

Deuxième partie :

PRESENTATION DES RECHERCHES

Introduction

Les 7 études sont présentées sous forme d'articles originaux publiés (études 4 et 5), acceptés (études 1, 2 et 3) ou soumis pour publications dans des revues scientifiques (études 6 et 7). Les études 1 et 2 font l'objet d'un seul article; les études 3, 4 et 5 ont été rédigées séparément et les études 6 et 7 sont présentées sous forme d'une série de 2 articles. Certaines études ont également été présentées sous forme d'abstract lors de congrès (études 1, 2, 4 et 5).

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Etudes 1 et 2

Dietary fibre in dog's diet : comparisons between cellulose, pectin, guar gum, and between two incorporation rates of guar gum.

Etude 3

The influence of sugar-beet fibre, guar gum and inulin on nutrient digestibility, water consumption and plasma metabolites in healthy Beagle dogs.

Etude 4

Influence of a blend of fructo-oligosaccharides and sugar beet fiber on nutrients digestibility and plasma metabolites concentrations in healthy Beagles.

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Influence de l'incorporation des pulpes de betterave ou de chicorée sur la digestibilité des nutriments et les concentrations plasmatiques de plusieurs métabolites.

Etudes 6 et 7

Influence of dietary fibers in healthy and obese Beagles :

I. Effects on feces and digestibility of the nutrients

II. Effects on plasma metabolites and insulin concentrations

ETUDES 1 et 2

Dietary fibre in dog's diet : comparisons between cellulose, pectin, guar gum, and between two incorporation rates of guar gum

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Introduction

Nowadays, most dogs receive exclusively commercial dog food as their sole diet. A complete and balanced diet is desirable for dogs at all life stages and must be offered to cover daily nutritional and energy requirements. Although dietary fibres (DF) are not considered as essential nutrients, they are nevertheless beneficial to the health (LEIBETSEDER 1982) and are incorporated at a low rate of 1 to 5 % dry matter in most dog foods. They are also used at higher rate, up to 20 % dry matter as an aid in the treatment of patients with obesity, diabetes mellitus, gastrointestinal diseases or hyperlipidaemia (BLAXTER et al. 1990; DIMSKI and BUFFINGTON 1991; NELSON 1992)

In a first study (Expt 1), we determined the effects of the incorporation of 3 purified fibres in healthy adult Beagle dogs on gastric emptying rate, xylose absorption, digestibility and postprandial plasma metabolites. Cellulose (CEL) was used as insoluble fibre and, guar gum (GG) and pectin (PEC) as soluble fibres. In a second study (Expt 2), the effects of two levels of GG were investigated. Pre- and postprandial plasma metabolites and nutrients digestibility were measured.

Material and methods

Animals and experimental set-up

Experiment 1

For the investigation, 4 young adult Beagle dogs (2 males and 2 neutered females) with an average age of 3 years and an average weight of 11.4 kg at the beginning of the experiment were used in a 4 X 4 Latin square design. A transition period of 1 week was used to adapt to new diets, each period of the Latin square lasted for one month. The dogs were kept separately in an outdoor kennel the first 3 weeks of one period of the Latin square, and then, placed in individual metabolism cages in a room during the last week for total collection of faeces. Room temperature was maintained at 18°C. They were offered water ad libitum during the whole trial. The composition of the control fibre-free diet (FF1), (g/kg dry matter basis) was : minced beef meat 389, cooked rice 472, maize oil 83 and minerals + vitamins 56; it was offered to provide 550 kJ of metabolizable energy (ME) per kg^{0.75} per day (NRC, 1974) to maintain a constant weight. The basal diet was then supplemented with CEL (Arbocell BE 600/30, Rettenmeier and Söhne, Germany), PEC (Pectin Rapid Set 150, Mero-Rousselot-Satia, France) or GG (Viscogum HV 3000A, Mero-Rousselot-Satia, France). The final concentration was 34 g of purified fibre per kg dry matter. The composition and the chemical analysis of the meals are presented in Table 1. The concentrations in the different nutrients in the fibre-supplemented diets were slightly reduced, compared with the control diets. Cooked rice and minced meat were stored in a freezer for the whole experiment. The ingredients and 200 ml of water were mixed daily in a blender during 2 min. When ready, food was offered to the animals after a delay of 5 min. The dogs were fed once a day at 0900 h and used to consume their whole meal within 10 min.

Experiment 2

Six adult Beagle dogs (2 males and 4 neutered females) were used in two combined 3 X 3 Latin square designs. The average age was 3 years and the mean weight was 10.3 kg. They were offered a fibre-free control diet (FF2) or a GG supplemented diet at a rate of 35 g/kg (3.5 % GG) or 70 g /kg dry matter (7 % GG). The composition of the diets are presented in Table 1. The management with the dogs was similar as in Expt 1.

Measurements

Expt 1. Gastric emptying rate was performed by sequential radiographs following a 24 h fast. Barium sulfate suspension was mixed to the diet at a rate of 3 ml/kg body weight. All animals belonged to our lab and thus were familiar with being examined clinically by students. For that reason, they were not sedated before radiological examinations. Radiographs were taken at different intervals postprandially (5, 15, 60, 120, 180, 360, 540, and 720 min) to monitor the transit in the stomach. The limit of the marker was defined on the plate and the area calculated with a computerised system. Gastric emptying was estimated by the changes in the area over time. Because the cross-sectional areas were not always similar, the data were corrected on the basis of the length of vertebra D 10.

The xylose test was used to monitor intestinal absorption following a 24 h fast. An indwelling sterile catheter was inserted into a cephalic vein. Catheters were filled with a heparinised saline (9 g NaCl/l) solution (120 U/ml) between sampling periods. Dogs were handled gently and did not appear excited during sampling. A blood sample was obtained and was considered as the zero-time. A 10 % xylose solution was then given by an intragastric tube at a rate of 0.5 g xylose/kg body weight. Blood was then serially collected into lithium-heparinized tubes after 30, 60, 90, 120, 180, 240, 300 and 360 min.

Digestibility measurements were carried out over 7 days during the last week of a period of the Latin square. Dogs were maintained in metabolism cages. Faeces were collected daily along with samples of the food mixtures. Water intakes were also noted. Metabolic and hormonal profiles were determined during the last day of the week, after the ultime collection of faeces. Blood was taken before feeding and then serially over a 6 h period at sampling times 20, 40, 60, 90, 120, 180, 240 and 360 min after feeding. The technique described for xylose test was used.

Expt 2. Digestibility measurements, metabolic and hormonal profiles were determined as in Expt 1.

Chemical analysis

Plasma xylose concentrations were determined by Autoanalyzer colorimetry using the ferric chloride-orcinol technique (ROBERTS and NORMAN 1979). Dry matter, ash and ether extract in feed and faeces were analysed by standard methods (AOAC 1975). Total dietary fibre was determined in food by a kit (Sigma, TDF-100) according method published by AOAC (1990). Kjeldahl nitrogen was estimated by block digestion and automatic

colorimetry using the Berthelot reaction (VAN EENAEME et al. 1969). Glucose in plasma was analysed by ortho-toluidine (CHARLIER et al. 1974), urea by diacetyl-monoxime (HENRY 1974) and α -amino-nitrogen by the trinitrobenzene-sulfonate (PALMER and PETERS 1969) methods using a Technicon Autoanalyser. Cholesterol and triglycerides were determined by kit procedures (Boehringer, Germany). Plasma insulin was estimated by a heterologous radioimmunoassay for ovine insulin (MICHAUX et al. 1981).

Statistical analysis

Analysis of variance was performed for the digestibility data according to a 4 X 4 Latin square design in Expt 1 and two combined 3 X 3 Latin square design in Expt 2 (DAGNELIE 1975). Data concerning gastric areas, xylose concentrations and plasma metabolites profiles were analysed using a dynamic linear model taking into account that the data were autocorrelated (JONES and BOADI-BOATENG 1991; LAMBERT 1996). The model allowed the inclusion of an autoregression and a random effect. Differences were accepted as significant when $p < 0.05$.

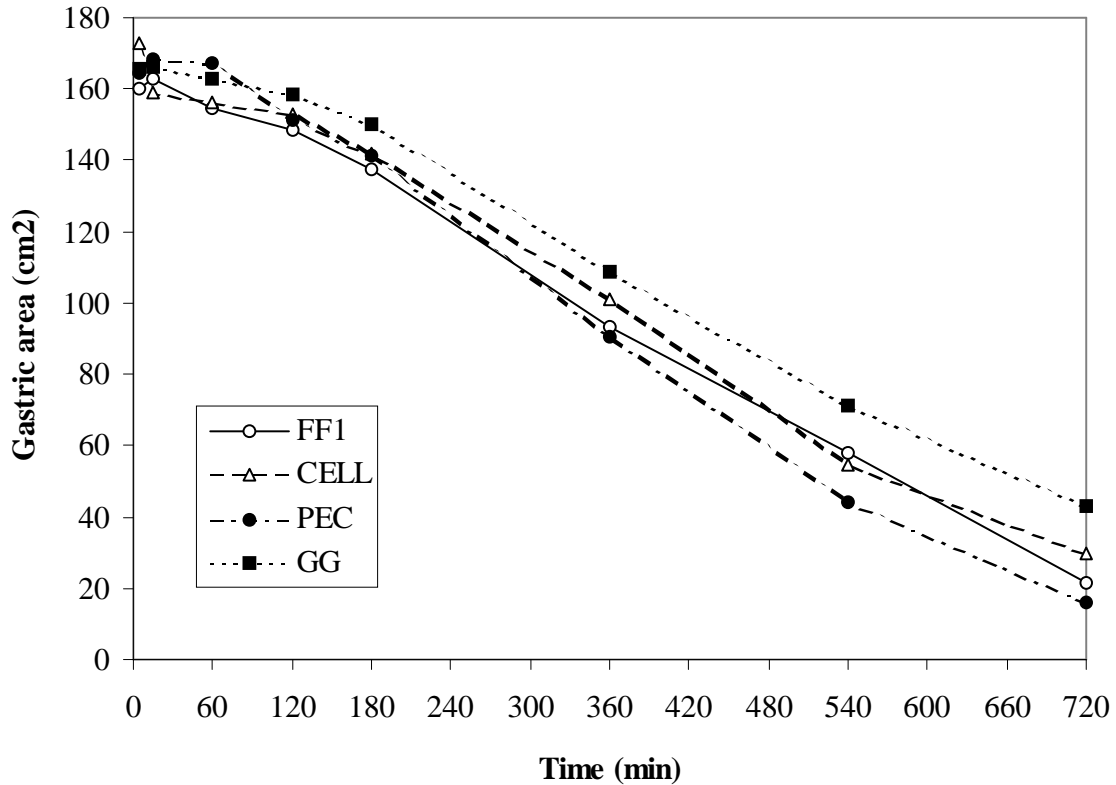
Results

Experiment 1

The daily average dry matter intake was 182.7 g/dog with FF1 diet and was 192.5, 188.6, 189.6 g/dog with CEL, PEC and GG, respectively ($p > 0.05$). The daily drinking water consumption was 170 ml/dog in the FF1 diet and was reduced in a non significant manner with the fibre supplementation (116, 110, and 134 ml/dog, respectively).

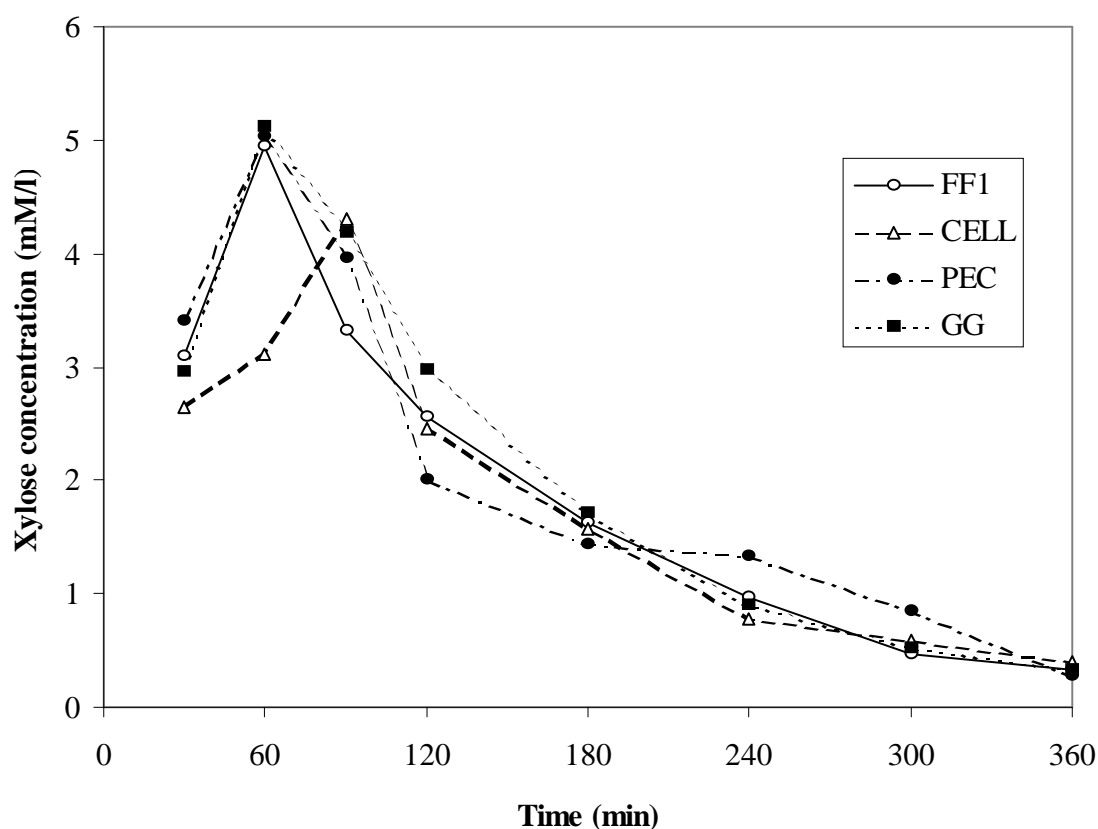
The gastric emptying rates are given in Figure 1. After feeding, the area with marker was approximately 160 cm² when the control diet was offered. It decreased slowly with time to 22 cm² 12 h after feeding. Although no significant differences were observed, the gastric emptying rate seemed to be faster during the first 3 h with the FF1 diet than with the diets enriched with fibre. Six hours after feeding, gastric emptying tended to be the fastest when PEC was added and the slowest with GG ($p < 0.10$).

Fig. 1.- Gastric emptying rates in 4 healthy Beagles offered diets containing different fibre sources.



The changes in the concentration of xylose in the plasma are shown in Figure 2. The concentration at zero-time was considered as a blank value and was subtracted from the values obtained after dosage. The concentration of xylose was 3.00 mM/l 30 min after the xylose solution was given to the dogs; it increased to 4.95 mM/l 60 min after dosage and then declined to 0.32 mM/l after 6 h. The pattern was similar when the diets were supplemented with GG and PEC. By contrast, xylose concentration increased more slowly when CEL was added so that 60 min after the meal, the xylose concentration was lower with CEL than with FF1 or PEC ($p < 0.05$). Furthermore, a 30 min delay was observed for the peak.

Fig. 2.- Changes in xylose concentration in 4 healthy Beagles offered diets containing different fibre sources.



On the whole experiment, digestibility coefficients were high (Table 2). The incorporation of DF reduced the digestibility of the different nutrients, the differences being significant with CELL for dry matter ($p < 0.05$), with GG for protein and ether extract ($p < 0.05$) and with PEC for protein ($p < 0.001$).

The concentrations in plasma insulin and metabolites are given in Table 3. The average plasma glucose concentration before feeding was 4.66 mM/l with no significant differences between treatments. After the meal, there were no significant differences between treatments although GG tended to induce lower glycaemia ($p < 0.10$). Insulin concentration was on average 12.1 mU/l in non fed dogs, with no significant differences between treatments. The inclusion of PEC resulted in higher postprandial values and reached the concentration of 50 mU/l ($p < 0.05$). Plasma α -amino-nitrogen and urea concentrations were similar in non fed animals but the inclusion of GG in the diet induced lower postprandial concentrations ($p < 0.05$ and $p < 0.01$, respectively).

Dietary fibres did not modify plasma triglyceride concentrations measured either in non fed or fed dogs. By contrast, when compared with FF1, inclusion of GG induced lower concentrations of plasma cholesterol in non fed or fed animals ($p < 0.05$).

Experiment 2

The concentrations of the nutrients in the fibre-supplemented diets were slightly reduced as compared with the FF2 diets. The daily average dry matter intake was 172.5 g /dog with the FF2 diet and 176.2 and 186.9 g/dog with 3.5 % GG and 7 % GG respectively ($p > 0.05$). The daily drinking water consumption was 177 ml/dog with the FF2 diet and was 153 and 221 ml/dog with 3.5 % GG and 7 % GG respectively.

The incorporation of GG reduced the digestibility of the different nutrients (Table 2). The differences were significant for crude protein with 3.5 % GG ($p < 0.01$) and 7 % GG ($p < 0.001$). By contrast, ash digestibility was significantly higher ($p < 0.05$ with 3.5 % and $p < 0.001$ with 7 % GG respectively).

The changes in plasma insulin and metabolites are given in Figure 3. There were no effects of increased incorporation rates of GG on pre- or postprandial plasma glucose concentrations (Fig. 3a). Plasma insulin fasting concentration (Fig. 3b) was 12.4 mU/l with FF2 diet; it gradually increased to reach a high value of 25.8 mU/l 3 h after feeding.

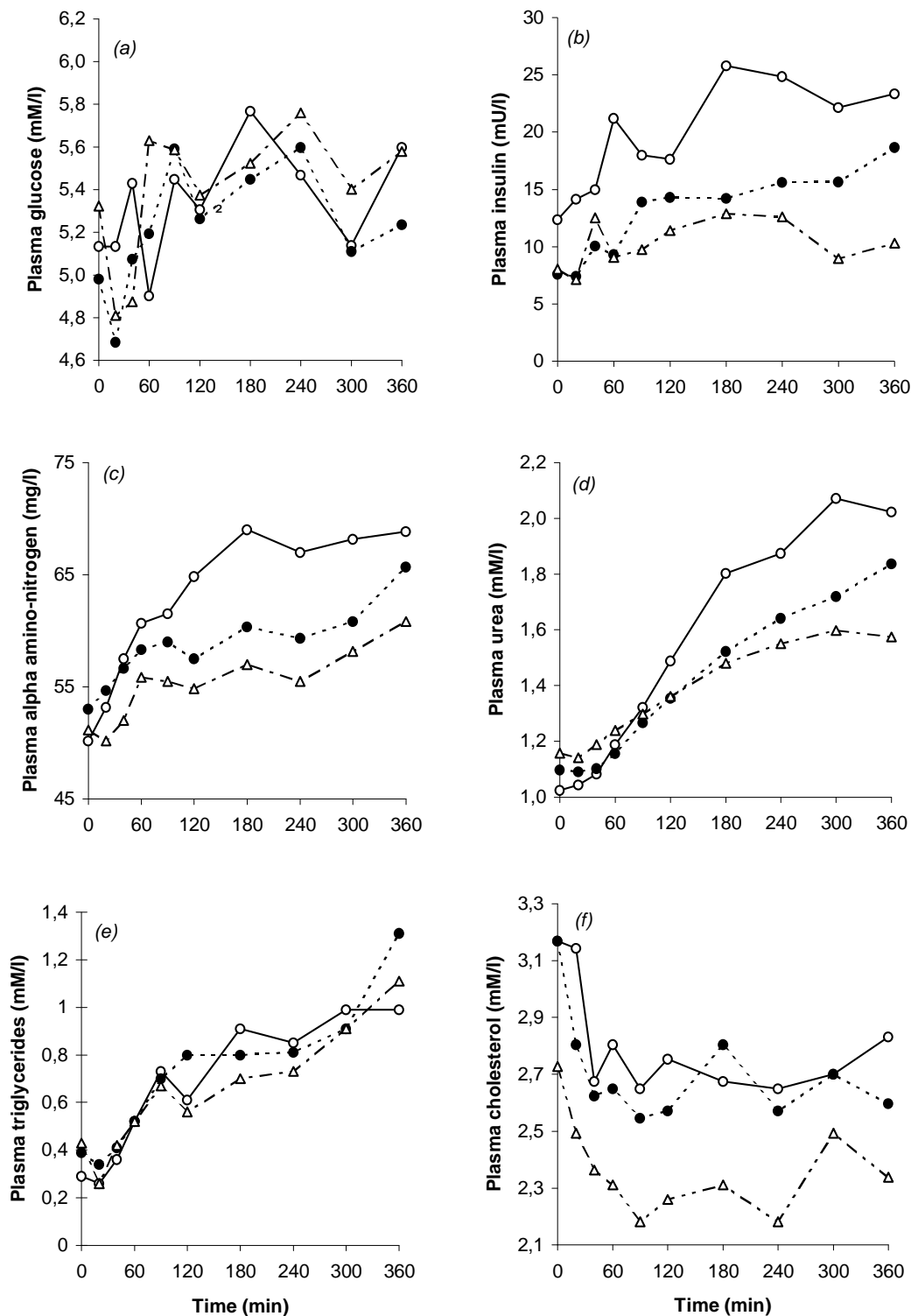
With 7 % GG, the extent of the postprandial rise was much smaller; the highest concentrations being less than twice before feeding. The pattern with 3.5 % GG was intermediate. Using Jones's statistical model, a dose-level effect was observed ($p < 0.011$ for 3.5 % GG *v.* FF2 and $p < 0.005$ for 7 % GG *v.* FF2). The inclusion of GG resulted in reductions of the concentrations in plasma α -amino-nitrogen (Fig. 3c) and urea (Fig. 3 d) in non fed animals ($p < 0.05$). Dose level effects were observed on postprandial plasma concentrations of α -amino-nitrogen ($p < 0.01$ and $p < 0.001$ for the 3.5 % and 7 % GG diets, respectively, *v.* FF2) and urea ($p < 0.001$ for 3.5 and 7 % GG *v.* FF2). The incorporation of GG did not change to a large extent the postprandial pattern of plasma triglycerides (Fig. 3e) although there was a trend for lower concentrations with 7 % GG ($p < 0.10$). The pattern of plasma cholesterol (Fig. 3f) was characterised by high value before feeding at 3.15 mM/l and then a decrease just after feeding to reach concentrations of approximately 2.66 mM/l for the FF2 diet. The inclusion of 7 % GG induced significant reductions both in pre- or postprandial cholesterol concentrations ($p < 0.01$).

TABLE 3.- *Plasma concentrations of metabolites and insulin (Expt 1).*

	FF1	CEL	GG	PEC	SED
Glucose, mM/l					
Before meal	4.84 ^a	4.63 ^a	4.52 ^a	4.65 ^a	0.20
Area under the curve	1722 ^a	1705 ^a	1697 ^a	1756 ^a	29
Insulin, mU/l					
Before meal	13.2 ^a	12.1 ^a	11.4 ^a	11.5 ^a	3.7
Area under the curve	7763 ^a	9323 ^a	8006 ^a	11878 ^b	1479
α-amino-nitrogen, mg/l					
Before meal	50.2 ^a	49.6 ^a	49.9 ^a	48.0 ^a	3.1
Area under the curve	24558 ^a	23473 ^a	22031 ^b	23752 ^a	1066
Urea, mM/l					
Before meal	1.44 ^a	1.13 ^a	1.12 ^a	1.16 ^a	0.16
Area under the curve	907 ^a	822 ^a	753 ^b	839 ^a	61
Triglycerides, mM/l					
Before meal	1.40 ^a	1.45 ^a	1.42 ^a	1.37 ^a	0.06
Area under the curve	608 ^a	547 ^a	551 ^a	612 ^a	33
Cholesterol, mM/l					
Before meal	3.28 ^a	3.05 ^a	2.87 ^b	3.08 ^a	0.16
Area under the curve	1141 ^a	1086 ^a	1041 ^b	1092 ^a	58

^{a,b} : Values in the same line with similar superscripts are not different (p > 0.05)

Fig. 3.- Changes in plasma concentration of glucose (a), insulin (b), α -amino-nitrogen (c), urea (d), triglycerides (e) and cholesterol (f) in 6 healthy dogs offered a control diet (○) or a diet supplemented with 3.5 (●) or 7 % (Δ) guar gum.



