average 26 weeks) and the average BW attained at the end of the WLP was 14.4 (SEM 1.1) kg (ranging from 12.7 to 15.8 kg). Before WLP, no differences in BW and blood parameters were detected either among sexes or between treatments; therefore, groups were considered homogenous.

The effects detected in the present study when values before and after the dietary treatment were considered are shown in Table 1. The dietary treatment (1 *v.* 3% DM sc-FOS) had a significant influence only on haptoglobin concentration (C group (1518 mg/l) *v.* T group (1075 mg/l), *P*<0.05), whereas time significantly affected dogs' BW, CHOL, TAG, insulin, leptin and haptoglobin, which were significantly lower after WLP; GLUC, on the contrary, was significantly higher and NEFA was unaffected by time. However, when blood parameter reductions after WLP were

Table 1. *P* values of the effects of dietary treatment (1 *v.* 3% DM short-chain fructooligosaccharide), time (before *v.* after weight loss program) and interaction of dietary treatment and time on body weight (BW) and blood parameters of the dog (Root mean squared errors (RMSE), goodness of fit (*R*²) and coefficients of variations)

Parameters	<i>P</i>				RMSE (df = 10)	<i>R</i> ²	CV
	Dog (df=10)	Dietary treatment (df = 1)	Time (df = 1)	Dietary treatment × time (df = 1)			
BW	NS	NS	<0.001	NS	1.50	0.95	8.25
NEFA	NS	NS	NS	NS	0.37	0.75	43.68
CHOL	<0.01	NS	<0.001	NS	0.20	0.93	9.79
TAG	NS	NS	<0.01	NS	0.59	0.70	48.20
Glucose	NS	NS	<0.001	NS	0.45	0.87	8.33
Insulin	NS	NS	<0.05	NS	4.73	0.67	38.56
Leptin	NS	NS	<0.001	NS	4.34	0.84	55.37
Haptoglobin	NS	<0.05	<0.01	<0.05	447.14	0.79	34.49

CHOL, cholesterol.

Table 2. Body weight (BW) and blood parameters resulting from the interaction between dietary treatment and time in test (T) and control (C) groups

(Estimated least squares mean values and standard error of the difference (SED))

Parameters	C before (n 6)*	C after (n 6)*	T before (n 6)†	T after (n 6)†	SED‡
BW (kg)	22.17 ^b	13.9 ^a	21.76 ^b	15.01 ^a	0.86
NEFA (mmol/l)	0.75	0.96	0.91	0.80	0.21
CHOL (mmol/l)	2.36 ^b	1.64 ^a	2.36 ^b	1.74 ^a	0.11
TAG (mmol/l)	0.83	0.39	0.79	0.35	0.17
Glucose (mmol/l)	4.70 ^b	5.85 ^a	4.99 ^b	6.27 ^a	0.25
Insulin (pmol/l)	16.10	11.23	13.81	7.95	2.73
Leptin (ng/ml)	11.93 ^{b,c}	1.05 ^a	14.42 ^c	3.98 ^{a,b}	2.50
Haptoglobin (mg/l)	1635 ^b	1400 ^{a,b}	1545 ^b	605 ^a	258.15

CHOL, cholesterol.

^{a,b,c} Values within a row with unlike superscript letters were significantly different ($P < 0.05$). P values have been adjusted by Bonferroni test.

* 1% DM short-chain fructooligosaccharide (sc-FOS).

† 3% DM sc-FOS.

‡ The SED shown is the maximal one based on between dog variation and was also used for the multiple comparisons.

compared between C and T groups, significant differences were detected only for haptoglobin, as the T group exhibited a significant reduction after WLP, which was not detected in the C group (T before *v.* T after: 1545 *v.* 605 mg/l, $P = 0.03$; C before *v.* C after: 1635 *v.* 1400 mg/l, $P = \text{NS}$; Table 2).