Discussion

If the area of the marker on the radiograph is considered as a measure of the volume in the stomach, approximately half of the food left the stomach within 6 h and one eighth was still present after 12 h. Such a pattern did not fully agree with the most commonly accepted concept that gastric emptying in dog occurred within a few hours after feeding (BÖRG et al. 1979; MIYABAYASHI et al. 1984). More recent data (ARNBJERG 1992) indicated an effect of the dry matter content of the diet on gastric emptying rate so that it is not unusual for foodstuff still to be present after 12 h. BURNS and FOX (1986), using a barium meal contrast procedure, reported a total gastric emptying time from 7 to 15 h in dogs. Although different authors such as RUSSELL and BASS (1985) with dogs and RAINBIRD and LOW (1986) with pigs reported some significant reduction on the gastric emptying rate with GG, only a non significant reduction was observed in the present experiment. It is likely that the small effect was associated with the quite low incorporation rate of GG.

Xylose test was proposed by HILL et al. (1970) to investigate, from blood rather than urine, malabsorption syndrome in the dog. The test was used in Expt 1 on the assumption that transport of pentose and hexose will be similarly affected by fibre supplementation. The pattern of xylose concentration in the dog was similar to that reported by HILL et al. (1970) and by STRADLEY et al. (1979). The supplementation with GG and PEC did not change the pattern of the curve. The difference in the pattern of xylose curve when CEL was given, could result from an alteration of the intestinal mucosa as observed in rats (CASSIDY et al. 1982), in pigs (MOORE et al. 1988) and in dogs (REINHART et al. 1994). When wheat bran or horse bean hulls were added to the diet of dogs, CHERBUT et al. (1986) reported a reduction in xylose concentration both in the interstitial fluid of the small intestine and in the plasma.

The high apparent digestibility coefficients obtained both in Expt 1 and 2 with the FF1 and FF2 diets were associated with the high quality of the ingredients : cooked or steam-treated rice and meat. The inclusion of fibre in the diet reduced the apparent digestibility of dry matter by approximately 3 % regardless of the fibre. The present decrease could also be accounted to a dilution effect of the fibre and not only to a specific fibre "bulking effect" as reported by BURROWS et al. (1982). They observed a 2.2 % decrease in dry matter digestibility for each percent of added cellulose. FAHEY et al. (1990a, 1990b, 1992) reported

a set of experiments in which dog's diets were supplemented with fibre from different foodstuffs as beet pulp, tomato pomace, peanut hulls, wheat bran or treated wheat straw. They observed slight reductions in digestibility coefficients regardless of the type of fibre source added in the diet. In the 2 experiments reported here, apparent digestibility of crude protein was significantly reduced with PEC and the 2 incorporation rates of GG although incorporation rates were quite low. The reductions in digestibility is the reason why manufacturers have to increase the concentrations of essential nutrients such as protein when formulating a high-fibre content dog food.

The lack of rapid increase in plasma glucose and insulin shortly after feeding in Expt 1 and to a lesser extent in Expt 2 was in line with the pattern reported in dogs by some authors (GORYA et al. 1981; NOMURA et al. 1985). By contrast, BLAXTER et al. (1990) reported reductions in hyperglycaemia for 240 min following a canned test meal containing 20 g guar gum in Beagle dogs. In that study, the concentration of guar gum was approximately 12 % in dry matter, which is higher than the concentration used in these experiments. Unexpected increases in plasma glucose and insulin were found with PEC, whereas CEL and GG did not show any particular effects in Expt 1. Accordingly to the technical note, sugar was added to PEC by the manufacturer to allow a standardised gel-forming reaction. The glucose content of PEC was 21.9 % in the dry matter, so that only 1.5 g of glucose was added to the diet; it was very unlikely that such a small amount could explain a large rise in both insulin and glucose concentrations. One of the most interesting property of GG is the insulin-lowering effect observed at the incorporation rate of 7 % on a dry matter basis. This is of interest for the formulation of specific-purpose dog food, especially for diabetic subjects. Decreases in plasma concentrations of α -amino-nitrogen and urea were observed with GG in the 2 studies. Amino acids are bound on the digesta in the small intestine, and therefore, the utilisation of dietary proteins is reduced, as indicated by the reduction of protein digestibility. The decrease in plasma urea concentration could be associated with the delay in the absorption of amino acids and their catabolism in the liver.

In humans, the effects on lipids metabolism are known to be properties of soluble fibres, the most efficacious being GG (LANDIN et al. 1992). In the studies reported here, GG had little effects on triglycerides but lowered cholesterol concentrations, both in non-fed or fed dogs. A practical application is suggested for clinical use in some diseases associated with disorders of lipid metabolism such as obesity or diabetes mellitus.

In conclusion, from the 3 purified fibres tested in Expt 1 -CEL, GG, and PEC-, it seemed that GG had most effects on plasma metabolites. The results of Expt 2 suggested that an incorporation rate of 7 % GG on dry matter basis is more appropriate.

Summary

The aim of the present experiments was to investigate the effects of adding dietary fibre (DF) in healthy dogs diets. In a first study, 4 young adult Beagle dogs were used in a 4 x 4 Latin square design. They were offered either a control diet (FF1) based on minced meat and cooked rice or the same diet supplemented with cellulose (CEL), pectin (PEC) or guar gum (GG) at an incorporation rate of 3.4 % on dry matter basis. Gastric emptying rate, measured by sequential radiographs during 12 h after the meal tended to be lowered when GG was added. The intestinal absorption of xylose measured on fasted animals was not affected by GG and PEC but was significantly delayed with CEL ($p < 0.05$). The incorporation of DF reduced the digestibility of the different nutrients, the differences being significant with CEL for dry matter ($p < 0.05$), with GG for protein and ether extract ($p < 0.05$) and with PEC for protein ($p < 0.001$). There were no effects of DF supplementation on plasma glucose, insulin, α -amino nitrogen, urea and triglycerides concentrations measured before the meal. PEC induced higher postprandial insulin concentration ($p < 0.05$). The postprandial rise of plasma α -amino nitrogen and urea concentrations were significantly reduced with GG ($p < 0.05$ and $p < 0.01$, respectively). GG induced lower concentrations of plasma cholesterol both in non-fed or fed animals ($p < 0.05$).

In the second study, 6 adult Beagle dogs were used in 2 combined 3 x 3 Latin square design. They were offered either a control diet (FF2) based on minced meat and steam-treated rice or a diet supplemented with 3.5 % and 7 % GG on dry matter basis. A dose level lowering-effect on the different nutrients digestibility and on plasma concentrations of insulin, α -amino-nitrogen and urea ($p < 0.01$ or $p < 0.001$) was also observed. Inclusion of 7 % GG decreased pre- and postprandial plasma cholesterol concentrations ($p < 0.01$).

Zusammenfassung

Ziel der folgenden Experimente war es die Wirkung der Zufuhr von Faserstoffen (FS) in einer Ration für gesunde Hunde zu untersuchen. In einer ersten Studie wurden 4 ausgewachsene Beagle Hunde in einen 4x4 lateinischen Quadrat Modell benutzt. Man verabreichte ihnen

entweder eine Kontrolldiät (FF1) basierend auf Hackfleisch und gekochtem Reis oder die gleiche Diät angereichert mit Zellulose (CEL), Pektin (PEC) oder Guar (GG), in einem Anteil von 3.4 % der Trockenmasse. Die Magenentleerungsrate, welche durch aufeinanderfolgende Röntgenaufnahmen während 12 Stunden gemessen wurde, tendierte zur niedriger, wenn GG hinzugefügt worden war. Die Xylose Absorption im Darmtrakt bei den Tieren mit der Diät ration wurde nicht beeinflusst durch GG und PEC, wurde aber signifikant verlangsamt mit CEL ($p < 0.05$). Das Hinzufügen von DF reduzierte der Verdaulichkeit der verschiedenen Nährstoffe, wobei die Diferenz signifikant war mit CEL für die Trockenmasse ($p < 0.05$), mit GG für Eiweiß und Ätherextrakt ($p < 0.05$) und mit PEC für Eiweiß ($p < 0.001$). Durch Hinzufügen von DF ergaben sich keine Veränderungen der Plasmakonzentrationen von Glukose, Insulin, α -Amino-Nitrogen; Harnstoff und Triglyzerid, im Vergleich zu denen, welche vor der Mahlzeit gemessen wurden. PEC verursachte eine höhere postprandiale Insulinkonzentrationen ($p < 0.05$). Der postprandiale Anstieg der Plasma α -Amino-Nitrogen und Harnstoffkonzentrationen wurde bedeutend reduziert durch GG ($p < 0.05$ und $p < 0.01$ jeweils). GG bewirkte eine geringere Konzentration von Plasma-Cholesterin, sowohl bei gefütterten als auch bei nicht gefütterten Tieren ($p < 0.05$).

In eine zweiten Untersuchung wurden 6 erwachsene Beagles in zwei kombinierten lateinischen Quadrat Modellen benutzt. Sie wurden entweder mit einer Kontrolldiät (FF2) auf Basis von Hackfleisch und gedämpftem Reis gefüttert, oder einer mit 3.5 % und 7 % GG angereicherten Diät, auf Trockenmassebasis. Ein die Dosis reduzierender Effekt auf die Verdaulichkeit der verschiedenen Nährstoffe auf die Plasmakonzentrationen von Insulin, α -Amino-Nitrogen und Harnstoff ($p < 0.01$ und $p < 0.001$) wurde ebenfalls beobachtet. Das Hinzufügen von 7 % GG reduziert die pre- und postprandialen Cholesterinplasmakonzentrationen ($p < 0.01$).

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ETUDE 3

The influence of sugar-beet fibre, guar gum and inulin on nutrient digestibility, water consumption and plasma metabolites in healthy Beagle dogs.

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SUMMARY

The aim of the present study was to evaluate in healthy dogs the effects of three fibres (sugar-beet fibre, guar gum and inulin) incorporated in a basal diet at 7 per cent of dry matter (DM). Parameters examined included stool output, water consumption, nutrient digestibility and fasting and postprandial plasma metabolites. All fibres increased wet faecal output; an increase in faecal DM output being observed with sugar-beet fibre only. Sugar-beet fibre and inulin increased daily water consumption. Sugar-beet fibre and guar gum decreased DM digestibility. The three fibres diminished organic matter and crude protein digestibility while ether extract digestibility was decreased by guar gum and inulin. Guar gum induced lower postprandial insulin, α -amino-nitrogen and urea plasma concentrations. Guar gum also lowered fasting cholesterolaemia. Sugar-beet fibre and inulin showed no metabolic effects. These physiological properties suggest that guar gum would be a suitable ingredient for dietary therapy of chronic diseases such as diabetes mellitus or hyperlipidaemia in the dog.

ALTHOUGH dietary fibres are not considered as essential nutrients, they are beneficial to health (Leibetseder 1982, Fahey et al 1990 a, b, 1992) and are incorporated at low rates of 1 to

5 per cent dry matter (DM) in most dog foods. They are also used at higher rates of up to 20 per cent DM as an aid in the treatment of chronic diseases such as obesity, diabetes mellitus, gastro-intestinal diseases or hyperlipidaemia (Blaxter et al 1990, Dimski and Buffington 1991, Dimski 1992, Nelson 1992, Graham et al 1994).

Beet fibre, a fibre source commonly incorporated in dog diets, is characterised by complementary viscous and nonviscous structural carbohydrates (Fahey et al 1990a). Guar gum is largely used as a source of soluble fibre although the insoluble fibre content is close to 30 per cent (Bauer and Maskell, 1996). Guar gum is a gel-forming galactomannan obtained from the cluster bean, *Cyanopsis tetragonoloba*, with potent short and long term effects on blood glucose and lipids (Jenkins et al 1978). Inulin contains fructans with a degree of polymerisation of 2 to 60. Due to the structural conformation of the osidic bridge (β 1-2), inulin resists hydrolysis by alimentary enzymes. Inulin has thus most of the characteristics of dietary fibres and Roberfroid (1993) proposed that it be classified as such. The purpose of this study was to assess the effects of these dietary fibres on nutrient digestibility and plasma metabolites in healthy Beagles. Supplementary dietary fibre was added daily to food at an incorporation rate of 7 per cent DM.

MATERIALS AND METHODS

Experimental animals

Eight Beagle dogs (two intact males and six neutered females), 5-years-old, weighing 11.3 to 13.4 kg were used. All dogs were healthy on the basis of results of physical examination, complete blood count, serum biochemical analysis (glucose, insulin, urea, creatinine, cholesterol, triglycerides concentrations and alkaline phosphatase and alanine transaminase activities). Dogs were weighed weekly. They were housed in outdoor kennels or in individual metabolic cages in a room with natural lighting during digestibility trials. Room temperature was maintained at 18 (\pm 2) °C. The protocol was approved by the university committee for care and use of laboratory animals.

Experimental design

For control diets, no supplementary fibre was added. In the other three regimes, fibre sources (beet fibre, guar gum or inulin) were added to the basal diet as a supplement at an incorporation rate of 7 per cent DM. The design was two 4X4 Latin squares (Dagnélie 1975). Each experimental diet was fed during 4 weeks. Each period of the Latin square was followed by an 1-week washout period in order to avoid residual metabolic effect of the fibre. The total length of the trial was 20 weeks.

Diet composition and feeding protocol

The basal diet was made of 20.8 per cent DM beef minced meat, 69.2 per cent DM flaked maize, 6.7 per cent DM maize oil and 3.3 per cent DM vitamin/mineral mixture (Radar, Belgium). The fibres used were beet fibre from British Sugar, guar gum -Viscogum HV 3000A- from Mero-Rousselot-Satia, France, and inulin -Raftiline- from Raffinerie Tirlemontoise, Belgium. Inulin was extracted from *Cichorium Intybus* roots. All ingredients, fibre sources included, were mixed with 400 ml water and were given to dogs after 5 minutes. The amount offered was based on the daily maintenance caloric requirements determined by body weight ($550 \text{ kJ/day/kg}^{0.75}$, NRC 1974). The dogs were fed once a day at 9.00 AM and they voluntarily consumed their whole meal within 5 minutes.

Digestibility trials

Digestibility measurements were carried out over 7 days during the last week of each period. Dogs were housed in metabolism cages. Faeces were collected twice daily and were stored at 4°C. Water intakes were recorded daily.

Plasma samples

Metabolic and hormonal profiles were determined in fasted dogs and postprandially during the last day of the week, after the last collection of faeces. An indwelling sterile catheter was inserted into a cephalic vein and filled with a heparinized (120 U/ml) saline solution to prevent formation of blood clots between sampling times. Dogs were handled gently and did not appear excited during sampling. Blood was taken before feeding; then dogs were fed their assigned diets. Serial postprandial blood samples (5 ml) were taken at 20, 40, 60, 90, 120, 180, 240, 300 and 360 minutes after feeding. The samples were centrifuged

at 3000 g for 15 minutes and the plasma was stored at -20°C until it was analysed for glucose, insulin, α -amino-nitrogen, urea, triglycerides and cholesterol.

TABLE 1.- *Chemical composition of the diets (g/kg DM).*

	Control	Beet fibre	Guar Gum	Inulin
Protein	244.5	232.8	239.1	236.1
Ether Extract	143.5	134.2	133.9	133.8
ADF	26.7	43.1	26.3	25.2
TDF	103.0	151.1	160.8	96.0
IDF	92.3	122.7	107.8	86.1
SDF	10.7	28.4	53.0	9.9
Calcium	6.0	6.3	5.6	5.6
Phosphorus	5.1	4.8	4.8	4.8
Metabolizable Energy, (KJoule/kg DM)	16741	15654	15616	15608

ADF, Acid Detergent Fibre; TDF, Total Dietary Fibre; IDF, Insoluble Dietary Fibre;
SDF, Soluble Dietary Fibre

Biochemical analysis

Dry matter, acid detergent fibre (ADF), ash and ether extract in feed and faeces were analysed by standard methods (Association of Official Analytical Chemists, 1975). Total dietary fibre (TFD) was determined in food using a kit (Sigma, TDF-100). This procedure was based on the method published by Association of Official Analytical Chemists (1990). Kjeldahl nitrogen was estimated by block digestion and automatic colorimetry using the Berthelot reaction (Van Eenaeme et al 1969). Organic matter content in feed and faeces was calculated by subtracting ash from DM. Plasma concentrations of glucose was analysed by ortho-toluidine (Charlier et al 1974), plasma urea by diacetyl-monoxime (Henry 1974) and plasma α -amino-nitrogen by the trinitrobenzene -sulfonate (Palmer and Peters 1969) methods using a Technicon Autoanalyzer. Plasma concentrations of cholesterol and triglycerides were determined by kit procedures (from Boehringer). Insulin were measured with an insulin RIA-100 kit (Medgenix Diagnostics, Biosource Europe, Fleurus, Belgium).

Statistics

Analysis of variance was performed for the digestibility trial data according to two combined 4X4 Latin squares design (Dagnélie 1975). Plasma metabolites data were analysed using a dynamic linear model taking into account that the data were autocorrelated (Jones and Boadi-Boateng 1991, Lindsey et al 1994, Lambert 1996). The model allowed the inclusion of an autoregression and a random effect. Differences were accepted as significant when $P < 0.05$.

RESULTS*Diet-induced variations in faeces, water consumption and digestibility of nutrients*

The concentration of protein, ether extract and minerals in the fibre-supplemented diets was reduced, compared with the control values (Table 1). By contrast, ADF and calcium concentrations were increased in the diet containing beet fibre. Total dietary fibre concentration in the four diets was greater than 10 per cent DM.

Acceptance of diet was good throughout the study; all dogs ate their entire ration at each meal. Each group of dogs lost weight; mean weight variations for dogs over the trial were - 0.075 kg for control, - 0.175, -0.038 and - 0.025 kg for diets containing beet fibre, guar gum and inulin, respectively.

Daily drinking water consumption was 7.0 ml/kg bodyweight on the control diet and 10.5 ($P < 0.01$), 8.3 ($P > 0.05$) and 9.9 ($P < 0.05$) ml/ kg bodyweight on the diets supplemented with beet fibre, guar gum and inulin respectively. These values seemed quite low. It should be noted that 400 ml water were added daily to the ingredients so that the total water intake ranged between 41.0 and 44.5 ml/kg bodyweight.

The quantity of wet faeces excreted (g/day) was significantly increased by the three sources of fibre (Table 2). The dry matter content of faeces in turn was decreased. The daily excretion of faecal dry matter was not significantly increased except when beet fibre was added ($P < 0.05$). Because TDF intake greatly affects wet faecal output, wet faecal output also is expressed per gram of TDF intake. In this case, dogs fed control and guar gum diets excreted the least amount of faeces/g of TDF intake (3.1 and 3.6, respectively) whereas dogs fed the beet fibre and inulin diets produced the greatest amount of faeces/g of TDF intake (4.0 and 4.8, respectively).

Apparent DM digestibility was significantly decreased for the diet containing beet fibre ($P<0.01$) and guar gum ($P<0.05$) but was unaffected for the diet containing inulin (Table 3). Apparent organic matter and protein digestibility were decreased with the three fibre-supplemented diets ($P<0.05$ or $P<0.01$). Apparent ash and nitrogen-free-extract digestibility were decreased only with the diet containing beet fibre ($P<0.05$). Apparent ether extract digestibility was decreased with the diet containing guar gum ($P<0.01$) and inulin ($P<0.05$). Apparent ADF digestibility was 23.3 per cent for control diet and was significantly increased with inulin ($P<0.05$).

TABLE 2.- Mean (SD) parameters of faeces characteristics after supplementation with 7 per cent DM dietary fibres in healthy dogs

Parameter	Control	Beet fibre	Guar Gum	Inulin
Faeces characteristics				
Wet faeces, g/d	65.6 (7.5) ^a	128.4 (27.3) ^{cd}	119.1 (39.8) ^{bc}	96.0 (19.3) ^b
Wet faeces, g/g of TDF intake	3.1 (0.4) ^a	4.0 (0.6) ^b	3.6 (0.7) ^{ab}	4.8 (0.6) ^c
Dry matter, %	34.4 (5.5) ^a	24.7 (2.9) ^{bc}	23.5 (2.3) ^{cd}	27.0 (2.4) ^b
Dry matter, g/day	22.6 (2.8) ^a	31.7 (5.4) ^b	28.0 (7.1) ^a	25.9 (5.1) ^a
^{a,b,c} Values with non-identical letters differ significantly within one line ($P<0.05$)				

Diet-induced variations of plasma metabolites

The concentrations of plasma metabolites were all with reference range (Kaneko 1980). Changes in glucose concentrations were characterised by hyperglycaemia 20 (control, inulin) or 40 minutes (guar gum, beet fibre) after the meal and followed by a slow decline (Fig 1). Glucose fasting concentration was 4.32 mmol/l with control diet. A higher fasting glucose concentration at 4.88 mmol/l was observed with guar gum inclusion ($P<0.05$). In contrast, during the whole postprandial curve, the three fibres did not induce changes in glucose concentrations.

TABLE 3.- Mean (SD) apparent digestibility coefficients after supplementation with 7 per cent dietary fibre in healthy dogs.

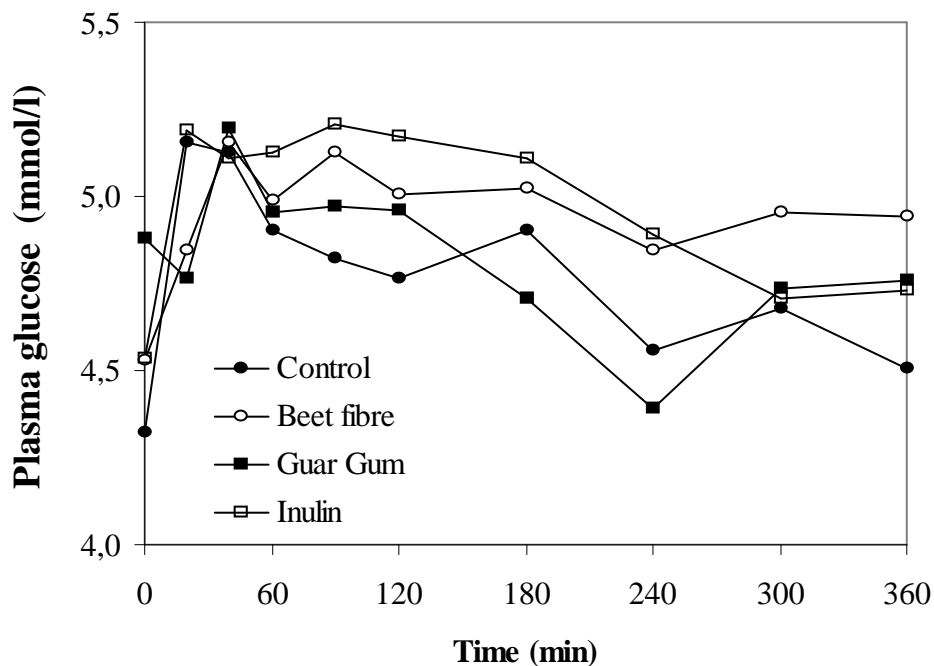
Parameter	Control	Beet fibre	Guar Gum	Inulin
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Digestibility

Dry matter, %	88.8 (1.0) ^a	85.3 (0.9) ^{cd}	86.6 (1.6) ^{bc}	87.5 (1.8) ^{ab}
Organic matter, %	91.1 (0.8) ^a	87.7 (0.7) ^c	88.8 (1.6) ^{bc}	89.6 (1.6) ^b
Ash, %	45.9 (8.7) ^a	39.2 (11.5) ^b	44.7 (14.0) ^{ab}	47.4 (9.4) ^a
Crude protein, %	90.9 (1.2) ^a	88.2 (1.6) ^b	85.6 (3.3) ^c	88.3 (1.5) ^b
Ether Extract, %	95.9 (1.8) ^a	95.8 (0.7) ^{ab}	91.0 (2.7) ^c	94.3 (1.6) ^b
Nitrogen-free extract, %	92.3 (0.8) ^a	89.9 (1.0) ^b	91.8 (1.6) ^a	91.3 (1.7) ^a
Acid detergent fibre, %	23.3 (8.4) ^{ab}	18.2 (8.2) ^a	28.2 (9.5) ^{ab}	37.9 (19.2) ^b

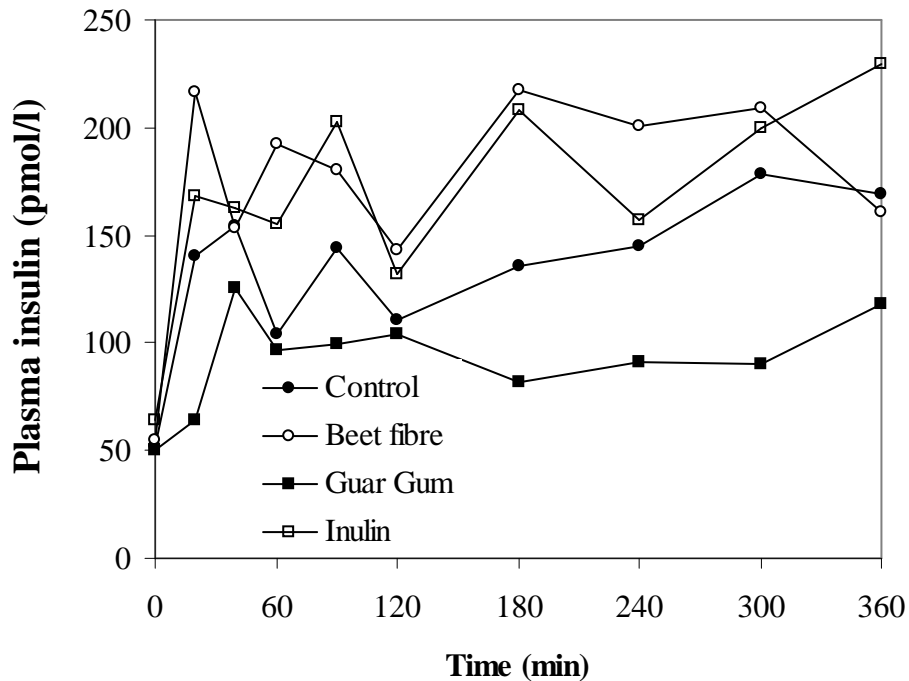
^{a,b,c} Values with non-identical letters differ significantly within one line ($P < 0.05$)

FIG 1.- Mean glucose plasma concentrations ($n=8$) obtained over a 6-h period in Beagles fed a meal containing no added fibre or 7 per cent DM beet fibre, guar gum or inulin.



Mean insulin concentration was 55 pmol/l before feeding for all dogs, with no significant differences between treatments (Fig 2). Concentrations increased to high values of 150 and 220 pmol/l after feeding and remained relatively high on beet fibre and inulin supplemented diets. Plasma insulin concentration on the control diet was intermediate while guar gum induced lower peak values and remained low over the 6 hours period ($P < 0.0001$).

FIG 2.- Mean plasma insulin concentrations ($n=8$) obtained over a 6-h period in Beagles fed a meal containing no added fibre or 7 per cent DM beet fibre, guar gum or inulin.



Fasting concentrations of plasma α -amino-nitrogen did not change among treatments (Fig 3). Plasma α -amino-nitrogen concentration rose rapidly after feeding and remained elevated over the sampling period. The inclusion of guar gum reduced the postprandial concentration ($P=0.012$). Changes in plasma urea concentrations were similar to those of α -amino-nitrogen (Fig 4). Fasted concentrations of plasma urea were similar among treatments but concentrations after guar gum were lower at each measurement ($P=0.0004$).

Cholesterol concentration before feeding was 3.6 mmol/l on the control diet and was significantly lower on guar gum, at 2.6 mmol/l ($P=0.00002$) (Fig 5). Beet fibre, guar gum and inulin had no effect on postprandial cholesterol concentrations. Fasting triglyceride concentration was 0.49 mmol/l for the control diet and was not significantly changed by inclusion of fibres (Fig 6) nor were there any significant effects of the dietary fibres on postprandial concentrations of triglycerides.

FIG 3.- Mean plasma α -amino-nitrogen concentrations ($n=8$) obtained over a 6-h period in Beagles fed a meal containing no added fibre or 7 per cent DM beet fibre, guar gum or inulin.

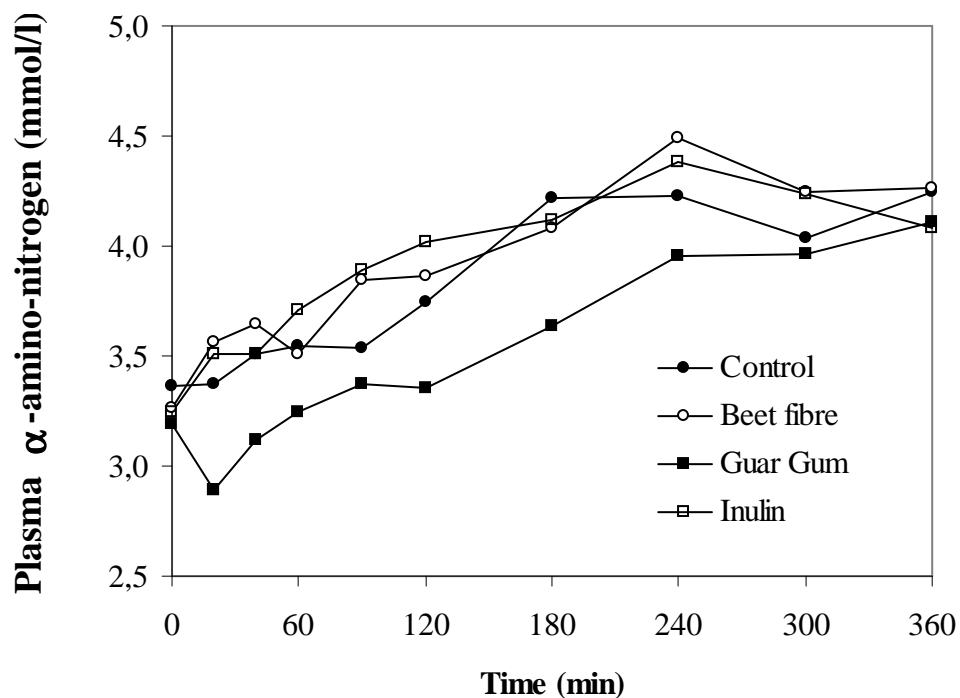


FIG 4.- Mean plasma urea concentrations ($n=8$) obtained over a 6-h period in healthy Beagles fed a single meal containing either no added fibre in the diet or 7 per cent DM beet fibre, guar gum or inulin.

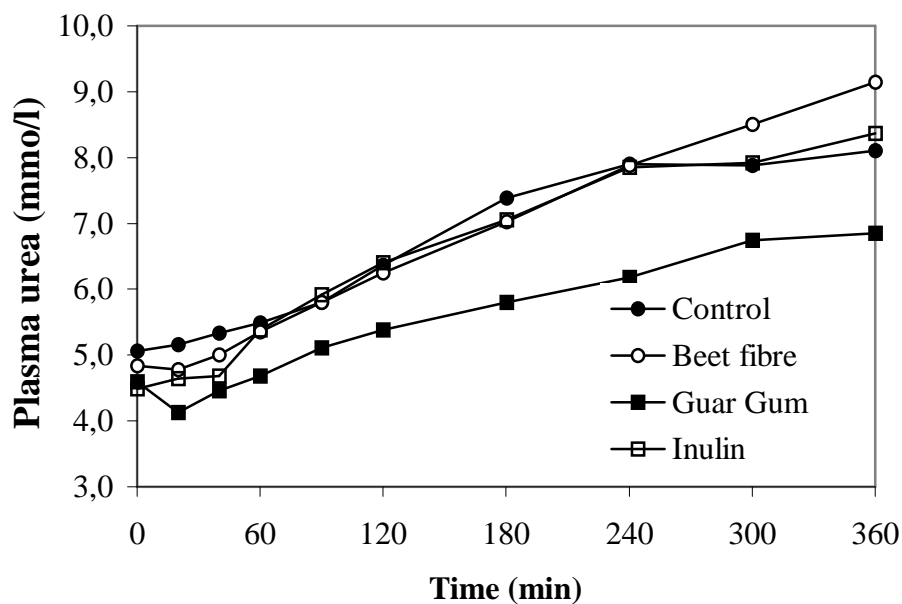


FIG 5.- Mean plasma cholesterol concentrations ($n=8$) obtained over a 6-h period in Beagles fed a meal containing no added fibre or 7 per cent DM beet fibre, guar gum or inulin.

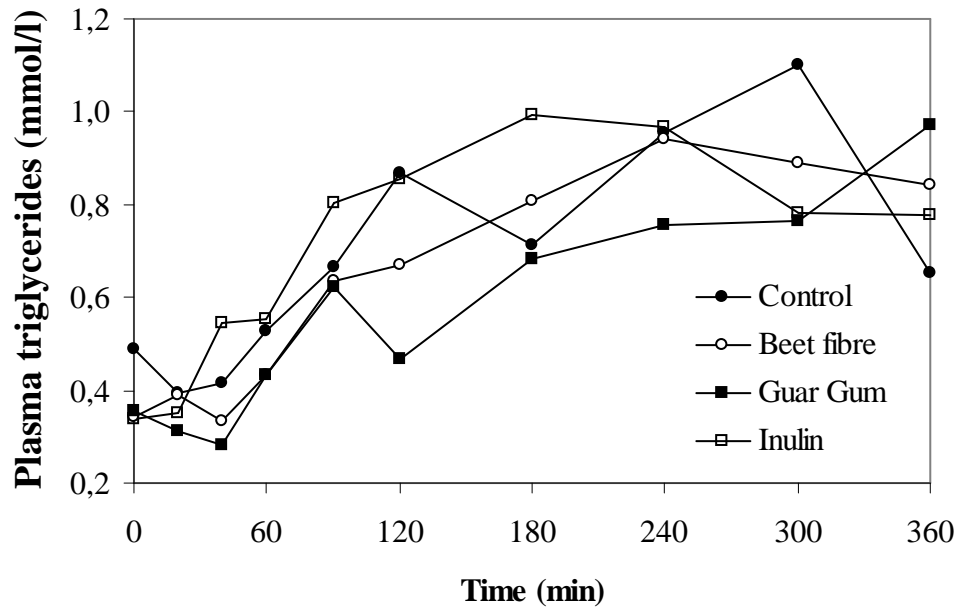
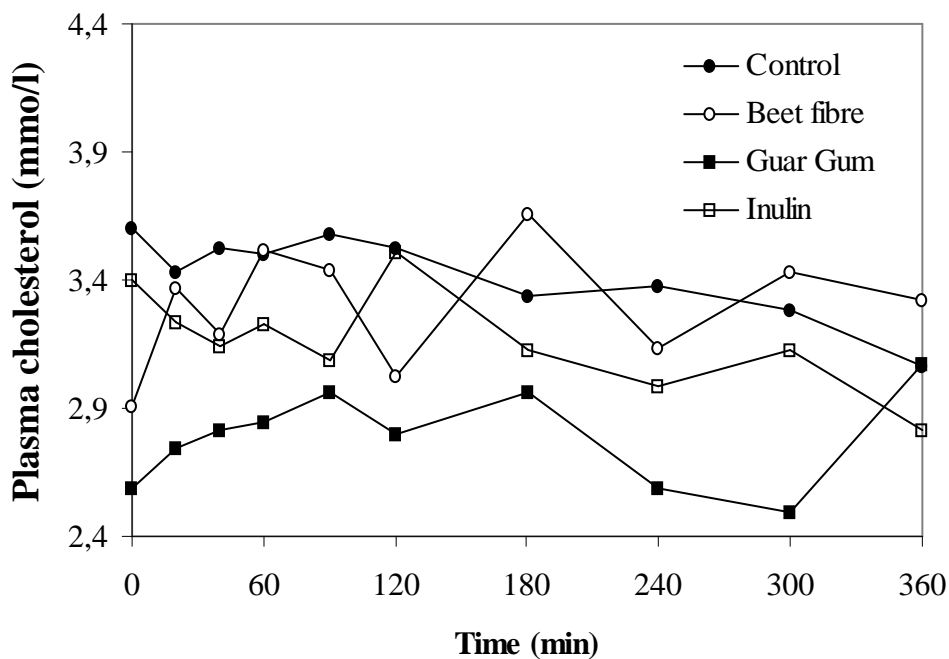


FIG 6.- Mean plasma concentrations ($n=8$) of triglycerides obtained over a 6-h period in Beagles fed a meal containing no added fibre or 7 per cent DM beet fibre, guar gum or inulin.



DISCUSSION

The TDF concentrations in the four diets were high at values close to or over 10 per cent DM. One contributor to the high fibre values obtained is the animal protein component

of the diet. Meat contains protein-polysaccharides of the connective tissue; these fibrous materials can escape from digestion by the enzymes of the digestive tract (Banta et al 1979) but are measured by the assays used to analyse TDF. Adding dietary fibre had a dilution effect on energy density but such an effect was of no importance since all dogs received their amount of feed based on individual energy requirements.

The amount of wet faeces excreted daily increased with the addition of fibre to the diet. Such an effect is called "bulking effect" of fibres, a property used for treatment of constipation. The faecal bulking effects appear to be most strongly associated with fibre sources which are insoluble, poorly fermentable and with good water-binding capacity. Although all sources produce some increases in stool weight, the increases with highly fermentable soluble forms are usually small (Southgate 1990). In this study, guar gum and inulin, the most soluble fibres induced smaller increases in fresh faecal weight than beet fibre characterised by a higher content of insoluble fibre. An increased excretion of fresh faeces has been reported in the dog either associated with various purified fibres such as cellulose (Burrows et al 1982), pectins (Lewis et al 1994), maize fibre (Egerton et al 1996) or foodstuffs high in fibre such beet pulp (Fahey et al 1990a, b, 1992, Sunvold et al 1995). When wet faeces excretion was expressed per gram of TDF intake, the greatest excretion surprisingly occurs on inulin. This was partly due to the water binding capacity of inulin associated with a lack in TDF content for this fibre.

The normal range of faecal DM content in dogs varies between 28 and 42 per cent (Griess and Enjalbert 1982). Fermentable fibres decrease the DM content of faeces (de Haan et al 1990, Fahey et al 1990a, b, Sunvold et al 1995). Adding beet pulp in a dog's diet (13.7 per cent TDF/DM), Fahey et al (1990a) reported DM content of 20.3 per cent as opposed to 38.2 per cent for a control diet. Our results are in line with the findings reported by others, that a significant decrease of faeces DM content is induced by the three sources of fibre. More interesting is the comparison of the total DM excreted. The inclusion of guar gum or inulin in the diet did not change the amount of DM excreted as compared to control diet. So, increased fresh faeces weight was really an increase in water content of faeces. By contrast, with beet fibre as a supplement, increased water excretion in faeces can't explain the increase in wet faeces weight alone although water-holding capacity of beet fibre is very high (Fahey et al 1990a). There should, therefore, be other mechanisms involved such as a greater amount of microbial cells and short chain fatty acids produced (Sunvold et al 1995) or a reduction in the

nutrients digestibility. Furthermore, it should be noted that the 100 % increase in wet faeces excretion with beet fibre is considered more as a disadvantage by most owners.

Although it is generally admitted that dietary fibres increase the excretion of water in faeces, measurement of drinking water consumption has never been reported with diets containing supplemental fibres in dog. It is therefore of interest to know if the effects are associated with just only redistribution of water excretion or with changes in water consumption. In the present study, daily water consumption was increased by 50 per cent on beet fibre, by 18 per cent on guar gum and by 39 per cent on inulin. These data imply that both mechanisms are involved : redistribution of excreted water towards faeces and increased water consumption.

The high apparent digestibility coefficients obtained in the study were associated with the high quality of the ingredients : flaked maize and fresh beef meat. The inclusion of dietary fibres in the diet reduced the apparent DM digestibility of 2.2 and 3.5 per cent for guar gum and beet fibre, respectively. The decrease could be accounted for by a dilution effect of the fibre, particularly for beet fibre and not to a specific "bulking effect". The apparent digestibility of crude protein was significantly reduced by the three fibres; the reduction was the greatest with guar gum inclusion (-5.3 %). This effect was due to a higher crude protein content of faeces. Although no microbial measurements were made in the present study, the high faecal protein content would indicate a microbial proliferation with fermentable fibres (Sunvold et al 1995). From Table 3, there were reductions in the apparent digestibility coefficients of the other chemical components of the diet. The effects were variable according to the fibre and the component. Beet fibre was the only fibre which reduced apparent digestibility coefficients of all nutrients. Thus, as a result of the reduction in nutrient digestibility when fibres are included, it is necessary to increase the concentration of different nutrients. These principles are applied in diet formulation of low-fat high-fibre diets for pets (Hand et al 1989).

Fasting plasma glucose concentration is normally maintained within a narrow range in dog regardless of the type of diet offered (Feldman and Nelson 1987). Adding dietary fibre did not affect fasting glucose and insulin concentration in this study nor in other published experiments in which cellulose, pectins or guar gum were incorporated at rates of 3.5 per cent DM (Istasse et al 1990) or with a blend of guar gum and pea fibre at 15 per cent DM (Graham et al 1994). In contrast, postprandial glucose concentrations can be modified to some extent by inclusion of soluble fibres in the diet. The addition of guar gum (15 to 20 per cent DM) in

one single meal to 6 dogs abolished postprandial hyperglycaemia in four cases and reduced the extent of hyperglycaemia in the remaining two animals (Blaxter et al 1990, Papasouliotis et al 1993). Similar effects, but to a lesser extent were observed with wheat bran by the same authors. In contrast, a mixture of guar gum and pea fibre did not modify postprandial glucose concentrations measured during 6 hours in dogs (Maskell et al 1994). In our study, guar gum was the only fibre to induce a significant decrease in plasma insulin. Postprandial decreases in plasma insulin were also reported with diets enriched with a mixture of pea fibre and guar gum (Maskell et al 1994) and diets with a high content of crude fibre (unknown fibre source) (Nguyen et al 1994).

Decreased glucose and/or insulin concentrations with guar gum have been shown in healthy humans (Jenkins et al 1977, Wolever et al 1979, Landin et al 1992, Fairchild et al 1996) and in diabetic subjects (Anderson et al 1980, Gatti et al 1984). The effects of guar gum on insulin metabolism could also be exploited in obese or diabetic dogs to improve glucose tolerance. There are no published report of the systemic effects of beet fibre in the dog. Beet fibre can improve glucose tolerance in man (Cherbut et al 1994) but the effects are less pronounced than with guar gum. There is no effect of beet fibre on insulin secretion according to Morgan et al (1988) and Frape et Jones (1995). In our study, beet fibre induced no effect on plasma metabolites at an inclusion rate of 7 per cent DM.

The inclusion of guar gum in the diet also reduced postprandial concentrations of α -amino-nitrogen and urea as previously described by Istasse et al (1990) and Delaunois et al (1990). Amino acids are bound on the digesta in the small intestine (Howard et al 1986) and therefore, binding of amino acids may contribute to the decrease in protein utilisation observed when fibre is added to the diet with, as result, a reduction of protein digestibility and lower plasma of α -amino-nitrogen concentrations. Plasma urea was reduced by addition of guar gum. Such effect could be associated with the delayed absorption of amino-acids and their catabolism in the liver.

In this study, guar gum induced lower fasted and postprandial cholesterol concentrations in plasma. In Blaxter's et al study (1990), no postprandial reductions in serum cholesterol and triglycerides were seen in the dog after a single dose of guar gum or wheat bran. The authors postulated that guar gum may still reduce blood lipids in the dog after long term administration. In our study, beet fibre had no effect. In humans, beet fibre was effective in reducing blood cholesterol in healthy (Morgan et al 1988, Tredger et al 1991) or hyperlipidaemic subjects (Frape and Jones 1996). The addition of beet fibre also increased net

cholesterol excretion in human subjects with an ileostomy (Langkilde et al 1993). According to Tredger et al (1991), the effectiveness of beet fibre to lower plasma cholesterol concentration is related to the fat intake of the subjects. In the present study, fat intake was quite low due to the use of low-fat meat and small amount of vegetable oil. The cholesterol lowering effect of guar gum is well established in healthy subjects (Morgan et al 1988, Landin et al 1992), in obese (Krotkiewski 1984) and in hyperlipidaemic patients (Gatti et al 1984). In contrast, the effect of guar gum on plasma triglycerides is much debated in humans (Landin et al 1992, Morgan et al 1993). So, in humans, hypolipidaemic effects are a property of soluble fibres, the most efficient being pectins and guar gum.

From the present trial it could be concluded that the main effects of supplementation with beet fibre, inulin and guar gum were increases in wet faecal output and reductions in apparent digestibility coefficients. Since guar gum induced metabolic effects on carbohydrate and lipid metabolisms after four weeks administration, this could be considered as an aid in dietetic treatments of chronic diseases such as hyperlipidaemia or diabetes mellitus. This is of further interest because fasting hyperlipidaemia occurs in 14.3 per cent of the dog population (Barrie et al 1992, 1993) and diabetes mellitus is frequently associated with disorders of lipid metabolism (Feldman and Nelson 1987).

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ETUDE 4

Influence of a blend of fructo-oligosaccharides and sugar beet fiber on nutrients digestibility and plasma metabolites concentrations in healthy Beagles

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Objective—To evaluate effects of a blend of fructo-oligosaccharides and sugar beet fiber (4:1) at 3 incorporation rates on nutrients digestibility and on plasma glucose, insulin, α -amino-nitrogen, urea, cholesterol and triglycerides concentrations measured weekly in non-fed dogs and during a 360-minute period after a meal.

Animals—8 castrated 1- to 1.4 -year-old young adult male Beagle weighing 10.0 to 13.5 kg.

Procedures—Diets containing 2 incorporation rates of a blend of fructo-oligosaccharides and sugar beet fiber (5 and 10 % on dry matter basis [diets B and C, respectively]) were compared with a control diet without additional fiber (diet A). The 3 diets were evaluated for ability to modify digestibility of dry and organic matter, protein, fat, and ash and for effects on plasma glucose, insulin, α -amino-nitrogen, urea, cholesterol and triglycerides concentrations. Each diet was fed for 6 weeks; plasma samples were collected weekly before feeding and after feeding on the last day of the period. During 1 week at the end of the 6-week period, dogs

were kept in metabolic cages. Each period of the block was followed by a 4-week washout period.

Results—Incorporating the blend of fructo-oligosaccharides and sugar beet fiber in the diet was associated with higher wet feces excretion (diets B and C) and lower protein digestibility (diet C). Postprandial glucose (diet C), urea (diets B and C) and triglycerides (diets B and C) concentrations were significantly ($P<0.01$) decreased. Weekly preprandial measurements were characterized by decreased urea (diets B and C), cholesterol (diet C) and triglycerides (diets B and C) concentrations ($P<0.001$).

Conclusion—Chronic consumption of fermentable fiber is associated with mildly decreased protein digestibility and with metabolic effects in nonfed or fed dogs.

Clinical Relevance—A blend of fructo-oligosaccharides and sugar beet fiber should be tested as a dietary aid for treatment of chronic diseases, such as diabetes mellitus or hyperlipidaemia, in dogs.