Significant positive correlations were observed before WLP between haptoglobin and TAG (0.65, $P < 0.05$) and between haptoglobin and CHOL (0.78, $P < 0.01$), but not after WLP (haptoglobin and TAG -0.15 , $P = \text{NS}$ and haptoglobin and CHOL 0.07, $P = \text{NS}$).

Discussion

sc-FOS are inulin-type fructans that are hydrolysed and completely fermented by the colonic microflora to produce gases and SCFA. By modulation of the composition of the microflora in the colon, sc-FOS have the potential to improve colonic health and consequently the well-being of the host⁽⁴⁾.

The use of a high-protein low-carbohydrate diet has already been demonstrated to be effective in ensuring canine weight loss, minimising lean body mass losses⁽¹³⁾ and reducing the level of plasma CHOL and TAG concentrations in obese dogs⁽¹⁴⁾. In the present study, the dogs were chronically obese, and the effect of sc-FOS included in an energy-restricted high-protein diet on BW loss and blood parameters was investigated.

A blend of fibers (5 and 10% DM), including sc-FOS, has already been shown to be efficient in lowering the post-prandial GLUC, urea and TAG concentrations, as well as the pre-prandial concentrations of urea, TAG and CHOL after a 6-week period of administration in a group of healthy dogs⁽¹⁵⁾. More recently, the 1% DM sc-FOS inclusion in a diet aiming to maintain dogs in an obese state decreased insulin resistance, although CHOL and TAG were not affected by the treatment (control diet *v.* sc-FOS diet: CHOL 5.4 (SEM 0.7) *v.* 5.5 (SEM 0.7) mmol/l and TAG 1.4 (SEM 0.6) *v.* 1.5 (SEM 0.5) mmol/l)⁽¹⁶⁾.

In the present study, a significant reduction of both BW and blood parameters was observed after WLP, and, although GLUC concentration significantly increased, its value was still within the physiological range. Notwithstanding this,

we observed that including either 1% DM or 3% DM sc-FOS to the energy-restricted diet induced no differences in either BW or biochemical parameters and this may be due to the small difference in the quantity of sc-FOS between the two diets.

Interestingly, when blood parameter values after WLP were compared between C and T groups, haptoglobin was found to be significantly lower in the group receiving 3% DM sc-FOS.

It is known that obesity promotes a low-grade inflammatory state, and in human subjects, concentrations of haptoglobin and other inflammatory markers have been shown to significantly increase with obesity⁽¹⁷⁾ and to decrease as a consequence of body fat mass reduction⁽¹⁸⁾. Therefore, recent attention has focused on the measurement of acute phase proteins in canine obesity. German *et al.*⁽¹⁹⁾ demonstrated a significant decrease of haptoglobin and C-reactive protein concentrations in twenty-six obese dogs after weight loss, suggesting a relationship between obesity and a subclinical inflammatory state. On the contrary, a recent study carried out on Beagle dogs failed at demonstrating an increase in haptoglobin and other acute phase protein concentrations after an experimentally induced fattening period⁽²⁰⁾; however, authors concluded that this was probably due to the short-term fattening period (10 weeks).

Our results confirm that the obese condition in dogs is characterised by a significantly higher haptoglobin concentration in the blood and they further demonstrate that haptoglobin is positively correlated to the lipaemic profile of the dogs, and particularly to TAG and CHOL concentrations in the blood. Surprisingly, in the present study, haptoglobin concentration before WLP was not correlated to leptin, which is a quantitative marker of adiposity in dogs.

Although the small number of dogs used in the present study certainly represents a limit, however, some conclusions can be drawn. Feeding obese dogs with an energy-restricted high-protein diet caused significant weight loss and reduction of obesity-related biochemical parameters such as TAG, CHOL, insulin and leptin, irrespective of the sc-FOS content in the diet (1% DM or 3% DM). Conversely, serum haptoglobin level, and the subclinical inflammatory state associated with it, was significantly lowered only when 3% DM sc-FOS

was included in the diet. Further studies involving a larger population will be needed to investigate the effect of including inulin-type fructans on canine obesity and metabolism.

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