Introduction

Fructo-oligosaccharides (FOS) are natural polymers of fructose found in various vegetables (banana, garlic, barley, onion)¹. These compounds are considered dietary fiber, according Trowell definition²; they are resistant to hydrolysis in the small intestine³ and are highly fermented in the large intestine, with production of short chain fatty acids⁴. They can modify microflora in dogs⁵ and cats⁵ and other species, such as rats⁶, pigs⁶, rabbits⁷ and human beings^{6,8,9}. They also can have metabolic effects in rats^{10,11}. Daily feeding of a 10% (w/w) oligofructose-containing diet to normolipidemic rats resulted in a decrease in plasma triglycerides and cholesterol concentrations¹²; the triglyceride-lowering effect was observed after 1 week. At present, FOS are incorporated in some commercial or specific-purpose dog foods to promote intestinal health or as an aid in the treatment of small intestinal bacterial overgrowth¹³. Sugar beet fiber is recognized as a fermentable fiber that promotes good-quality stools and is frequently used in commercial dog food^{14,15}. The purpose of the study reported here was to evaluate effects of a blend of FOS and sugar beet fiber on digestibility of

major nutrients and on plasma metabolites to assess potential use in pets with chronic diseases, such as diabetes mellitus or hyperlipidemia.

Materials and Methods

Dogs—Eight 1- to 1.4-year-old adult male Beagles weighing 10.0 to 13.5 kg, were obtained for the study^a. All dogs were healthy on the basis of results of physical examination, CBC^b and serum biochemical analysis (glucose^c, insulin^d, urea^c, creatinine^c, cholesterol^c and triglyceride^c concentrations and alkaline phosphatase^c and alanine transaminases^c activities). The dogs had never been used for experiments before this study. All dogs were castrated 2 months before entry in the study. Dogs were weighed weekly and were housed in outdoor kennels or, during digestibility trials, in individual metabolic cages in a room with natural lighting. Room temperature was maintained at 18 ± 2 C. Water was offered ad libitum. The protocol was approved by the university's committee for care and use of laboratory animals, and all experiments were carried out according to Belgian regulations for animal research and experimentation.

Diet composition—The basal diet (diet A) was composed of minced meat (beef), flaked maize, maize oil and a vitamin/mineral mixture^e (Table 1). In a preliminary study of 2 dogs, consumption of FOS (Raftilose)^f at an incorporation rate of 10 % dry matter in the diet was associated with runny feces. To avoid such inconvenience, FOS was mixed with sugar beet fiber^g in a 4-to-1 ratio. This blend of fermentable fiber was incorporated into diets B and C at proportion of 5 or 10 % on a dry matter basis, respectively. The FOS product used in this study contained 92.6 % FOS, with 3.9 % glucose, fructose and sucrose and 3.5 % water. The blend of FOS and sugar beet fiber was substituted to an equal proportion of flaked maize in each diet. All ingredients were mixed with 400 ml water and were given to dogs 5 minutes after preparation. Diet A (control) contained no additional fiber.

TABLE 1.- *Ingredients and nutrient composition of diets*

Ingredients, %	Diet					
	A		B		C	
	AF	DM	AF	DM	AF	DM
Beef protein (minced raw meat)	38.1	14.5	38.1	14.5	38.1	14.5
Flaked maize	56.8	78.1	53.1	73.1	49.4	67.9
Maize oil	2.6	3.8	2.6	3.8	2.6	3.8
Vitamins and minerals	2.5	3.6	2.5	3.6	2.5	3.6
Sugar beet fiber	-	-	0.8	1.0	1.5	2.0
Fructo-oligosacharides	-	-	2.9	4.0	5.9	8.2
Nutrients, g/1000 kcal	A		B		C	
Protein	65.3		66.9		68.8	
Fat	22.5		23.1		23.7	
Fiber (ADF)	7.7		8.4		9.1	
Calcium	2.0		2.0		2.0	
Phosphorus	1.3		1.3		1.3	
Sodium	0.8		0.8		0.8	

AF, as fed; DM, on dry matter basis

Experimental design—A randomized block experiment was chosen to allow comparisons between diets consumed by the same dog¹⁶. Dogs were randomly assigned to 1 of the 3 diet groups. To avoid carry over effects between feeding periods, a 4-week washout period was allowed between each of the 3 feeding periods during which dogs were fed diet A to avoid residual metabolic effect of the fiber.

Feeding protocol—Each diet was fed for 42 days. Amount fed was determined on the basis of daily maintenance caloric requirements for body weight ($132 \text{ kcal/day/kg}^{0.75}$)¹⁷; water was always available. Dogs were fed once a day at 9 AM, and voluntarily consumed their whole meal within 5 minutes.

Digestibility trials—Digestibility measurements were carried out during the last 7 days of each period when dogs were housed in metabolism cages. During the collection phase, total fecal output was collected twice daily and stored at 4 C. At the end of the week, feces were dried in an oven at 60 C to reach constant weight. After complete drying, feces were ground through a 2 mm screen by use of a mill. Feeds and feces were analyzed according to official procedures¹⁸.

Plasma samples—Preprandial metabolic and hormonal profiles were determined on day 0, 7, 14, 21, 28 and 35 in nonfed dogs. Five milliliters of blood was obtained by venipuncture from each dog. Postprandial profiles were also determined on day 42, the last day of the feces collection period. An indwelling sterile catheter was inserted in a cephalic vein. Catheters were filled with heparinized (120 U/ml) saline solution to prevent formation of a blood clot between sample collection periods. Dogs were handled gently and did not appear excited during sample collection. Blood was taken before feeding; then dogs were fed their assigned diet as a single meal. Serial postprandial blood samples (5 ml) were taken 20, 40, 60, 90, 120, 180, 240, 300 and 360 minutes after feeding. Plasma samples obtained from blood were stored at -18 C. All samples were analyzed the same day for plasma glucose^c, insulin^d, urea^c, α -amino-nitrogen^c, triglycerides^c and cholesterol^c concentrations.

Statistical evaluations—Digestibility data were analyzed, using a software package Statgraphics^h and a desktop computerⁱ. Mean (\pm SD) values were calculated for all data. Two-ways ANOVA was used, with dietary treatments and periods as variables. If ANOVA revealed differences in a single digestibility result attributable to diet consumed, comparisons between mean results of diet groups were performed, using a multiple range test¹⁶, a P value of < 0.05 was considered significant. Plasma metabolite data were analyzed, using a dynamic linear model, taking into account that data were autocorrelated^{19,20}. Mean (\pm SEM) values were reported for plasma metabolite data.

Results

Diet-induced variations in body-weight, feces, and digestibility of nutrients—

Acceptance of diet was good throughout the study; all dogs ate their entire ration at each meal. At initiation of the study, body weight ranged from 10 to 13.5 kg. Mean weight gains for dogs during the 42-day trial periods were 0.41, 0.65 and 0.24 kg for diets A, B and C, respectively.

Quantity of wet feces excreted (g/day) increased linearly ($P < 0.00025$) with increasing amount of fermentable fiber in the diet (Table 2). Dry matter content of feces, in turn, decreased linearly ($P < 0.00001$) from 29 % in dogs fed diet A to 22 % in dogs fed diet C.

TABLE 2.- *Characteristics of feces from and digestibility of dry and organic matter, protein, ether extract, and ash in 8 healthy Beagles fed diets containing different incorporation rates of a blend of fructo-oligosaccharides and sugar beet fiber*

	Diet		
	A	B	C
Feces characteristics			
Wet weight, g/day	139 ± 9^a	180 ± 10^{ab}	222 ± 20^b
Dry matter, %	29.0 ± 0.8^a	25.3 ± 0.9^b	22.0 ± 0.8^c
Dry matter, g/day	40.3 ± 2.4^a	45.0 ± 2.4^a	48.0 ± 3.0^a
Digestibility, %			
Dry matter	86.5 ± 0.5^a	86.0 ± 0.5^a	84.8 ± 0.7^a
Organic matter	88.1 ± 0.4^a	87.5 ± 0.5^a	86.2 ± 0.6^a
Protein	87.8 ± 0.5^a	86.3 ± 0.5^{ab}	83.8 ± 1.0^b
Ether Extract	92.0 ± 0.6^a	91.4 ± 0.5^a	89.7 ± 0.8^a
Ash	47.4 ± 1.7^a	48.4 ± 2.8^a	47.4 ± 3.2^a

Values with different superscripts differ ($P < 0.05$) significantly. Values are expressed as mean \pm SD.

Apparent dry and organic matter, fat and ash digestibilities were unaffected by incorporation of the blend of fiber. By contrast, protein digestibility decreased from 87.8 % in diet A to 83.8 % in diet C ($P < 0.004$).

Diet-induced variations of plasma metabolites in samples obtained up to 6 hours after the meal—All results were within référence ranges²¹ (Table 3). Postprandial insulin, α -amino-nitrogen and cholesterol concentrations were not affected. By contrast, glucose (Fig 1) and triglycerides (Fig 2) concentrations were significantly ($P < 0.009$ and $P < 0.001$, respectively) reduced by consumption of diet C during the 6-hour period. A dose effect was also observed for plasma urea concentration ($P < 0.011$ and $P < 0.001$ for diets B and C, respectively, vs diet A).

Figure 1.- Postprandial plasma glucose concentration (mean \pm SEM) in 8 healthy dogs fed diets containing different incorporation rates of a blend of fructo-oligosaccharides and sugar beet fiber

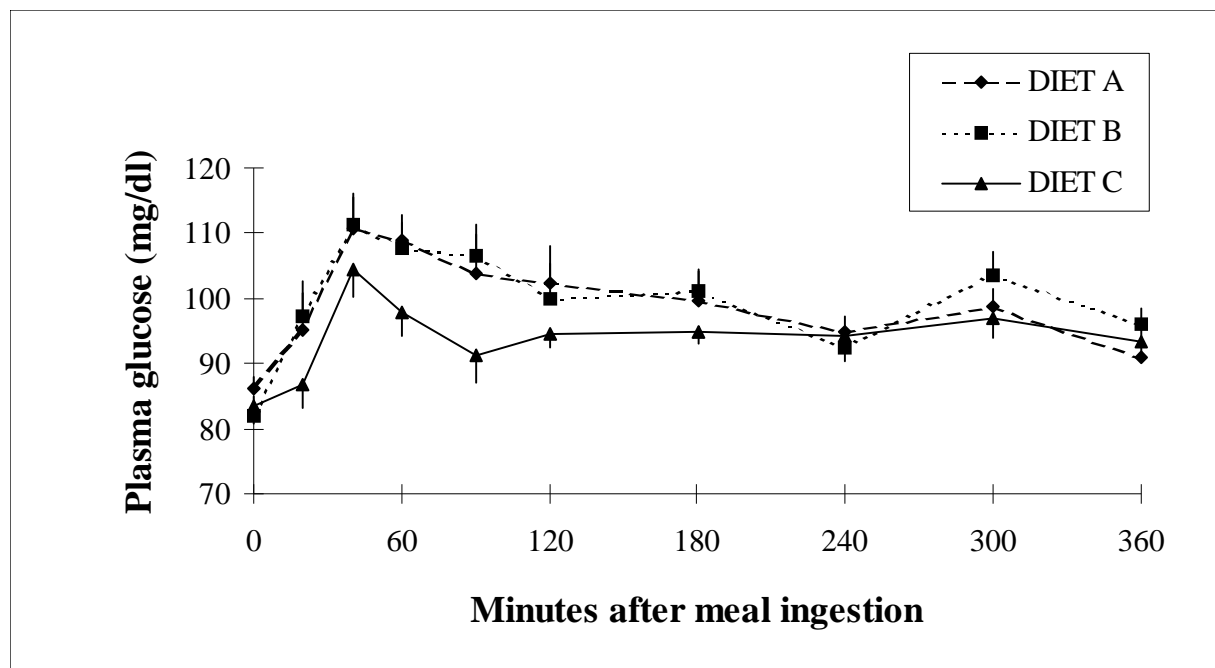
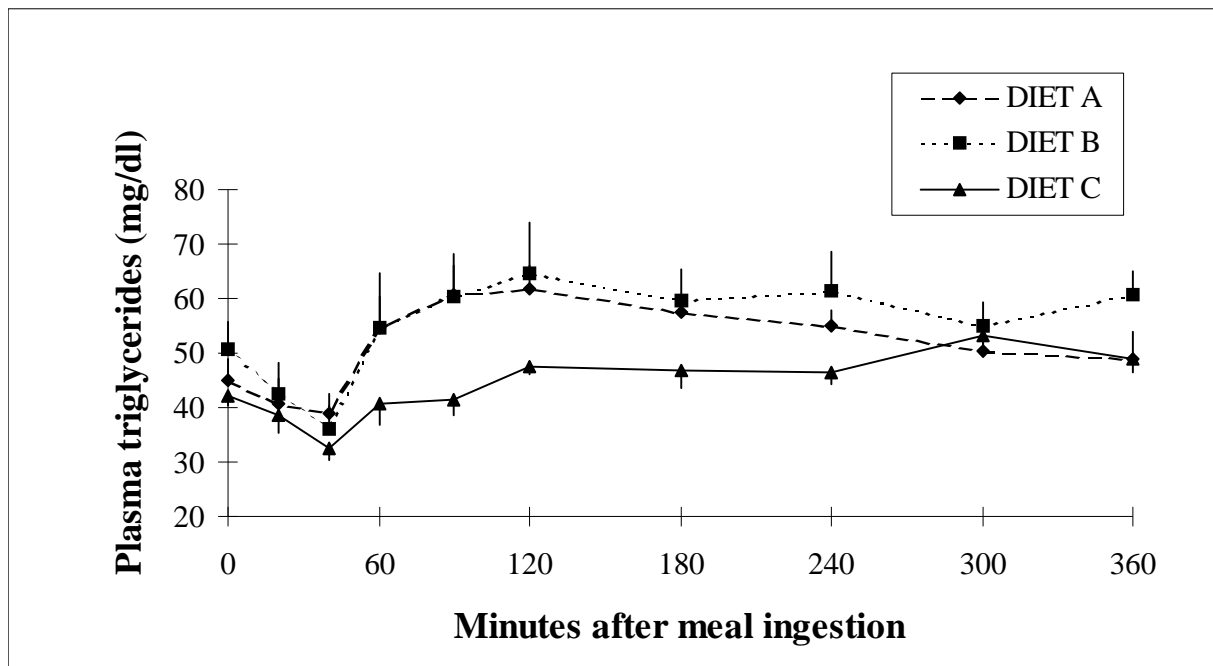


Figure 2.- Postprandial plasma triglycerides concentration (mean \pm SEM) in 8 healthy dogs fed diets containing different incorporation rates of a blend of fructo-oligosaccharides and sugar beet fiber



Diet-induced variations in plasma metabolites in weekly measurements—

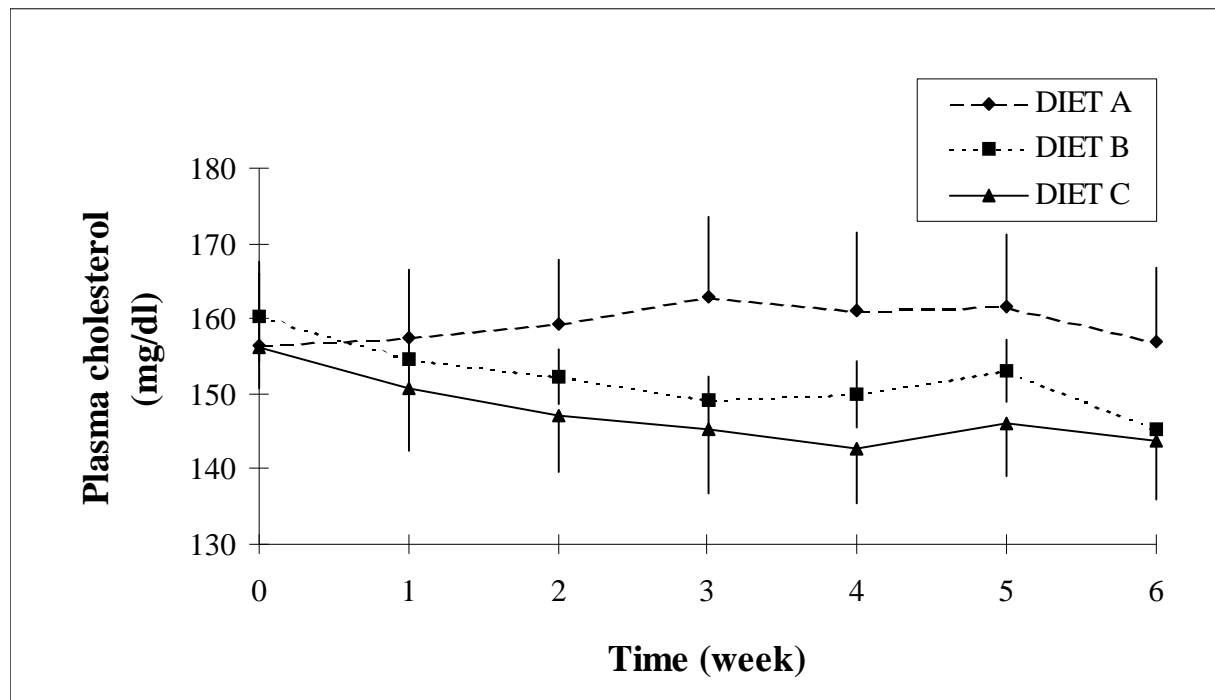
Preprandial glucose, insulin, α -amino-nitrogen concentrations were not affected in dogs fed diets B and C (Table 4). A dose effect was observed for urea ($P < 0.002$ for diet A vs diet B and $P < 0.001$ for diet C vs diet A) and triglycerides ($P < 0.008$ for diet A vs diet B and $P < 0.001$ for diet C vs diet A) concentrations. Feeding diet C to dogs led to a significant ($P < 0.009$) decrease in cholesterol concentration, compared with that in dogs fed diet A (Fig 3).

TABLE 4.- Average weekly plasma concentration in glucose, insulin, α -amino-nitrogen, urea, triglycerides and cholesterol in 8 healthy Beagle dogs offered diets containing different incorporation rates of a blend of fructo-oligosaccharides and sugar beet fiber

Variable	Time (week)						
	0	1	2	3	4	5	6
Glucose, mg/dl							
A	86 ± 2	88 ± 3	86 ± 1.	83 ± 4	83 ± 3	83 ± 3	86 ± 2
B	82 ± 4	89 ± 2	84 ± 3	89 ± 1	80 ± 3	79 ± 3	82 ± 1
C	83 ± 2	84 ± 3	86 ± 2	85 ± 3	83 ± 3	86 ± 2	83 ± 3
Insulin, mU/l							
A	7.5 ± 0.6	6.3 ± 1.0	6.7 ± 0.6	7.9 ± 0.7	12.3 ± 5.1	7.0 ± 0.6	6.5 ± 1.3
B	8.0 ± 0.6	8.8 ± 1.8	6.4 ± 1.1	10.0 ± 2.5	7.3 ± 0.7	14.1 ± 4.4	6.2 ± 1.9
C	5.2 ± 0.7	5.4 ± 2.7	5.0 ± 0.8	7.0 ± 3.0	8.0 ± 0.7	6.0 ± 0.6	6.3 ± 0.6
α-amino-nitrogen, mg/dl							
A	5.6 ± 0.3	5.3 ± 0.1	5.9 ± 0.2	5.5 ± 0.3	5.9 ± 0.4	5.5 ± 0.1	5.6 ± 0.4
B	5.6 ± 0.3	5.5 ± 0.3	5.8 ± 0.2	5.7 ± 0.2	6.0 ± 0.1	5.6 ± 0.2	5.6 ± 0.2
C	4.8 ± 0.2	5.6 ± 0.2	5.6 ± 0.3	5.6 ± 0.2	5.8 ± 0.2	5.0 ± 0.1	5.9 ± 0.1
Urea, mg/dl							
A ^a	13.6 ± 0.7	14.6 ± 0.9	15.3 ± 0.8	14.2 ± 0.8	14.3 ± 0.6	15.0 ± 0.5	15.0 ± 0.5
B ^b	13.1 ± 0.4	13.7 ± 0.5	13.9 ± 0.4	12.2 ± 0.6	14.4 ± 0.8	14.8 ± 0.9	14.8 ± 0.4
C ^c	15.2 ± 1.8	12.0 ± 0.9	13.2 ± 0.7	13.4 ± 0.6	12.5 ± 0.6	13.9 ± 0.5	13.6 ± 0.5
Triglycerides, mg/dl							
A ^a	47 ± 3	51 ± 3	50 ± 3	55 ± 3	52 ± 4	48 ± 3	51 ± 2
B ^b	47 ± 3	45 ± 3	44 ± 2	48 ± 3	49 ± 4	49 ± 3	45 ± 3
C ^b	49 ± 4	47 ± 3	44 ± 4	45 ± 4	43 ± 2	42 ± 2	42 ± 2

Variables with different superscripts differ at the P < 0.05 level. Values are expressed as mean ± SEM.

Figure 3.- Evolution in plasma cholesterol concentration (mean \pm SEM) in 8 healthy Beagle dogs fed diets containing different incorporation rates of a blend of fructo-oligosaccharides and sugar beet fiber



Discussion

Inclusion of fermentable fiber in the diet induced, on the whole, similar effects on feces content and apparent nutrient digestibility, to those described in dogs fed other dietary fibers. Increased feces production has been associated with various fibers, such as cellulose²², beet pulp^{14,15}, pectins²³ or maize fiber²⁴. In the study reported here, the main effect was an increase in water content of feces, the increase in dry matter excretion being insignificant. Increased water content of feces could be attributable, to some extent, to beet fiber, a component with a high water-holding capacity¹⁴. Digestibility of nutrients was high, owing to the high quality of basal ingredients. Thus, fermentable fiber supplementation had no effects on apparent digestibility of dry matter. Such results are consistent with reports in literature, because a systematic decrease in digestibility has not been associated with feeding of dietary fibers in dogs^{15,25}. Minimal incorporation rate of dietary fibers appears to be necessary to modify digestibility²⁶; furthermore, effects on digestibility of various nutrients are dependent on type of fiber used²⁵. In this study, the only significant effect of diet C was on digestibility of protein. Similar results in dogs were reported for incorporation of 3.5 % guar gum^{25,26} or 7

% guar gum²⁶ in the diet. Protein digestibility of a high-quality commercial diet ranges between 80 and 90 %²⁷. Protein digestibility coefficients of 87.8 % (diet A) and 83.8 % (diet B) can be considered acceptable. Thus, the present lowering effect of fiber did not influence nitrogen balance, which is known to be affected by dietary factors, such as protein quality, amino acids composition, protein digestibility and energy density^{27,28}. When protein digestibility is markedly decreased, protein content of the diet must be increased to ensure an adequate supply of amino acids.

Although FOS are incorporated at low rates in various commercial normal and specific-purpose diets, to the author's knowledge, metabolic effects in dogs have not been reported. The blend of FOS and sugar beet fiber had no effect on plasma insulin concentration before or after the meal. Lack of effect of dietary fiber on insulin concentration in nonfed dogs has been reported for other fibers such as guar gum²⁹. Individual changes in plasma insulin concentration in nonfed dogs are large³⁰. It appeared that glucose is not the only stimulus of insulin secretion in dogs, because meat intake also induces insulin production³¹. In this study, diet C limited the postprandial increase in glucose concentration in fed dogs but had no effect on plasma glucose concentration in nonfed dogs. Lack of effect of fiber on plasma glucose concentration in healthy nonfed dogs is not surprising because of the ability of dogs to maintain plasma glucose concentration within a narrow range, regardless the type of diet consumed³². For treatment of diabetic dogs, a diet low in soluble sugar, high in starch, and supplemented with dietary fiber^{33,34} is recommended to decrease postprandial hyperglycemia induced by food or to limit the degree in glucose concentration³⁵. This diet enhances action of insulin and reduces derangements in lipids metabolism frequently associated with diabetes mellitus^{32,36}. In some instances, this sort of diet can also allow reduction in daily requirements of diabetics for exogenous insulin³⁷. Our results suggest that this blend of FOS should be tested as a dietary aid in diabetic dogs.

Effects of a blend of FOS and sugar beet fiber on protein metabolism and on urea production in dog are unknown. Plasma α -aminonitrogen, considered an indicator of the adequacy of dietary protein, is closely related to individual plasma amino acids profiles³⁸. In this study, plasma α -aminonitrogen concentration was not affected; however, apparent digestibility of protein was significantly reduced. Various investigators reported a rapid increase in plasma urea concentration toward a plateau lasting for many hours before return to baseline concentration when a meal high in protein was offered once a day^{39,40}. Postprandial plasma urea concentration, however, was reduced when protein intake was lower⁴¹. In this

study, postprandial changes in plasma urea concentration recorded during a 6-hour period were in agreement with the previously described pattern. Furthermore, the blend of fiber had a dose effect on pre- and postprandial plasma urea concentrations.

Lipid metabolism also was modified by supplementation with fermentable fiber. Main effects were a large reduction in preprandial plasma cholesterol concentration associated with diet C and a tendency for lower postprandial cholesterol concentration. Such effects have been reported in other species such as rats, with larger inclusion rates of FOS^{11,42,43} than those in the study reported here. Although it was not significant, a similar effect was observed for plasma triglycerides concentration. Decreased preprandial concentration of cholesterol was observed in dogs receiving 3.5 % guar gum⁴⁴. In this study, the hypolipidemic effect could not be related to reduction in energy supply, which was similar, because all diets provided equivalent amounts of energy. Furthermore, apparent digestibility of ether extract of diets B and C was not affected. Other mechanisms could be involved. From studies in other monogastric species⁴⁵, it has been suggested that fermentation products in the large intestine could affect lipid metabolism in the liver. Support for such mechanisms was provided by a reduction in serum and liver cholesterol, but not triglycerides, concentrations in rats fed diets supplemented with 0.5 % propionate, a metabolic product of fiber fermentation. It was concluded that propionate may mediate part of the hypocholesterolemic effects of some soluble plant fibers⁴⁵. It also has been reported that propionate was produced in large amounts by *in vitro* fermentation of FOS from canine fecal inoculum⁴⁶. Although fermentation products were not measured during this study, one can speculate that propionate production from fermentable fiber in the large intestine could have mediated hypocholesterolemic effects.

In conclusion, at incorporation rates of 5 or 10 %, blends of FOS and sugar beet fiber did not change apparent digestibility of nutrients, except for protein, but induced metabolic effects. Incorporation of a blend of FOS and sugar beet fiber in industrial pet food may, therefore, be useful as an aid in dietary treatment of chronic diseases, such as diabetes mellitus and dyslipidemia.

^a Animal Reproduction Department, University of Liege, Belgium.

^b Cell-Dyn 3500, Abbott Laboratories, Chicago, Ill.

^c Technicon RA 1000, Technicon Autoanalyzer, Technicon Instruments, Tarrytown, N.J.

^d Insulin RIA-100, manufacturer's literature, Medgenix Diagnostics, Biosource Europe, Fleurus, Belgium.

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- ^e Minerals and Vitamins for dogs 9205, Premix, RADAR, Deinze, Belgium.
- ^f Raftilose, Raffinerie Tirlemontoise, Tirlemont, Belgium.
- ^g Betafibre, British Sugar, United Kingdom.
- ^h Statgraphics, Microsoftware Publishing Division, STSC Inc., Rockville, Md.
- ⁱ IBM, model 6322-002, IBM United Kingdom Ltd, Greenock, Scotland, United Kingdom
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ETUDE 5**FIBRES ALIMENTAIRES CHEZ LE CHIEN :****Influence de l'incorporation des pulpes de betterave ou de chicorée sur la digestibilité des nutriments et les concentrations plasmatiques de plusieurs métabolites.**

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RESUME

Cette étude relate les effets de la distribution de deux rations enrichies en pulpes de betterave ou de chicorée (7 p.100 ADF dans la matière sèche) -en comparaison à une ration témoin- sur les paramètres fécaux, la digestibilité des principaux nutriments et plusieurs paramètres biochimiques (glucose, insuline, azote alpha-aminé, urée, cholestérol et triglycérides) mesurés à jeun ou pendant six heures après le repas chez huit chiens adultes en bonne santé. Les régimes enrichis en pulpes ont entraîné une augmentation significative de l'excrétion fécale totale, de l'excrétion de MS dans les matières fécales, une diminution de la teneur en MS des fèces ainsi qu'une diminution des coefficients de digestibilité des principaux nutriments. Le régime contenant la pulpe de betterave a entraîné une diminution de la glycémie à jeun, de l'insulinémie et des concentrations en triglycérides postprandiales, des

concentrations en urée et en cholestérol mesurées avant et après le repas. Le régime contenant la pulpe de chicorée a provoqué une diminution de l'insulinémie postprandiale, des concentrations en urée et en cholestérol à jeun et après le repas. Ces deux types de pulpes, semblables par leur composition, induisent les mêmes types d'effets sur les paramètres fécaux et la digestibilité des nutriments, mais les effets systémiques sont plus importants lors de la distribution de pulpes de betterave.

MOTS-CLÉS : fibres alimentaires - digestibilité - paramètres sanguins - chien.

SUMMARY

Dietary fibre in the dog - Influence of diets containing sugar beet- or chicory pulps on nutrients digestibility and on plasma metabolites. By M. DIEZ, J.L. HORNICK, C. VAN EENAEME, P. BALDWIN and L. ISTASSE.

Two diets containing sugar beet pulp or chicory pulp (7 p. 100 ADF on dry matter basis) were compared with a control diet without additional fibre in eight adult healthy dogs. The three diets were evaluated for their ability to modify faecal parameters, nutrients digestibility and blood metabolites (glucose, insulin, alpha-amino nitrogen, urea, cholesterol and triglycerids) measured on fasted dogs or postprandially during six hours. Incorporating sugar beet pulp or chicory pulp in the diet was associated with increased wet faeces excretion, increased excretion of faecal dry matter and lower nutrients digestibility. Consumption of sugar beet pulp containing diet was associated with decreased fasted glucose, cholesterol and urea concentrations and with decreased postprandial insulin, urea, cholesterol and triglycerids concentrations. Chicory pulp containing diet led to significant decrease of fasted urea and cholesterol concentrations and with postprandial decreased of insulin, urea and cholesterol. Sugar beet pulp and chicory pulp, similar in composition, produced similar effects on faecal parameters and nutrients digestibility but systemic effects were more important with beet pulp.

KEY WORDS : dietary fibre - digestibility - blood parameters - dog.

Introduction

Le marché des aliments industriels pour les carnivores domestiques est en constante progression en Europe. Actuellement, le volume de vente de ces aliments permet de nourrir en moyenne 55 p. cent des chiens européens. Les contraintes des fabricants sont de proposer des aliments complets et équilibrés en nutriments à des coûts intéressants. La valorisation de sous-produits industriels comme les farines animales et les sous-produits végétaux permet la production annuelle de plusieurs centaines de milliers de tonnes d'aliments en France [14].

Parmi les nutriments indispensables à la santé de l'animal, les protéines, les minéraux, les vitamines ou les acides gras ont fait l'objet de nombreuses publications [30,38,39]. Les besoins journaliers sont de mieux en mieux définis. Par contre, les fibres alimentaires ne constituent pas des nutriments indispensables au sens strict mais il est généralement admis qu'elles exercent des effets favorables sur le transit intestinal [11, 12], le bon fonctionnement du colon [23] et même sur la tolérance au glucose [21,36,40]. Elles sont incorporées à faible concentration (1 à 5 p. cent de la matière sèche -MS) dans les aliments physiologiques et à doses plus élevées (jusqu'à 20 p. cent MS) [33] dans les aliments diététiques pour la prévention de la constipation [31], le traitement de l'obésité [24], du diabète [21,36,40] ou de certaines colopathies [32]. Bien que l'on ait souvent opposé les fibres insolubles représentées par la cellulose aux fibres solubles représentées par les gommages ou pectines, plusieurs auteurs préconisent actuellement l'utilisation de fibres mixtes, contenant à la fois des fibres solubles et insolubles [15,17,21]. La pulpe de betterave est une source de fibre mixte largement utilisée dans les aliments industriels en raison de son faible coût et de ses propriétés physico-chimiques; elle est constituée d'un mélange de fibres solubles et insolubles caractérisé par une bonne capacité de rétention d'eau [15,17]. La chicorée (*Cichorium intybus*) est cultivée pour la production de l'inuline et de ses produits d'hydrolyse comme les fructo-oligosaccharides. Le traitement de la chicorée en usine laisse un résidu appelé ,comme pour la betterave sucrière, pulpe. La pulpe constitue donc le sous-produit de la culture de chicorée. Cette étude avait pour but de comparer les effets de ces deux types de pulpes qui présentent des compositions chimiques comparables [45]. Les investigations ont porté sur les effets de l'incorporation de ces deux sources de fibres - à raison de 7 p. cent d'ADF (Acid Detergent Fibre - fibre détergent acide) - dans un régime complet et équilibré distribué à des chiens

adultes en bonne santé. Les paramètres étudiés étaient les modifications biochimiques plasmatiques et les modifications fécales.

Matériel et méthodes

A) LES ANIMAUX

Nous disposions de 8 chiens de race Beagle, dont 2 mâles non castrés et 6 femelles stérilisées, âgés de 5 ans, identifiés, vermifugés (Praziquantel, Embonate de Pyrantel, Febantel) et vaccinés (maladie de Carré, hépatite, parainfluenza, parvovirose, leptospirose et rage). Tous les chiens étaient en bonne santé sur base d'un examen clinique, d'une numération sanguine et des analyses biochimiques (glucose, insuline, urée, créatinine, cholestérol, triglycérides, phosphatases alcalines et alanines amino-transférases). Le poids des animaux variait de 10 à 14,4 kg au début de l'expérience. Les animaux étaient pesés une fois par semaine. Les chiens étaient dans un chenil extérieur pourvu d'un abri, en groupe de 2 ou 3, ou dans des cages à métabolisme durant les mesures de digestibilité. Les cages à métabolisme étaient placées dans un local pourvu d'une lumière naturelle. Le protocole expérimental a été approuvé par le comité d'éthique responsable des soins et de l'utilisation des animaux de laboratoire.

B) LES ALIMENTS

Les compositions des 3 régimes testés sont présentées dans les Tableaux I et II.

TABLEAU I.- *Composition des rations*

Ingrédients en p. cent de la ration	A	B	C
Viande de boeuf	42,3	36,2	37,5
Maïs floconné	50,6	43,4	45,0
Huile de maïs	4,2	3,6	3,7
Complexe minéral et vitaminé	2,9	2,5	2,6
Pulpes	-	14,3	11,2

TABLEAU II. - *Composition chimique moyenne des rations*

Analyses en p. cent de la MS	A	B	C
Protéines brutes	24,5	22,6	22,9
Matières grasses	14,3	11,8	12,3
Calcium	0,6	0,7	0,7
Phosphore	0,5	0,4	0,5
Fibre détergent acide ADF	2,7	7,0	7,3
Fibres alimentaires totales TDF	10,3	23,0	19,9
Fibres insolubles	9,2	19,8	15,8
Fibres solubles	1,1	3,2	4,0
Densité énergétique, kcal/kg MS *	4005	3239	3421

* Calculée en attribuant un coefficient de 3.52 kcal/g pour les glucides digestibles et les protéines et un coefficient de 8.46 kcal/g pour les lipides.

La ration de base (aliment A) était composée de viande de boeuf hachée, de maïs floconné moulu, d'huile de maïs et d'un complexe minéral et vitaminé (Radar, Belgique) spécialement formulé pour le test. Les pulpes de betterave (aliment B) ou de chicorée (aliment C) étaient ajoutées à la ration de base en quantité suffisante pour apporter 7 p. cent de fibres ADF dans la MS. Toutes les rations contenaient de la fibre de maïs, à prédominance insoluble. Les compositions en fibres des pulpes étaient sensiblement différentes (Tableau III). La pulpe de betterave contenait 25,4 p. cent ADF et 76,5 p. cent de fibre alimentaire totale - Total Dietary Fiber (TDF) dont 11,9 p. cent de fibres solubles dans la MS tandis que la pulpe de chicorée contient 34,4 p. cent ADF et 76,1 p. cent TDF dont 21,5 p. cent de fibres solubles. L'examen de ces compositions permet d'expliquer les différences entre les rations. D'autre part, la pulpe de chicorée contenant plus de fibres ADF, des quantités moindres ont été ajoutées pour obtenir 7 p. cent d'ADF dans la MS. Les rations expérimentales étaient préparées chaque matin en mélangeant les ingrédients et en ajoutant 400 ml d'eau. Les animaux recevaient en un seul repas une quantité fixe de nourriture correspondant à leurs besoins énergétiques calculés selon la formule 132 kcal/kg PM (P.V. kg^{0.75}) [38]. Les chiens étaient habitués à consommer la totalité de leur repas en 5 minutes.

TABLEAU III.- *Composition chimique des pulpes de betterave et de chicorée*

Analyses en p. cent de la MS	Pulpes de Betterave	Pulpes de Chicorée
Protéines brutes	14,8	14,1
Matières grasses	0,7	0,2
Cendres brutes	6,8	6,6
Calcium	1,2	1,1
Phosphore	0,1	0,2
Fibre détergent acide ADF	25,4	34,4
Fibres alimentaires totales TDF	76,5	76,1
Fibres insolubles	64,6	54,6
Fibres solubles	11,9	21,5

C) CONDUITE DE L'EXPÉRIMENTATION

Afin de minimiser les différences individuelles ou de période, un schéma expérimental de bloc aléatoire complet à 3 périodes a été choisi pour permettre les comparaisons entre les régimes consommés par le même animal [8]. Les chiens ont été attribués à un des groupes de façon aléatoire. Une période de transition d'une semaine a été instaurée entre chaque période expérimentale qui durait 4 semaines. Pendant cette période, les chiens recevaient la ration A. La durée totale de l'expérience était donc de 15 semaines. Chaque aliment testé a été distribué pendant un mois. Au cours de la dernière semaine du mois, les animaux ont été placés en cage à métabolisme pour la récolte totale des matières fécales pendant 7 jours successifs. Les chiens adultes étaient habitués aux cages et aucun problème comportemental n'a été observé. Les chiens recevaient également de l'eau *ad libitum*. A la fin de cette dernière semaine, après la dernière récolte de matières fécales, un cathéter était inséré dans la veine céphalique chez les animaux à jeun. Un premier prélèvement de 5 ml de sang était réalisé avant la distribution du repas et ensuite, 20, 40, 60, 90, 120, 180, 240, 300 et 360 minutes après le repas. Les cathéters étaient remplis avec une solution saline héparinée (120U/ml) pour prévenir la formation de caillots entre les prélèvements. Les échantillons étaient immédiatement centrifugés à 3000 tours pendant 15 minutes et le plasma était congelé à -20°C. Tous les

échantillons ont été analysés en même temps pour les dosages des métabolites suivants : glucose, insuline, azote alpha-aminé, urée, triglycérides et cholestérol.

D) ANALYSES BIOCHIMIQUES

La MS, les cendres, l'ADF et l'extrait éthéré ont été déterminés par les procédures standards dans les aliments et dans les matières fécales [1]. La TDF a été analysée avec un kit (Sigma TDF-100) selon la méthode publiée par l'AOAC [2]. La détermination de la fibre insoluble a également été réalisée et la fibre soluble a donc été calculée par différence. L'azote a été déterminé par la méthode de Kjeldahl par digestion et colorimétrie automatique selon la réaction de Berthelot [44]. Les concentrations en glucose et en urée ont été analysées sur un analyseur Technicon. L'insuline a été mesurée avec un kit insuline RIA 100 (Medgenix Diagnostics, Biosource Europe, Fleurus, Belgium). Le cholestérol et les triglycérides ont été analysés avec des kits (Boehringer).

E) ANALYSE STATISTIQUE

Les données de digestibilité ont été analysées avec le programme Statgraphics (Statgraphics, STSC, SNC, Microsoftware Publishing Division, Rockville, Maryland). Les moyennes et l'écart-type ont été calculés pour chaque donnée. Une analyse de la variance à 2 critères a été réalisée en utilisant les traitements alimentaires et les périodes comme facteurs. Lorsque l'analyse de la variance révélait des différences, les comparaisons entre moyennes étaient calculées avec le test de Scheffé, une valeur de $P < 0.05$ étant considérée comme significative. Les données concernant les métabolites plasmatiques ont été analysées selon un modèle linéaire dynamique prenant en compte l'autocorrélation des données [28,35].

Résultats

A) CARACTÉRISTIQUES DES MATIÈRES FÉCALES ET DIGESTIBILITÉ DES NUTRIMENTS.

Préalablement, il faut souligner que les différentes rations ont été bien acceptées par les animaux; les chiens mangeaient la totalité de leur ration à chaque repas. Les variations moyennes de poids au cours de l'essai étaient de + 38 g pour les animaux témoins, de -262 et -125 g pour les animaux recevant respectivement les rations enrichies en pulpes de betteraves et de chicorées. Les principales caractéristiques des matières fécales sont présentées dans le Tableau IV.

TABLEAU IV.- *Caractéristiques des matières fécales (Moyenne et écart-type)*

	A	B	C
Excrétion fécale, g/jour	72,8 (14,7) ^a	368,0 (100,3) ^b	351,6 (87,4) ^b
Teneur en MS, p. cent	34,4 (4,4) ^a	15,6 (2,5) ^b	17,3 (3,0) ^b
Excrétion fécale, g MS/jour	25,0 (4,7) ^a	57,4 (11,8) ^b	60,8 (16,5) ^b

^{a,b} Les valeurs portant une lettre différente en exposant différent à l'intérieur d'une ligne.

La comparaison de l'excrétion fécale en termes de poids frais lors de l'incorporation des pulpes de betterave ou de chicorée a montré une augmentation significative ($P < 0,001$). L'utilisation des pulpes a provoqué une diminution de 55 p. cent et de 50 p. cent de la teneur en MS des matières fécales ($P < 0,001$), respectivement pour les aliments B et C. Il en résulte une augmentation de la quantité de MS excrétée journallement dans les fèces (augmentation significative de 129 et 143 p. cent, respectivement pour les rations B et C). Enfin, il n'existe aucune différence significative entre les 2 types de pulpes quant à leurs effets sur les matières fécales.

Les coefficients de digestibilité apparente de la ration témoin étaient relativement élevés en raison de la bonne qualité des ingrédients utilisés dans la ration de base. La digestibilité apparente de la MS, de la matière organique, des protéines brutes, des matières

grasses et des cendres a diminué lors de l'incorporation des pulpes de betterave ou de chicorée ($P < 0.01$ ou $P < 0.001$) (Tableau V).

TABLEAU V.- *Coefficients de digestibilité apparente des principaux nutriments (Moyenne et écart-type)*

Digestibilité apparente, en p. cent	A	B	C
Matière sèche	88,1 (1,9) ^a	77,3 (4,0) ^b	74,3 (5,7) ^b
Matière organique	90,6 (1,4) ^a	80,7 (3,6) ^b	77,8 (4,8) ^b
Protéines brutes	90,7 (2,1) ^a	79,6 (5,0) ^b	76,7 (7,1) ^b
Matières grasses	95,7 (0,8) ^a	93,6 (2,2) ^b	93,3 (1,7) ^{bc}
Cendres	41,9 (15,2) ^a	24,2 (13,6) ^{bc}	25,0 (10,8) ^b

^{a,b} Les valeurs portant une lettre différente en exposant différent à l'intérieur d'une ligne.

B) MODIFICATIONS BIOCHIMIQUES DANS LE PLASMA SANGUIN

Les concentrations plasmatiques des différents métabolites mesurés à jeun se situaient dans la limite de la normalité [29]. Le profil glycémique (Figure 1) était caractérisé par un pic 20 minutes après le repas pour les traitements A et C et 40 minutes après le repas pour le traitement B. La glycémie à jeun était significativement plus faible pour le traitement B par rapport au témoin ($P < 0.05$). Par contre, il n'existait pas de différence significative dans l'évolution postprandiale des profils glycémiques.

L'insulinémie moyenne (Figure 2) à jeun était de 53 pmol/l, pour les 3 traitements. L'examen de l'évolution postprandiale de la concentration en insuline a révélé des différences entre les traitements. L'incorporation des 2 types de pulpes a induit une insulinémie postprandiale significativement plus faible sur l'ensemble de la période d'observation ($P < 0.01$). L'ingestion de rations enrichies en pulpes n'a pas induit de différence dans les concentrations plasmatiques en azote alpha-aminé mesurées à jeun et après le repas (Figure 3).

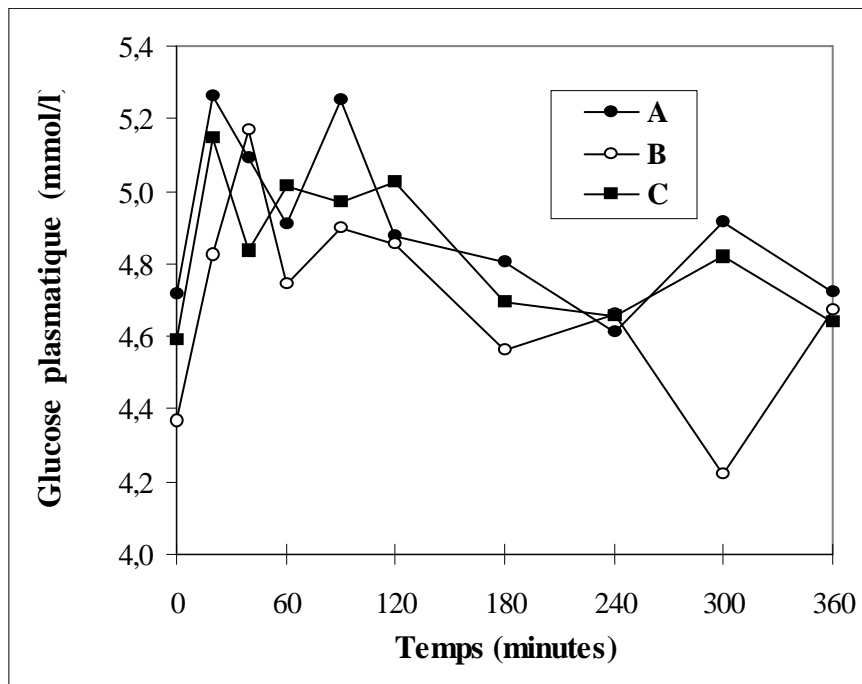
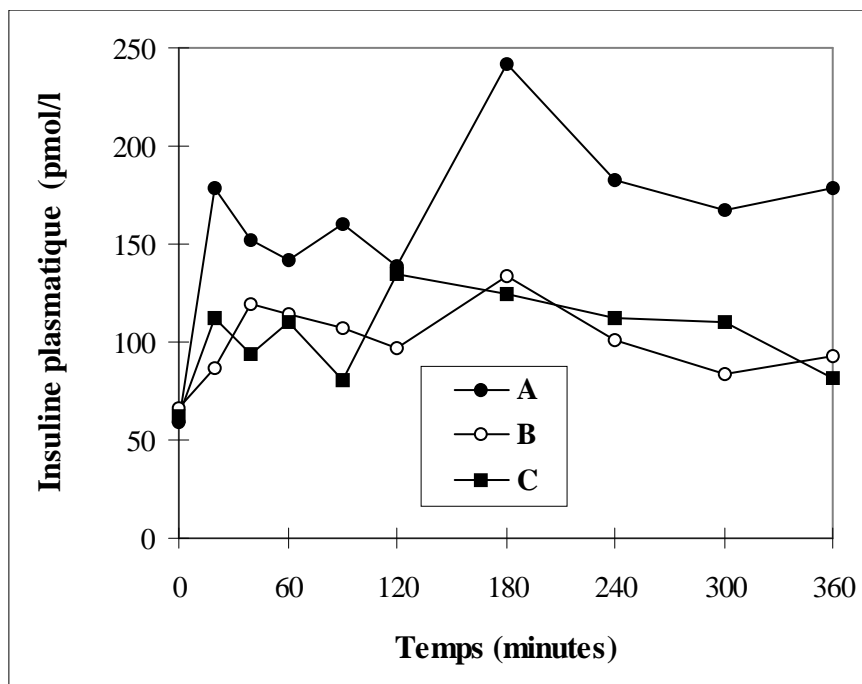
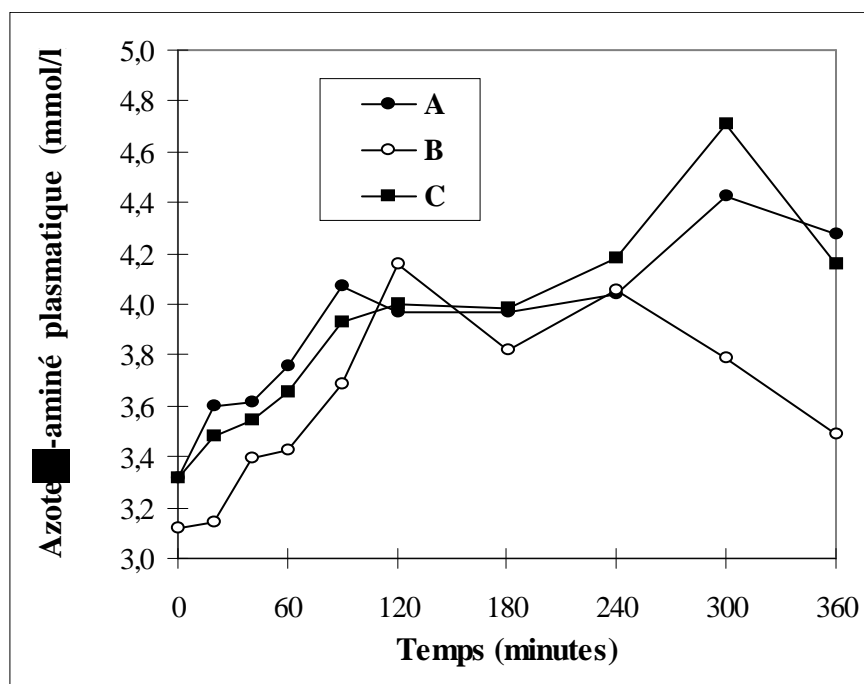
Fig. 1.- Evolution de la concentration plasmatique en glucose.**Fig. 2.-** Evolution de la concentration plasmatique en insuline.

Fig. 3.- Evolution de la concentration plasmatique en azote alpha-aminé.

L'examen des concentrations plasmatiques en urée mesurées à jeun ou après le repas a révélé des différences significatives entre la ration témoin et les rations enrichies en pulpes (Figure 4). La concentration postprandiale en urée a été la plus faible lors de l'ingestion de la ration B ($P < 0.001$) et intermédiaire avec la ration C ($P < 0.01$).

La cholestérolémie à jeun a été significativement diminuée lors de l'ingestion des repas B et C (Figure 5). La cholestérolémie postprandiale a été réduite également lors de l'ingestion des 2 types de pulpes ($P < 0.01$).

La concentration en triglycérides à jeun n'a pas été modifiée lors de l'ingestion des régimes B et C par rapport au régime A (Figure 6). Par contre, la concentration plasmatique en triglycérides a été modifiée après le repas par l'ingestion de pulpe de betterave ($P < 0.05$) alors que les pulpes de chicorée n'ont pas eu d'effet.

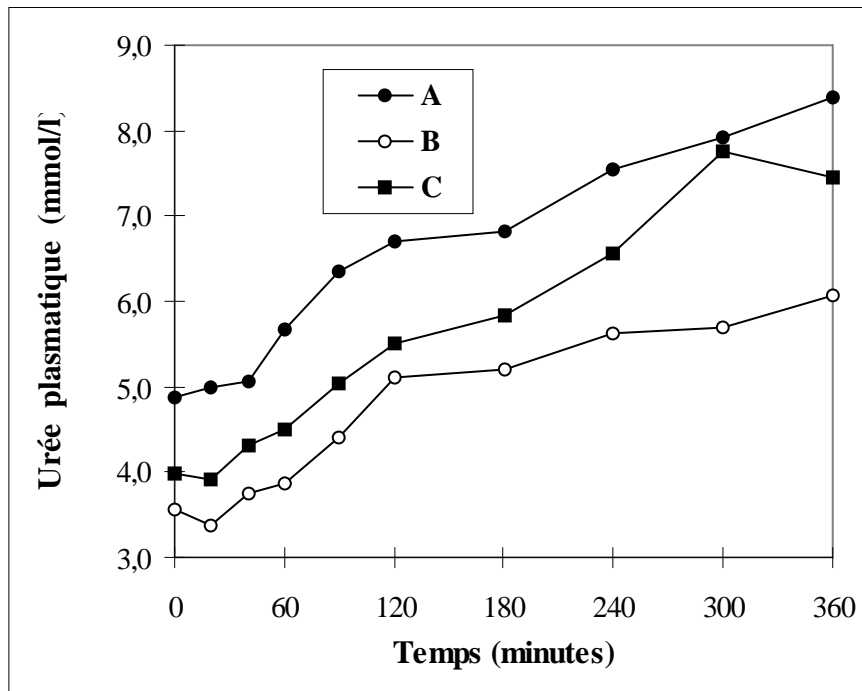
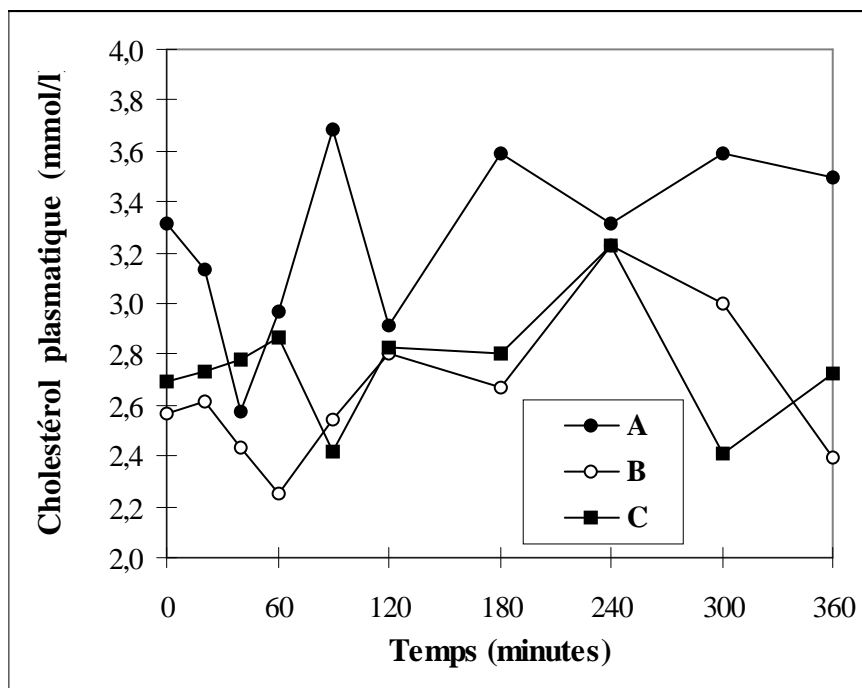
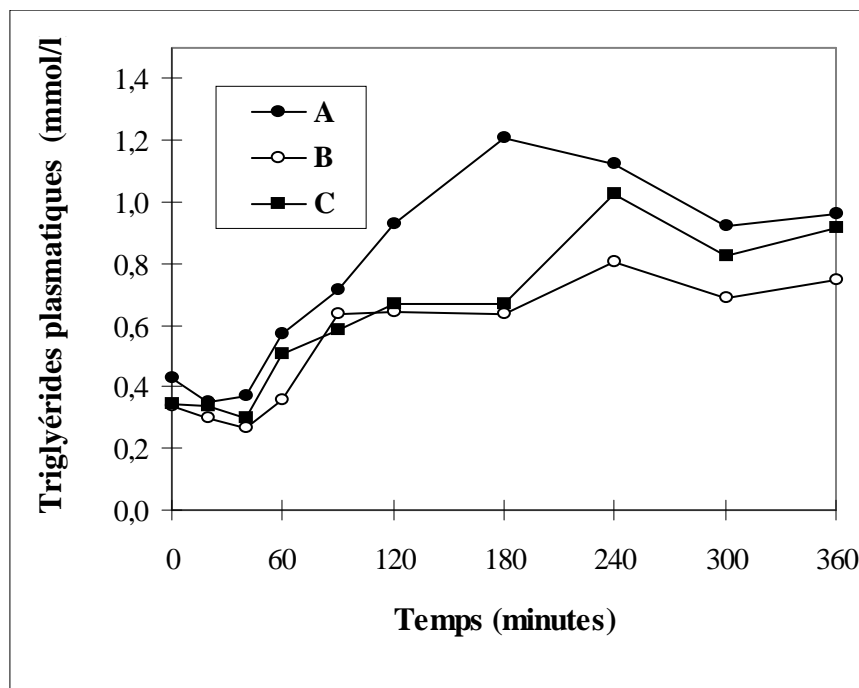
Fig. 4.- Evolution de la concentration plasmatique en urée.**Fig. 5.-** Evolution de la concentration plasmatique en cholestérol.

Fig. 6.- Evolution de la concentration plasmatique en triglycérides.

Discussion

L'incorporation de fibres alimentaires dans les aliments pour chien est actuellement une pratique courante aussi bien pour les aliments physiologiques que pour les diététiques. Les exigences légales en matière d'étiquetage des aliments destinés aux carnivores sont limitées aux nutriments essentiels et à la cellulose brute. Les dosages de la cellulose brute ainsi que celui de la fibre ADF sous-estiment grandement les teneurs réelles en fibres des aliments, et ce d'autant plus que des quantités importantes de fibres solubles sont ajoutées à la ration. Ainsi dans notre étude, une ration contenant 7 p. cent de fibres ADF dans la MS contenait en réalité 23 p. cent de fibres totales. C'est-à-dire que environ un quart de la MS du mélange était constitué d'un résidu indigestible. En réalité, la différence entre ADF et fibre totale était déjà importante pour le régime A. Une des explications précédemment proposée est que des quantités importantes de fibres sont amenées par les aliments vecteurs de protéines [3]. En effet, la viande contient des complexes protéino-polysaccharidiques, notamment au niveau des tissus de soutiens (tendons, aponévroses). Ces matériaux fibreux peuvent échapper à la digestion enzymatique et être dosés lors des mesures de la fibre totale [15]. Cette hypothèse peut expliquer la quantité importante de fibre totale présente dans le régime de base

utilisé dans cette étude. D'autre part, l'addition de fibres a entraîné une dilution des nutriments, d'où une diminution de la densité énergétique pour les régimes B et C. Néanmoins, cet effet n'a pas eu d'influence sur les paramètres mesurés puisque les chiens recevaient une quantité de nourriture basée sur leurs besoins énergétiques journaliers et qu'ils consommaient la totalité de leur ration.

Les quantités de matières fécales excrétées quotidiennement ont augmenté suite à l'incorporation des pulpes dans les rations. Cette propriété est exploitée pour prévenir la constipation et est généralement associée à l'ingestion de fibres insolubles, peu fermentées et présentant des capacités de rétention d'eau [42]. Bien que toutes les sources de fibres entraînent des augmentations du poids fécal, les fibres solubles ont généralement un effet moins prononcé. Dans notre étude, les pulpes de betterave et de chicorée ont entraîné les mêmes conséquences sur les paramètres fécaux. L'augmentation du poids fécal a été précédemment décrit chez le chien suite à l'ingestion de diverses fibres purifiées comme la cellulose [5], les pectines [34], la fibre de maïs [13] ou d'ingrédients riches en fibres comme les pulpes de betterave² [15, 16, 17,]. La teneur moyenne en MS des fèces varie entre 28 et 42 p. cent [22]. La teneur en MS des fèces des animaux recevant le régime de base dans cette expérience est situé dans cette fourchette. Par contre, chez les animaux recevant les pulpes en supplément, les teneurs en MS étaient réduites de moitié. Cependant, il ne faut pas conclure que ces animaux présentaient de la diarrhée. Au contraire, le volume de fèces était important mais elles étaient fermes et bien moulées. La teneur en MS n'est donc pas un critère absolu de détermination de la qualité des matières fécales. L'observation de la consistance est un meilleur critère que la simple détermination de la teneur en MS [34]. Malheureusement, l'augmentation du volume des fèces est considéré plus comme un inconvénient par de nombreux propriétaires.

L'altération de la digestibilité des principaux nutriments suite à l'ingestion de régimes riches en fibres a été rapportée à de nombreuses reprises chez les carnivores [9,10,15,16,17]. La diminution de la digestibilité des protéines est due à une augmentation de l'excrétion fécale. Comme aucune mesure bactérienne n'a été réalisée dans cette étude, il n'est pas possible de déterminer la nature de l'azote fécal : protéines alimentaires non digérées, pertes endogènes ou protéines microbiennes. En effet, la teneur importante en protéines dans les fèces pourrait indiquer une prolifération induite par les fibres solubles et fermentescibles contenues dans les pulpes. Bien que significatifs, les effets sur la digestibilité des matières grasses ont été de moindre ampleur. A la lecture de ces résultats, il apparaît que lors de la

formulation des aliments, il faut, soit fixer un niveau de fibres inférieur qui n'altérera pas la digestibilité des nutriments, soit augmenter les concentrations en protéines et en minéraux de façon à contrecarrer ces effets négatifs. La dernière suggestion est rencontrée dans la formulation de régimes hypocaloriques à haute teneur en fibres insolubles [24].

Bien que les fibres soient principalement incorporées dans les aliments pour chiens en raison de leurs effets sur les matières fécales et la santé du côlon, elles peuvent aussi induire des effets systémiques. Le chien présente une glycémie extrêmement stable, généralement comprise dans une fourchette de 0.8 à 1.2 g/l [18]. La teneur en glucides digestibles de la ration peut varier de 0 à 62 p. cent sans influencer de façon significative la glycémie à jeun et l'évolution postprandiale du glucose et de l'insuline [41]. Néanmoins, les pulpes de betterave ont induit une diminution de la glycémie à jeun; des tendances vers une diminution étant observées avec les pulpes de chicorée. Ces résultats sont en contradiction avec d'autres expériences rapportées chez le chien. Aucun effet n'a été rapporté avec des rations enrichies en cellulose, pectines ou gomme de guar à des taux d'incorporation de 3.5 p. cent dans la MS [10,26], des rations enrichies en gomme de guar utilisée à raison de 7 p. cent dans la MS [10] ou d'un mélange de gomme de guar et de fibres de pois à une dose de 15 p. cent de la MS [21]. Il semblerait donc que l'utilisation de grandes quantités de fibres puissent induire une diminution de la glycémie à jeun, tout en la maintenant dans les limites normales. Paradoxalement, l'étude des profils postprandiaux n'a pas révélé pas de différence significative bien que l'ingestion de pulpes de betterave tendait à induire une glycémie et un pic plus faibles.

L'ingestion du régime enrichi en pulpes a également entraîné une diminution de l'insulinémie postprandiale, ainsi que précédemment rapporté avec d'autres types de fibres alimentaires [36]. Les effets des pulpes de betterave sont comparables en certains points à ce qui a été rapporté chez l'homme [31,37]. La fibre de betterave peut améliorer la tolérance au glucose mais elle n'influence pas la sécrétion d'insuline [7]. Les doses de fibres utilisées dans ces expériences chez l'homme étaient inférieures aux concentrations que nous avons testées.

L'absence d'effets de l'ingestion des pulpes sur la concentration plasmatique en azote alpha-aminé peut sembler en contradiction avec les résultats de la digestibilité des protéines. En effet, le dosage de l'azote alpha-aminé est un indicateur du statut nutritionnel protéique de l'animal. Une diminution de la concentration en azote alpha-aminé aurait pu être interprétée comme le signe d'une liaison protéines-fibres entraînant une diminution de la digestibilité

protéique. Les effets négatifs sur la digestibilité ne se traduisent donc pas sur la concentration en azote alpha-aminé. Par contre, l'ingestion des pulpes s'est accompagnée d'une diminution des concentrations en urée plasmatique mesurées à jeun et après le repas. Une telle variation pourrait être due à une diminution du catabolisme des acides aminés au niveau hépatique [10].

Les effets sur le métabolisme lipidique sont également intéressants. L'ingestion des 2 types de pulpes a entraîné une diminution des concentrations plasmatiques en cholestérol mesurées chez l'animal à jeun ainsi qu'après le repas. A ce jour, l'utilisation de fibres alimentaires à faibles doses n'a pas permis de mettre en évidence des modifications de la cholestérolémie chez le chien [26]. Cependant, l'incorporation de fibres de maïs (24 p. cent), a induit une diminution de la cholestérolémie et de la triglycéridémie à jeun [13]. Il apparaît donc d'après cette étude et selon nos propres résultats que des taux élevés de fibres (supérieurs à 20 p. cent TDF dans la MS) soient nécessaires pour modifier le profil lipidique chez le chien. Par contre, chez l'homme [19,20,43] et le rat [27], la plupart des fibres solubles ainsi que la fibre de betterave permettent de réduire les concentrations plasmatiques en cholestérol. Selon Chen et al. [6], le propionate, produit de fermentation des fibres, pourrait entraîner des modifications hépatiques susceptibles de modifier le métabolisme du cholestérol. Chez le chien, les hyperlipémies ne sont pas rares; lors d'une étude épidémiologique [4], des hyperlipémies à jeun ont été constatées chez 14,3 p. cent de la population. Dans la plupart des cas, l'hyperlipémie était secondaire à d'autres désordres métaboliques tels que le diabète mellitus et l'hypothyroïdie. La pulpe de betterave qui, utilisée à concentration élevée, induit des effets systémiques sur le métabolisme des glucides et des lipides pourrait donc être efficacement utilisée pour le traitement diététique de ces troubles métaboliques. Une telle utilisation a d'autant plus d'intérêt que le diabète est fréquemment associé à des troubles du métabolisme lipidique [40].

En conclusion, il est apparu lors de cet essai que les principaux effets de l'ingestion de grandes quantités de pulpes étaient des modifications des caractéristiques des matières fécales et de la digestibilité des nutriments associés à certains effets systémiques exploitables pour le traitement des maladies chroniques. La comparaison des 2 types de pulpes montre que leurs effets sur les fèces et les digestibilités sont comparables mais que les effets systémiques sont moins prononcés lors de l'utilisation des pulpes de chicorées.

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ETUDES 6 et 7

Influence of dietary fibers in healthy and obese Beagles :**I. Effects on feces and digestibility of the nutrients**

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Submitted (1997)

Objective—To evaluate the influence of 4 dietary fiber sources added in the diet on feces characteristics and nutrient digestibility in 5 healthy (Experiment 1) and 5 obese (Experiment 2) Beagles.

Animals—5 healthy adult male Beagles, 1.8 to 3 years old, weighing 11.7 to 14.5 kg (Experiment 1) and 5 obese adult male Beagles, 2.8 to 3 years old, weighing 18.0 to 24 kg (Experiment 2).

Procedures— Diets containing 9-11 % total dietary fiber on dry matter basis (guar gum, cellulose, sugar beet fiber and a blend of guar gum and cellulose, respectively called diets B, C, D and E) were compared with a control diet without additional fiber (diet A) in 5 healthy (Experiment 1) and 5 obese (Experiment 2) Beagles. The fiber-enriched diets were evaluated for their ability to modify feces characteristics and apparent digestibility of dry matter, organic matter, protein, ether extract, ash, and total, insoluble and soluble dietary fibers. Each diet was fed for 4 weeks in a 5X5 Latin square design. During the last week of the 4-week period, dogs were kept in metabolism cages for total collection of feces. Each period of the Latin square was followed by an 1-week washout period.

Results—Experiment 1. Compared to diet A, incorporating the 4 fiber sources in the diet was associated with greater excretion of wet feces (all diets; $P<0.05$ or $P<0.001$), higher dry matter content of feces (diet C; $P<0.01$), lower dry matter content (diets B and D; $P<0.001$) and increased daily excretion of dry matter (diets C, D and E; $P<0.001$). Dry matter and organic matter digestibility coefficients were decreased with diets C, D and E ($P<0.01$). Protein digestibility was decreased with diets B ($P<0.01$) and D ($P<0.001$) and ether extract digestibility was decreased by diets B and E ($P<0.05$). Ash digestibility was decreased only with diet D ($P<0.05$). Total dietary fiber digestibility was the highest for diet B and was decreased with diets C, D and E ($P<0.05$, 0.01 or 0.001). Soluble dietary fiber digestibility was the lowest for diet D ($P<0.001$) and insoluble dietary fiber digestibility was increased for diet B ($P<0.01$).

Experiment 2. Inclusion of the 4 fibers was associated with greater wet feces excretion ($P<0.05$, 0.01 or 0.001), lower dry matter content of feces (diets B, D and E; $P<0.01$ or $P<0.001$) and increased excretion of daily feces dry matter (all diets; $P<0.05$ or $P<0.001$). Dry matter and organic matter digestibility coefficients were decreased with all diets ($P<0.01$). Protein digestibility was decreased with diets B, D and E; ($P<0.01$ or $P<0.001$); ether extract digestibility was lower with diets B and E ($P<0.01$ and $P<0.001$). Ash digestibility was decreased only with diet D ($P<0.05$). Total dietary fiber digestibility was the highest for diet B and was significantly decreased with diets C, D and E ($P<0.01$ and $P<0.001$). Soluble dietary fiber digestibility was the lowest for diets D and C and insoluble dietary fiber digestibility coefficients were not significantly different ($P<0.01$).

Conclusion—Chronic consumption of dietary fibers was associated with changes of feces characteristics and nutrient digestibility coefficients.

Clinical Relevance—The different extents of changes in dry matter content of feces and in daily wet feces excretion when guar gum, cellulose or sugar beet fiber are included in the diet could be tested for adequate treatment of constipation.

Although dietary fibers are not considered as essential nutrients, they are beneficial to health and are incorporated at low rates of 1 to 5 % dry matter in most dog foods.^{1,2,3} They are also used at higher concentrations up to 25 % dry matter as an aid in the treatment of chronic diseases such as obesity⁴, diabetes mellitus⁵, or gastro-intestinal diseases.⁶

Cellulose is used as the typical insoluble fiber source. Sugar beet fiber, is sometimes incorporated in dog diets and is characterized by complementary viscous and nonviscous structural carbohydrates.² Guar gum is a gel-forming galactomannane obtained from the cluster bean, *Cyanopsis tetragonoloba*, with potent short and long term effects on blood glucose and lipids in human subjects.⁷ A blend of cellulose and guar gum could imitate the ratio of soluble-to-insoluble fiber of the beet fiber.

The purpose of the two studies reported here was to assess the effects of these dietary fibers on fecal characteristics and nutrient digestibility in healthy and obese Beagles.

Materials and Methods

Dogs—Experiment 1. Five adult castrated male Beagles, 1.8 to 3 years old, weighing 11.7 to 14.5 kg were used. All were healthy on the basis of results of physical examination, CBC^a, serum biochemical analysis (glucose^b, insulin^c, urea^b, creatinine^b, cholesterol^b, and triglycerides^b concentrations and alkaline phosphatase^b and alanine transaminases^b activities).

Experiment 2. Five adult obese castrated male Beagles, 2.8 to 3 years old, weighing 18.0 to 24 kg were used for the second study. When the dogs were aged of one year, their weight ranged between 13.0 and 14.2 kg, which was optimal as determined by a 9-points-body condition score (BCS).^{8,9} At that time, they were offered a highly palatable high-fat balanced and complete home-made food. All dogs gained weight and were considered as obese from at least 1 year, with a BCS over than 6 (Table 1). Health status was assessed by physical examination, CBC^a, serum biochemical analysis, complete urine analysis, radiography of the thorax, TSH stimulation and dexamethasone suppression tests.¹⁰ All dogs received routine vaccination^d and were dewormed^e two months before entry in the study. Dogs were weighed weekly and were housed in outdoor kennels or, during digestibility trials, in a room with natural lighting, in individual metabolism cages. Room temperature was maintained at 18 ± 2 C. Water was offered ad libitum. All dogs belonged to the Animal Nutrition Unit and the protocols of the 2 studies were approved by the university committee for care and use of laboratory animals, and all experiments were carried out according to the Belgian regulations for animal research and experimentation.

Table 1—*Individual body weight changes in five obese Beagles used in Experiment 2 during overnutrition period*

Dog	Age	Optimum	Obese	Weight changes	
		weight	weight	kg	%
	year	kg	kg	kg	%
1	3	13.6	24.0	+ 10.4	+ 76.5
2	3	13.7	20.6	+ 6.9	+ 50.4
4	2.9	14.0	18.0	+ 4	+ 28.6
7	2.9	14.2	19.9	+ 5.7	+ 40.1
10	2.8	13.0	22.6	+ 9.6	+ 73.8

Diet composition—The same diets were offered to the dogs in the 2 studies. The basal diet was made of minced beef meat, gelatinised corn starch^f, maize oil, and a vitamin/mineral mixture^g (Table 2). Control diet (diet A) contained no additional fiber source. Preliminary study of 6 dogs receiving more than 5 % dry matter guar gum in the diet was associated with runny feces. To avoid such inconvenience, the incorporation rate of guar gum^h was limited to 4.3 % dry matter in diet B and the total dietary fiber concentration was 9.1 % on dry matter basis. Celluloseⁱ and beet fiber^j were added respectively in diet C and D to reach a concentration of 11 % total dietary fiber on dry matter basis. In diet E, a blend of guar gum and cellulose was used in a 0.48-to-0.52 ratio in order to obtain a similar soluble/insoluble ratio as in diet D, and therefore to compare the effects of the blend with diet containing beet fiber. All ingredients were mixed with 600 or 400 ml of water, respectively in Experiments 1 and 2 and were given to dogs 5 minutes after preparation.

Experimental design—The design used was a 5X5 Latin square¹¹ in both Experiments 1 and 2. Each experimental diet was fed for 4 weeks. Each period of the Latin square was followed by an 1-week washout period during which dogs were fed diet A to avoid residual metabolic effect of the fiber. The duration of each study was 25 weeks.

Feeding protocol—The amount fed was based on daily maintenance caloric requirements determined by body weight ($132 \text{ kcal/kg}^{0.75}$)¹² in non-obese Beagles in Experiment 1. In Experiment 2, obese Beagles were fed at an energy level of 40 kcal/kg bodyweight/day. They were offered this lower level of energy and they maintained their overweight for more than one year. The dry matter intakes, similar in Experiments 1 and 2, were on average 250 g per day and per dog. Water was offered ad libitum. Dogs were fed once a day at 9 AM, and they voluntarily consumed their meal within 5 minutes.

Digestibility trials—Digestibility measurements were carried out over 7 days during the last week of each period. Dogs were housed in metabolism cages. During the collection phase, total fecal output was collected twice daily and stored at 4 C. At the end of the week, feces were dried to reach a constant weight in a 60 C oven. After complete drying, feces were ground through a 2 millimeters screen in a mill. Feeds and feces were analyzed according to official procedures.¹³ Total dietary fiber was determined in food and feces using a kit^k. This procedure was based on the method published by Association of Official Analytical Chemists.¹⁴ Insoluble fiber was also measured and soluble fiber content was calculated by subtracting insoluble from total dietary fiber.

Statistical evaluations—ANOVA was performed on the fecal and digestibility data according to a 5X5 Latin square design¹¹, using a software package^l and a desktop computer.^m Mean (\pm SD) values were calculated for all data. If ANOVA revealed differences in a single digestibility result attributable to diet consumed, comparisons between differences of mean results of diet groups were performed using a Student t test; a *P* value < 0.05 was considered significant. Data of Experiments 1 and 2 were treated separately, according the same procedure. Since the trials were conducted as Latin square designs, comparisons between the two trials and therefore the two types of dogs, were not statistically valid.

Results

The protein and ether extract concentrations in the fiber-supplemented diets were slightly reduced, compared with diet A (Table 2). Calcium concentration was slightly increased in diet D, containing beet fiber. Total dietary fiber concentration was increased by more than 100 % in diets C, D and E. Diet B contained the largest level of soluble fiber while diet C contained the lowest. Total dietary fiber and the ratio soluble-to-insoluble fiber were similar for diets D and E.

Acceptance of diets was good throughout the two studies. However, if occasionally one dog refused part of its diet, it was systematically withdrawn within 10 minutes, and the weight of leftover recorded. The refused feed was always less than 15 %.

Diet-induced variations in feces—Experiment 1. In healthy dogs, quantity of wet feces excreted (g/day) was significantly increased with diets B, C ($P<0.01$), D ($P<0.001$) and E ($P<0.05$) (Table 3). The dry matter content of feces was not modified with diet E but was decreased with diets B and D ($P<0.001$). By contrast, diet C increased the dry matter content of feces, compared with diet A ($P<0.01$). The daily excretion of fecal dry matter was the lowest for diet A; all fiber sources increased the excretion; differences being significant for diets C ($P<0.001$), D and E ($P<0.01$).

Experiment 2. In obese dogs, daily excretion of wet feces was significantly increased with the 4 fiber-supplemented diets, compared with diet A ($P<0.05$ for diet C, $P<0.01$ for diet E, and $P<0.001$ for diets B and D) (Table 3). The dry matter content of feces was not significantly modified with diet C but was decreased with diets B, D ($P<0.001$) and E ($P<0.01$). Daily fecal dry matter was increased with diet B ($P<0.05$) and with diets C, D and E ($P<0.001$).

Diet-induced variations in digestibility of nutrients—Experiment 1. Apparent dry matter and organic matter digestibility coefficients were affected by the inclusion of cellulose, beet fiber and the blend of fibers in the diets ($P<0.001$ for diets C, D and E). By contrast, apparent protein digestibility was only decreased by diet B ($P<0.01$) and diet D ($P<0.001$). Apparent digestibility of ether extract was the largest for diets A and C, and was slightly decreased for diets B and E ($P<0.05$). Apparent ash digestibility was characterized by large individual variations and was only decreased by diet D ($P<0.05$). Apparent total dietary fiber digestibility was the greatest for diet B ($P<0.01$) and was diminished with diets C, D and E ($P<0.001$; $P<0.01$ and $P<0.05$, respectively). Apparent digestibility of soluble dietary fiber was high for all diets; it was only decreased with addition of sugar beet fiber in diet D

($P < 0.001$). Individual variations in the apparent digestibility of insoluble dietary fibers were large and it was only with diet B that an increase was observed ($P < 0.01$).

Table 3—*Characteristics of feces from 5 healthy (Experiment 1) and 5 obese (Experiment 2) Beagles fed diets containing different dietary fibers*

	Expt 1	Expt 2
Feces characteristics		
Wet weight (g/d)		
Diet A	68 ± 27 ^a	43 ± 11 ^a
Diet B	116 ± 34 ^b	106 ± 40 ^{bc}
Diet C	114 ± 43 ^b	81 ± 26 ^b
Diet D	164 ± 38 ^c	122 ± 42 ^c
Diet E	111 ± 17 ^b	93 ± 35 ^b
Dry matter (%)		
Diet A	27.7 ± 7.4 ^a	38.6 ± 3.8 ^a
Diet B	18.4 ± 5.6 ^b	22.7 ± 5.0 ^c
Diet C	35.3 ± 8.5 ^c	42.5 ± 7.0 ^a
Diet D	18.6 ± 2.4 ^b	25.3 ± 5.3 ^c
Diet E	25.5 ± 6.4 ^a	30.8 ± 5.9 ^b
Dry matter (g/d)		
Diet A	17.2 ± 2.5 ^a	16.6 ± 4.3 ^a
Diet B	20.1 ± 4.7 ^a	22.8 ± 6.7 ^b
Diet C	37.3 ± 6.0 ^c	33.6 ± 7.7 ^d
Diet D	30.1 ± 5.6 ^{bc}	29.3 ± 4.9 ^{cd}
Diet E	27.6 ± 4.2 ^b	27.0 ± 5.5 ^{bc}

Values with different superscripts differ within one column ($P < 0.05$). Values are expressed as mean ± SD.

Table 4—*Apparent digestibility coefficients from 5 healthy (Experiment 1) and 5 obese Beagles offered diets containing different fiber sources*

	Expt 1	Expt 2
Digestibility (%)		

Dry matter		
Diet A	93.5 ± 1.0 ^a	93.5 ± 1.5 ^a
Diet B	92.7 ± 1.5 ^a	91.2 ± 2.0 ^b
Diet C	86.8 ± 1.4 ^c	87.2 ± 1.8 ^d
Diet D	88.0 ± 1.2 ^{bc}	89.3 ± 0.8 ^c
Diet E	89.5 ± 1.7 ^b	89.1 ± 0.7 ^c
Organic matter		
Diet A	95.5 ± 1.0 ^a	95.5 ± 1.1 ^a
Diet B	94.4 ± 1.1 ^a	93.1 ± 1.9 ^b
Diet C	88.5 ± 1.4 ^c	89.1 ± 1.7 ^d
Diet D	90.0 ± 1.0 ^b	91.3 ± 0.7 ^c
Diet E	91.2 ± 1.3 ^b	90.9 ± 1.1 ^c
Protein		
Diet A	94.4 ± 2.8 ^a	93.2 ± 2.1 ^a
Diet B	91.4 ± 4.8 ^b	87.2 ± 3.3 ^c
Diet C	94.4 ± 2.2 ^a	92.1 ± 1.8 ^a
Diet D	88.9 ± 5.1 ^c	89.5 ± 2.4 ^b
Diet E	92.6 ± 4.0 ^{ab}	89.1 ± 1.6 ^{bc}
Ether Extract		
Diet A	96.6 ± 1.6 ^a	96.7 ± 1.4 ^a
Diet B	93.1 ± 3.4 ^{bc}	91.0 ± 3.3 ^b
Diet C	96.0 ± 0.6 ^{ab}	95.2 ± 1.3 ^a
Diet D	93.6 ± 2.1 ^{abc}	96.3 ± 0.5 ^a
Diet E	92.6 ± 2.5 ^c	92.8 ± 2.1 ^b

Table 4. (p2/2)

	Expt 1	Expt 2
Digestibility (%)		
Ash		
Diet A	40.7 ± 12.2 ^a	47.7 ± 12.4 ^a
Diet B	46.6 ± 13.2 ^a	48.1 ± 9.2 ^a
Diet C	38.3 ± 5.8 ^{ab}	41.0 ± 7.2 ^{ab}
Diet D	27.7 ± 3.4 ^b	39.4 ± 5.2 ^b
Diet E	41.5 ± 12.9 ^a	43.9 ± 6.3 ^{ab}
Total dietary fiber		
Diet A	64.6 ± 4.5 ^a	69.4 ± 10.8 ^a
Diet B	78.0 ± 7.6 ^d	77.0 ± 6.7 ^a
Diet C	24.7 ± 7.5 ^c	29.1 ± 13.4 ^d
Diet D	55.1 ± 2.5 ^b	56.0 ± 4.3 ^b
Diet E	53.0 ± 9.4 ^b	52.3 ± 3.5 ^{bc}
Soluble dietary fiber		
Diet A	97.0 ± 4.2 ^a	96.7 ± 2.5 ^a
Diet B	97.8 ± 2.0 ^a	95.5 ± 1.8 ^{ab}
Diet C	93.8 ± 5.4 ^a	89.3 ± 9.1 ^{bc}
Diet D	85.8 ± 6.7 ^b	87.0 ± 4.2 ^c
Diet E	92.1 ± 2.9 ^a	90.6 ± 5.9 ^{abc}
Insoluble dietary fiber		
Diet A	19.5 ± 11.6 ^{ab}	21.5 ± 15.5 ^{ab}
Diet B	44.4 ± 20.8 ^c	38.0 ± 23.6 ^a
Diet C	5.4 ± 4.5 ^a	6.7 ± 8.2 ^b
Diet D	31.3 ± 7.5 ^{bc}	29.6 ± 10.8 ^a
Diet E	29.0 ± 16 ^{bc}	21.0 ± 9.5 ^{ab}

Values with different superscripts differ within one column ($P < 0.05$). Values are expressed as mean ± SD.

Experiment 2. Apparent dry matter and organic matter digestibility coefficients were similarly decreased by the inclusion of fibers as compared with diet A ($P < 0.001$ for diets C, D, E and $P < 0.01$ for diet B). Apparent protein digestibility was decreased with diets B, E ($P < 0.001$) and D ($P < 0.01$). Apparent ether extract digestibility was decreased with diets B ($P < 0.001$) and E ($P < 0.01$) but ash digestibility was decreased only with diet D ($P < 0.05$). Apparent digestibility of total dietary fiber, characterized by large individual changes, was high at 69.4 % with diet A and was non significantly increased with diet B. By contrast, diets C, D and E induced lower total dietary fiber digestibility coefficients, compared with diet A ($P < 0.001$ or $P < 0.01$). Apparent digestibility of soluble dietary fiber was decreased with diets C and D ($P < 0.01$) while digestibility of insoluble fiber was characterized by large changes and no significant differences, compared with diet A.

Discussion

The total dietary fiber concentration of 5.1 % dry matter in the control diet was due to the animal protein component. Meat contains protein-polysaccharides of the connective tissues; these fibrous materials escape from digestion by the enzymes of the digestive tract¹⁵ but are measured by the assays used to analyse total dietary fiber. Adding dietary fiber had a dilution effect on energy density but such an effect was of no importance since all dogs received their amount of feed based on individual energy requirements.

The amount of wet feces excreted daily increased with the addition of fiber to the diet. Such an effect is called "bulking effect" of fiber, a property used for treatment of constipation. In man, the fecal bulking effects appear to be most strongly associated with fiber sources which are insoluble, poorly fermentable and with good water-binding capacity.¹⁶ In the two studies reported here, sugar beet fiber induced the largest excretion of feces and was followed by guar gum which has the highest soluble fiber content. Cellulose and the blend induced similar effects as guar gum on wet feces weight. In dogs, it can be concluded that the bulking effect is a property of both the highly soluble or insoluble fiber contents as reported by others with various purified fibers such as cellulose,¹⁷ pectins,¹⁸ maize fiber¹⁹ or foodstuffs high in fiber such as beet pulp^{2,3} or citrus pulp.²⁰

The normal range of fecal dry matter content in dogs is between 28 and 42 %.²¹ Fermentable fibers decrease the dry matter content of feces^{3,22}, and a similar finding is reported here with guar gum and beet fiber. By contrast, adding cellulose increased the dry matter content of feces, in a significant manner in Experiment 1. This has been precedently reported by

others.^{17,18} More interesting is the comparison of the total dry matter excretion. Except for guar gum in Experiment 1, all fiber sources increased the amount of dry matter excreted compared to the control diet. This effect is attributable partly to the slight increase of ingested dry matter as dietary fiber and also to a reduction in nutrient digestibility, mainly the fiber fraction. There are also other mechanisms involved such as greater amounts of microbial cells and of short chain fatty acids produced²⁰ in the hindgut. Furthermore, it should be noted that the 150 % increase in wet feces excretion with beet fiber is considered more as a disadvantage by most dogs owners.

Comparison between diets D and E shows that beet fiber and the blend did not induce the same effects on daily wet feces weight and dry matter content, beet fiber being characterized by a higher water-holding capacity than the blend. For the characteristics of the feces, the specific effects of the fibers were similar both with the healthy and obese dogs.

The high apparent digestibility coefficients obtained in the 2 studies were associated with the high quality of the ingredients : fresh beef meat and corn starch. Guar gum decreased apparent protein digestibility in the 2 studies. This effect was due to a high protein content of feces which could have come from increased microbial protein. Although no microbial measurements were made in the present studies, the high fecal content would indicate a microbial proliferation with fermentable fibers.²⁰ This could also explain the decreased protein digestibility induced by sugar beet fiber. Diet B containing guar gum was characterized by the highest apparent digestibility of total dietary fiber. Such effects are explained by the high content of soluble fiber in the guar gum supplemented diet and the high digestibility coefficient of the soluble fiber (Table 4).

The inclusion of cellulose reduced the apparent dry matter digestibility by 6.7 and 6.3 % units, respectively in Experiments 1 and 2. Similar effects were also induced by cellulose on apparent organic matter digestibility. By contrast, protein, ether extract and ash digestibility coefficients were not modified both in Experiments 1 and 2, when cellulose was added in the diets. Thus, the decrease in dry matter and organic matter digestibility coefficients observed with inclusion of cellulose can be explained by the low digestibility coefficient of this fiber, present in large quantities in the feces but without major effects on digestibility of the main nutrients.

Sugar beet fiber and the blend induced similar effects on dry matter, organic matter, total dietary fiber and insoluble fiber digestibility coefficients, both in Experiment 1 and 2. By contrast, in Experiment 1, blend did not modify protein digestibility as opposed to sugar beet

fiber. Ether extract apparent digestibility coefficients were reduced in Experiment 2 with the blend, and not with beet fiber. Apparent ash digestibility was the lowest with beet fiber in the 2 studies. Soluble dietary fiber digestibility was lower with beet fiber in Experiment 1 than with the blend. On the whole, however, it could be concluded that, except for ash, the apparent digestibility coefficients are quite close when beet fiber and the blend are supplemented in similar amounts. The determination of total dietary fiber allows a better understanding of the mode of action of dietary fiber in the digestive tract of the dog. The two experiments reported here indicated that total dietary fiber was relatively well digested in the control diet when total dietary fiber concentrations were close to 5 %; the apparent digestibility coefficients of 64.6 and 69.4 % obtained in the two studies being comparable to data previously reported.² In the two experiments, it was when guar gum was added that total dietary fiber digestibility was the highest. It was due to the soluble fraction which, in guar gum, was the largest fraction in total dietary fiber, the soluble fraction being also the more digestible. Although not significant in both experiments, the supplementation with guar gum induced also the largest digestibility for the insoluble fraction. This could be rather surprising, but the more insoluble fibers are present, the lower are their digestibility coefficients. The very low total dietary fiber digestibility coefficients observed when cellulose was added are similar to figures previously reported by others^{17, 18} when fibers were measured as neutral detergent fiber or as crude fiber. It should also be noted that the soluble fiber fraction was characterized by high digestibility coefficients in the two experiments, the coefficients being not different from the control values, except when beet fiber and cellulose were used in Experiment 2.

Although it was not statistically possible to compare the two studies, the effects of fiber supplementation appeared similar in healthy and obese dogs. There are thus actually no convincing evidences to indicate that nutrient digestibility is reduced in obese dogs. One can therefore use digestibility data obtained in healthy dogs offered fiber supplemented diets to assess the effects on obese subjects.

^a Cell-Dyn 3500, Abbott, Abbott Park, IL 60064, USA

^b Technicon RA 1000, Technicon Autoanalyzer, Technicon Instruments, Tarrytown, NJ.

^c Insulin RIA-100, manufacturer's literature, Medgenix Diagnostics, Biosource Europe, Fleurus, Belgium.

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- ^d Vanguard[®] da2pi-CPV-Lepto, Smithkline Beecham A.H., Louvain-La-Neuve, Belgium.
- ^e Drontal[®], BAYER s.a.-n.v., Bruxelles, Belgium.
- ^f Merigel A, Amylum N.V., Aalst, Belgium.
- ^g Minerals and Vitamins for dogs, Premix, ALFRA, Horion Hozémont, Belgium.
- ^h Viscogum HV 3000A, Mérot Rousselot Satia, France.
- ⁱ Arbocell BE 600/30, Rettenmeier and Söhne, Germany.
- ^j Betafibre, British Sugar, United Kingdom.
- ^k TDF-100, Sigma Chemical CO, St Louis, Mo.
- ^l Excel 5.0[®], Microsoft Corporation, IL.
- ^m IBM, model 6322-002, IBM United Kingdom Ltd, Greenock, Scotland, United Kingdom.
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Influence of dietary fibers in healthy and obese Beagles :
II. Effects on plasma metabolites and insulin concentrations

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Submitted (1997)

Objective—To evaluate the influence of 4 dietary fiber sources added in the diet on plasma metabolites and insulin concentrations in 5 healthy (Experiment 1) and 5 obese (Experiment 2) Beagles.

Animals—5 healthy adult male Beagles, 1.8 to 3 years old, weighing 11.7 to 14.5 kg (Experiment 1) and 5 obese adult male Beagles, 2.8 to 3 years old, weighing 18.0 to 24 kg (Experiment 2).

Procedures—Diets containing 9-11 % total dietary fiber on dry matter basis (guar gum, cellulose, sugar beet fiber and a blend of guar gum and cellulose, respectively called diets B, C, D and E) were compared with a control diet without additional fiber (diet A) in 5 healthy (Experiment 1) and 5 obese (Experiment 2) Beagles. The fiber-enriched diets were evaluated for their ability to modify plasma glucose, insulin, α -aminonitrogen, urea, triglycerides and cholesterol concentrations. Each diet was fed for 4 weeks in a 5X5 Latin square design. At the end of the 4 weeks period, plasma samples were collected before feeding and after feeding during 360 minutes. Each period of the Latin square was followed by an 1-week washout period.

Results—Experiment 1. Diets containing cellulose and beet fiber induced no effects on pre- or postprandial plasma concentrations. Diet containing guar gum was associated with lower

pre- and postprandial plasma cholesterol concentrations ($P<0.001$) and a trend to lower plasma glucose concentration within 180 minutes after feeding ($P<0.10$). Inclusion of the blend in the diet induced lower pre- and postprandial cholesterol concentrations ($P<0.05$ and $P<0.01$) and diminished plasma glucose concentration during 180 minutes after feeding ($P<0.05$).

Experiment 2. Adding cellulose in the diet induced no metabolic effects. Incorporating fiber sources in the diet was associated with higher postprandial plasma glucose concentration (diet D; $P<0.001$), lower postprandial insulin concentrations (diet B, $P<0.01$, diet E, $P<0.001$), lower pre- and postprandial cholesterol concentrations (diet B, $P<0.05$ and $P<0.001$; diet E, $P<0.05$ and $P<0.001$).

Conclusion—Chronic consumption of guar gum or a blend of cellulose and guar gum was associated with reductions in pre- and postprandial plasma cholesterol concentrations in 5 healthy dogs and reductions in pre- and postprandial cholesterol concentrations and reductions in insulin and urea concentrations measured postprandially in 5 obese dogs.

Clinical Relevance—Guar gum or a mixture of guar gum and cellulose should be tested as an aid for dietary therapy of chronic diseases such as hyperlipidemia or diabetes mellitus in dogs.

The interest of adding dietary fibers in commercial^{1,2,3} or specific-purpose⁴ dog food is well demonstrated in dog. Although their beneficial effects on the digestive tract are more and more exploited⁵, their applications in the treatment of disorders in lipid and glycosidic^{6,7} metabolisms are less frequent than in human patients.⁸ Furthermore, there is no agreement to use either soluble or insoluble dietary fibers.

The aim of the 2 experiments reported here was to assess the effects of 4 dietary fibers on the major plasma metabolites in the normal healthy dog and in the obese dog. The 4 fibers were guar gum, cellulose, sugar beet fiber and a blend of guar gum and cellulose in a ratio to obtain the soluble-to-insoluble fiber concentrations found in sugar beet fiber. These fibers were tested because they are largely used by feed manufacturers although their metabolic effects are not well documented. Obese dogs were used since it is known that metabolic disorders such as glucose intolerance⁹ or hyperlipidemia¹⁰ could be observed in these animals.

Materials and Methods

Dogs—Five adult healthy castrated male Beagles, 1.8 to 3 years old, weighing 11.7 to 14.5 kg were used in Experiment 1. Five adult obese castrated male Beagles, 2.8 to 3 years old, weighing 18.0 to 24 kg were used for Experiment 2. The optimum weight of the obese Beagles, determined by a 9 points body condition score¹¹, ranged between 13.0 and 14.2 kg. At the beginning of Experiment 2, all dogs were considered as obese after voluntary consumption of a highly palatable home-made dog food and the subsequent weight gain. Individual characteristics of the dogs were precedently reported (Diez et al, part I)¹². The dogs used in the 2 studies reported here were healthy on the basis of results of physical examination, CBC^a and serum biochemical analysis.¹²

In the obese dogs group, complete urine analysis, radiography of the thorax, TSH stimulation and dexamethasone suppression tests¹³ were also performed. All dogs entered the 2 studies two months after receiving routine vaccination^b and being dewormed^c. Dogs were weighed weekly and were housed in outdoor kennels or in a room with natural lighting, in individual metabolism cages during digestibility trials and plasma collection. Room temperature was maintained at 18 ± 2 C and water was offered ad libitum.

Diet composition—Similar diets were offered to the dogs in the 2 experiments. Briefly summarized, a basal diet made of minced beef meat, corn starch, maize oil and a vitamin/mineral mixture^d was used as a control diet (diet A). Guar gum^e, cellulose^f, beet fiber^g and a blend of guar gum and cellulose in proportion allowing a soluble-to-insoluble fiber ratio similar to that of beet fiber were added to the basal diet to reach a concentration of 11 % total dietary fiber on a dry matter basis; diets were respectively called diets B, C, D and E. All ingredients were mixed with 600 or 400 ml of water, respectively in experiment 1 and 2 and were given to dogs 5 minutes after preparation.

Experimental design—Each experiment was designed as a 5X5 Latin square¹⁴ with periods of 4 weeks and an 1-week washout period.

Feeding protocol—The amount fed was calculated on daily maintenance requirements of 132 kcal/kg^{0.75} in non-obese Beagles¹⁵ and on 40 kcal/ kg^{0.75} for the obese dogs.¹² Dogs were fed once a day at 9 AM, and they voluntarily consumed their meal within 5 minutes.

Plasma samples—Preprandial and postprandial profiles were determined at the end of each 4-week period of the Latin square. An indwelling sterile catheter was inserted in a cephalic vein. Catheters were filled with a heparinized (120 U/ml) saline solution to prevent blood clotting between sampling periods. Dogs were handled gently and did not appear excited during sampling. Blood was taken before feeding; then the dogs were fed their assigned diets, as a single meal. Serial postprandial blood samples (5 ml) were taken at 20, 40, 60, 90, 120, 180, 240, 300 and 360 minutes after feeding. Plasma samples obtained from blood were stored at -18 C. All samples were analyzed on the same day for plasma glucose^h, insulinⁱ, urea^h, α -amino-nitrogen^h, triglycerides^h and cholesterol^h.

Statistical evaluations—Data were analyzed using a software package^j and a desktop computer^k. Plasma metabolites data obtained in nonfed dogs were analyzed, according to a 5X5 Latin square design¹⁴. The area under the curve was calculated for the evaluation of postprandial plasma metabolites and the data were analyzed according to a 5X5 Latin square design. If ANOVA revealed differences among treatment, comparisons between mean results of diet groups were performed using a Student t test. Means (\pm SEM) were reported for preprandial plasma metabolite data. For presentation in Table 1, the data related to the area under the curve for postprandial metabolites were divided by 360 which was the duration of the sampling period.

Results

Diet induced variations of plasma metabolites in samples obtained in fasted animals—

Experiments 1 and 2. All results were within reference ranges¹⁶ (Table 1). There were no effects on preprandial glucose, insulin, α -aminonitrogen, urea and triglycerides concentrations. By contrast, preprandial cholesterol concentrations were significantly reduced by diet B both in Experiments 1 ($P<0.001$) and 2 ($P<0.05$) and by diet E both in Experiments 1 ($P<0.01$) and 2 ($P<0.05$).

Diet induced variations of plasma metabolites in samples obtained up to 6 hours after the meal —All results were within reference ranges.¹⁶

Experiment 1. There were no effects on postprandial insulin, α -aminonitrogen, urea and triglycerides concentrations (Table 1). Feeding diet E to dogs led to a significant ($P<0.05$) decrease in glucose concentration but only during the first 180 minutes after the meal. Feeding diet B tended ($P<0.10$) to decrease glucose concentration during the same time interval. Feeding diets B and E to dogs led to significant decreases ($P<0.001$ and $P<0.01$, respectively) in cholesterol concentration measured during 360 min after the meal, as compared with diet A (Fig 1).

Experiment 2. There were no effects on postprandial α -aminonitrogen concentrations. Feeding diet D induced an increase ($P<0.001$) in postprandial glucose concentrations. Plasma insulin and urea concentrations were decreased by diets B ($P<0.01$) and E ($P<0.001$ and $P<0.01$). Feeding diet E was associated with a trend ($P<0.10$) to decrease plasma triglycerides concentrations. Postprandial plasma cholesterol concentration was reduced by diets B and E ($P<0.001$) (Fig 2).

Table 1—*Plasma biochemical variables measured before and after the meal during a 360-min period in 5 healthy (Experiment 1) and 5 obese dogs (Experiment 2) offered diets containing different fiber sources*

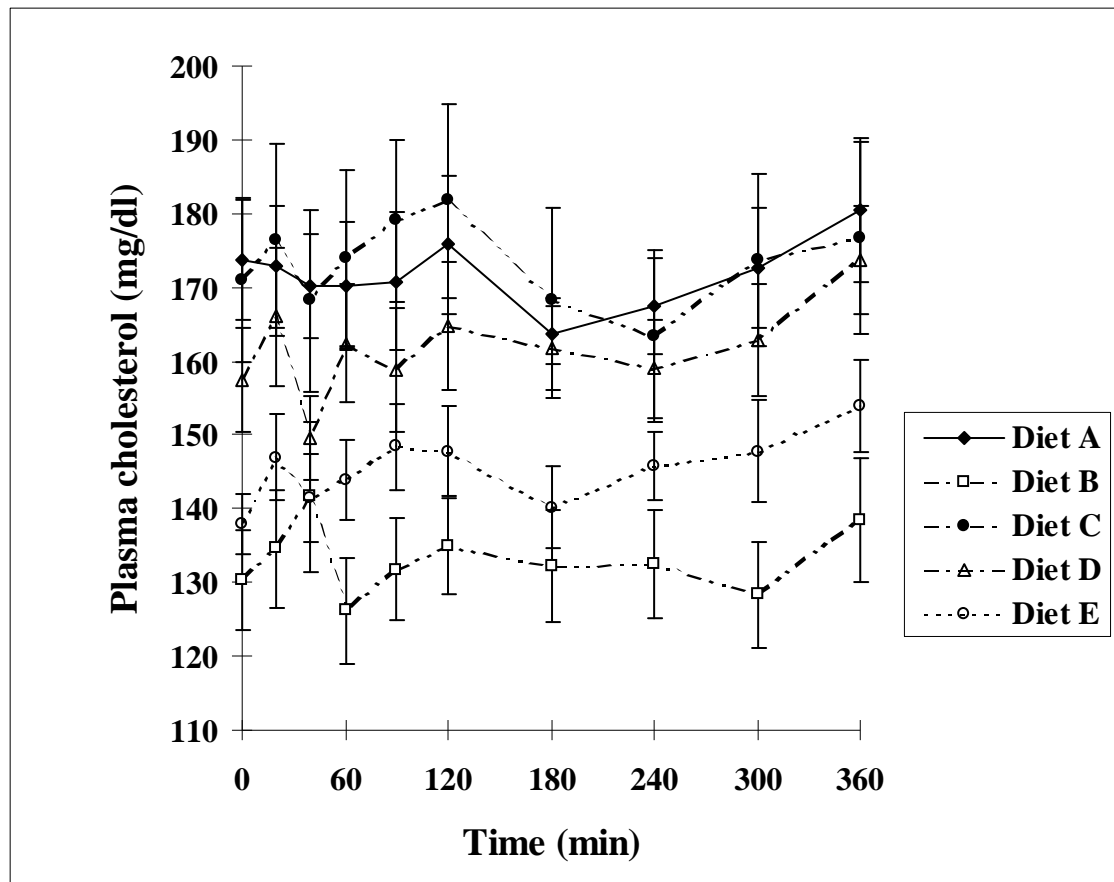
Variable	Experiment 1		Experiment 2	
	Preprandial values	Area under curves	Preprandial values	Area under curves
Glucose, mg/dl				
Diet A	88.1 ± 3.6 ^a	89.3 ± 3.8 ^a	94.4 ± 3.1 ^a	90.6 ± 4.4 ^a
Diet B	86.7 ± 6.0 ^a	86.8 ± 2.8 ^{ab†}	99.6 ± 5.7 ^a	89.5 ± 5.0 ^a
Diet C	84.7 ± 3.0 ^a	91.5 ± 2.9 ^a	97.8 ± 3.9 ^a	93.5 ± 4.2 ^a
Diet D	84.0 ± 7.0 ^a	89.9 ± 5.4 ^a	94.6 ± 2.1 ^a	101.0 ± 5.8 ^b
Diet E	86.7 ± 4.3 ^a	85.6 ± 3.3 ^{b‡}	97.4 ± 6.3 ^a	93.7 ± 5.6 ^a
Insulin, mU/L				
Diet A	5.4 ± 2.3 ^a	35.6 ± 8.6 ^a	15.2 ± 5.1 ^a	73.3 ± 8.2 ^a
Diet B	11.3 ± 2.3 ^a	28.1 ± 5.6 ^a	12.2 ± 3.4 ^a	45.8 ± 15.3 ^b
Diet C	11.8 ± 5.5 ^a	41.1 ± 8.3 ^a	15.5 ± 2.5 ^a	62.3 ± 6.3 ^a
Diet D	10.0 ± 3.5 ^a	33.4 ± 6.9 ^a	14.6 ± 1.7 ^a	70.6 ± 10.7 ^a
Diet E	13.1 ± 8.6 ^a	32.2 ± 1.7 ^a	14.3 ± 2.4 ^a	40.7 ± 6.3 ^b
α-aminonitrogen, mg/dl				
Diet A	6.0 ± 0.2 ^a	8.8 ± 0.7 ^a	5.9 ± 0.2 ^a	9.0 ± 0.1 ^a
Diet B	5.9 ± 0.3 ^a	8.3 ± 1.0 ^a	5.6 ± 0.2 ^a	8.8 ± 0.3 ^a
Diet C	5.8 ± 0.2 ^a	9.0 ± 0.2 ^a	6.1 ± 0.2 ^a	8.9 ± 0.5 ^a
Diet D	5.6 ± 0.2 ^a	8.8 ± 0.5 ^a	5.8 ± 0.3 ^a	9.1 ± 0.6 ^a
Diet E	5.9 ± 0.4 ^a	8.7 ± 0.6 ^a	5.8 ± 0.3 ^a	8.4 ± 0.5 ^a
Urea, mg/dl				
Diet A	12.3 ± 0.8 ^a	21.1 ± 2.3 ^a	12.2 ± 0.8 ^a	22.4 ± 1.7 ^a
Diet B	12.1 ± 0.6 ^a	21.5 ± 2.2 ^a	11.5 ± 1.1 ^a	19.0 ± 1.2 ^c
Diet C	12.7 ± 1.0 ^a	23.6 ± 2.1 ^a	10.6 ± 1.1 ^a	20.9 ± 1.8 ^{ab}
Diet D	12.7 ± 0.6 ^a	21.8 ± 0.5 ^a	13.4 ± 2.2 ^a	20.8 ± 1.7 ^{ac}
Diet E	12.8 ± 1.0 ^a	22.9 ± 1.3 ^a	13.2 ± 2.4 ^a	19.2 ± 0.8 ^{bc}

Table 1. (p2/2)

Variable	Experiment 1		Experiment 2	
	Preprandial values	Area under curves	Preprandial values	Area under curves
Triglycerides, mg/dl				
Diet A	50.0 ± 5.9 ^a	59.9 ± 2.2 ^a	63.6 ± 9.3 ^a	76.5 ± 9.5 ^a
Diet B	39.2 ± 5.6 ^a	51.6 ± 7.1 ^a	54.2 ± 4.0 ^a	67.7 ± 7.2 ^a
Diet C	51.4 ± 6.3 ^a	61.9 ± 7.7 ^a	58.8 ± 3.5 ^a	76.3 ± 8.0 ^a
Diet D	49.4 ± 9.6 ^a	60.3 ± 10.5 ^a	58.6 ± 5.7 ^a	78.3 ± 4.9 ^a
Diet E	51.2 ± 1.7 ^a	60.4 ± 3.5 ^a	50.4 ± 5.8 ^a	63.8 ± 10.0 [‡]
Cholesterol, mg/dl				
Diet A	173.6 ± 16.3 ^a	170.9 ± 14.4 ^a	198.8 ± 14.5 ^a	190.5 ± 11.4 ^{ab}
Diet B	130.4 ± 13.7 ^c	132.7 ± 14.6 ^c	154.6 ± 8.0 ^b	147.9 ± 6.6 ^d
Diet C	171.0 ± 22.3 ^a	172.6 ± 24.1 ^a	207.0 ± 8.1 ^a	187.5 ± 8.2 ^b
Diet D	157.4 ± 14.0 ^{ab}	162.1 ± 14.3 ^{ab}	185.0 ± 15.1 ^{ab}	202.1 ± 10.6 ^a
Diet E	138.0 ± 8.2 ^{bc}	145.8 ± 11.2 ^{bc}	164.6 ± 11.8 ^b	161.2 ± 12.5 ^c

Values with different superscripts differ ($P < 0.05$) within one column. Values are expressed as mean ± SEM. † Significant difference at $P < 0.10$. ‡ Differences were significant on a 180-min period.

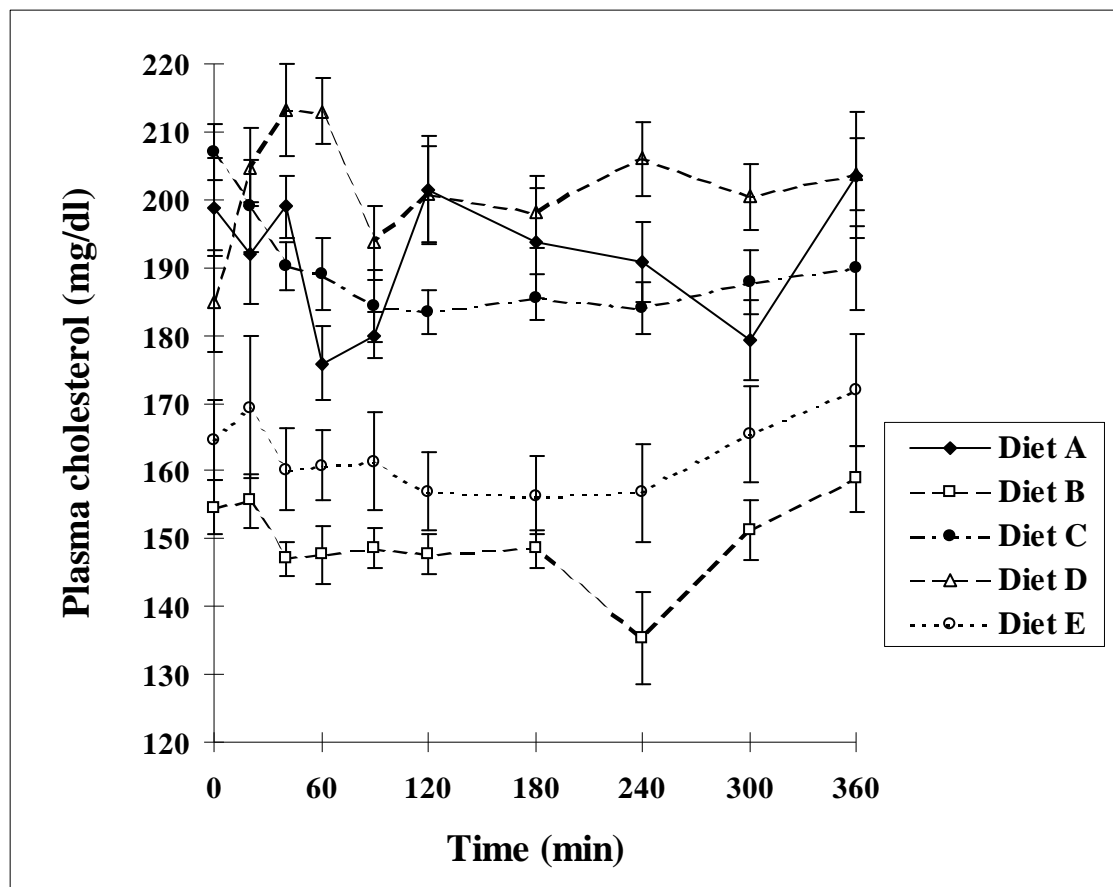
Figure 1—Postprandial plasma cholesterol concentration (mean \pm SEM) in 5 healthy dogs offered diets containing different fiber sources



Discussion

All results were within normal ranges in both healthy and obese dogs.¹⁶ Although no statistical comparisons were made between the 2 studies¹², it appeared that plasma glucose, insulin and lipids concentrations were slightly higher in obese dogs. Diabetes-obesity interactions are well documented in the dog. Even though hyperglycemia may not exist, either glucose intolerance or hyperinsulinemia, or both are present in 61 % of obese dogs⁹. The greater the degree of obesity and the longer its duration, the more severe are the glucose intolerance and hyperinsulinemia. In Experiment 2, the dogs used were quite young, being 2 years of age when they became obese, so hyperinsulinemia or glucose intolerance could not be diagnosed in any one of them. Their responses to supplemental fiber in the diet were however different, compared with healthy dogs.

Figure 2—Postprandial plasma cholesterol concentration (mean \pm SEM) in 5 obese dogs offered diets containing different fiber sources



Fasting plasma glucose concentration is normally maintained within a narrow range in dogs regardless of the type of diet offered.^{13, 17} Adding dietary fiber did not affect fasting glucose and insulin concentrations in these studies nor in other published experiments in which cellulose, pectins or guar gum were incorporated at rates of 3.5 % dry matter¹⁸ or with a blend of guar gum and pea fiber at 15 % dry matter.⁷ In contrast, some authors have shown postprandial glucose concentrations to be modified by inclusion of soluble fibers in the diet¹⁹, but others have found soluble fiber to have no effect on postprandial glucose.²⁰ In healthy dogs, only guar gum and the blend containing guar gum decreased postprandial plasma glucose concentrations but only during the first three hours after the meal. On the whole of the 360-min observation period, none of the fibers induced any significant effects on blood glucose. In obese dogs, postprandial plasma glucose concentration increased with diet containing beet fiber. This was not surprising since this supplement contains small quantities of saccharose and obese dogs may be more sensitive than healthy dogs to saccharose in the diet.⁹ By contrast, decreases in postprandial plasma insulin concentration occurred over the

360-min sampling period in obese dogs offered guar gum and the blend of guar gum and cellulose. One of the properties of this blend of fiber is to minimize postprandial variations of insulin. Postprandial decreases in plasma insulin were also reported with diets enriched with a mixture of pea fiber and guar gum and diets with a high content of crude fiber of unknown sources.²⁰ Decreased glucose and/or insulin concentrations with guar gum have been shown in healthy human beings^{21,22} and diabetic subjects.²³ The effects of guar gum on insulin metabolism could also be exploited in obese or diabetic dogs to improve glucose tolerance.

The inclusion of guar gum and the blend of guar gum and cellulose in the diet also reduced postprandial concentrations of plasma urea in obese dogs without changing plasma α -aminonitrogen. Plasma α -aminonitrogen considered an indicator of the adequacy of dietary protein is closely related to individual plasma amino acids profiles.²⁴ The reduction of plasma urea could not be associated with a lower protein intake but could reflect either a delay in absorption of amino-acids, or their catabolism in the liver. Decreased postprandial concentrations of plasma urea were previously described in dogs receiving guar gum in the diet.^{18,25} Because the reduction of plasma urea concentration with guar gum appears to be consistent, guar gum could also be suggested as an aid in the treatment of chronic renal diseases.

In this study, guar gum and the blend of fibers induced lower pre- and postprandial cholesterol plasma concentrations both in healthy and obese dogs. Previously, no postprandial reductions in serum cholesterol and triglycerides were observed in dog after a single dose of guar gum or wheat bran.¹⁹ The authors postulated that guar gum may still reduce blood lipids in the dog after long term administration. In man, the cholesterol lowering effect of guar gum is well established in healthy and obese subjects²⁶⁻²⁸, and in hyperlipidemic patients.^{23, 29} In contrast, the effect of guar gum on plasma triglycerides of people is much debated.^{27,30,31} In people, hypolipidemic effects are a property of soluble fibers, the most efficient being guar gum. In dogs, since guar gum induces metabolic effects on carbohydrate and lipid metabolism after four weeks administration, this could be considered as an aid for dietetic treatments of chronic diseases such as hyperlipidemia or diabetes mellitus. This is of further interest because fasting hyperlipidemia occurs in 14.3% of the dog population³² and diabetes mellitus is frequently associated with disorders of lipid metabolism. Furthermore, the blend of guar gum and cellulose is suggested since it does not induce diarrhea and since incorporation of 3.4 % guar gum is sufficient to induce the beneficial effects. In our study, beet fiber and cellulose had no effect on plasma metabolites. The lack of effect of cellulose on plasma metabolites in

human beings and rats is well documented, cellulose being used as a negative control in most studies concerning metabolic effects of dietary fibers.³³ In contrast, beet fiber is known to improve glucose tolerance³⁴ and was effective in reducing blood cholesterol in healthy³⁰ or hyperlipidemic³⁵ human subjects. The effectiveness of beet fiber to lower plasma cholesterol concentration in man was related to the fat intake of the subjects.²⁶ In the present study, fat intake was quite low due to the use of a low-fat meat and a small amount of vegetable oil.

One of the purposes of the two studies reported here was a comparison between two fibers supplements with a similar soluble-to-insoluble fiber ratio either as a single component or as a blend of two different supplements. Because the metabolic effects were not similar, it seems more appropriate to characterize and debate on the metabolic effects of a supplement as a whole rather than to associate the effects on the degree of solubility of the fibers.

Finally, we can conclude that all the metabolic effects observed in healthy dogs were also observed in obese dogs. Nevertheless, in obese dogs, additional effects appeared so that transposition of data obtained in healthy dogs to obese animals leads to an incomplete description of the effects of dietary fibers.

^a Cell-Dyn 3500, Abbott, Abbott Park, IL 60064.

^b Vanguard[®] da2pi-CPV-Lepto, Smithkline Beecham A.H., Louvain-La-Neuve, Belgium.

^c Drontal[®], BAYER s.a.-n.v., Bruxelles, Belgium.

^d Minerals and Vitamins for dogs, Premix, ALFRA, Horion Hozémont, Belgium.

^e Viscogum HV 3000A, Mérot Rousselot Satia, France.

^f Arbocell BE 600/30, Rettenmeier and Söhne, Germany.

^g Betafibre, British Sugar, United Kingdom.

^h Technicon RA 1000, Technicon Autoanalyzer, Technicon Instruments, Tarrytown, NJ.

ⁱ Insulin RIA-100, manufacturer's literature, Medgenix Diagnostics, Biosource Europe, Fleurus, Belgium.

^j Excel 5.0[®], Microsoft corporation, IL.

^k IBM, model 6322-002, IBM United Kingdom Ltd, Greenock, Scotland, United Kingdom.

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