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Dietetic combination of mannan-oligosaccharides and fructooligosaccharides modifies nitrogen metabolism in dogs

A associação dietética de mananoligossacarídeos e frutoligossacarídeos modifica o metabolismo nitrogenado em cães

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Abstract

The goals of this study were to evaluate the effects of a mannan-oligosaccharide (MOS) and a frutoligosaccharide (FOS) mixture in a 1:1 ratio added to a commercial diet for dogs before the extrusion process on the coefficient of total tract apparent digestibility (CTTAD) of the diets and on the metabolizable energy (ME), nitrogen metabolism (postprandial blood urea curve, organic nitrogen balance, fecal concentration of ammonia and excretion of urinary urea) and fecal fermentation parameters (fecal pH and concentrations of short chain fatty acids). Eighteen adult Beagle dogs were used and randomly distributed into two treatments, being a control diet plus one diet in which the mixture of prebiotics was added before the extrusion of the ration. There was no difference in digestibility, metabolizable energy, fecal ammonia content and short-chain fatty acids (acetic, propionic and butyric) between the control diet and the one with the addition of prebiotics. However, the dogs supplemented with prebiotics before extrusion, had a lower fecal pH ($p < 0,05$) compared to those in the control diet and a reduction in the post prandial blood urea concentration ($p < 0,01$) was also observed in the animals receiving prebiotics, observed by the areas below the curve of urea and its increment. The possible mechanism involved in the effect of prebiotics on the reduction in the concentrations of blood urea is the reduction in the formation and intestinal absorption of ammonia, favored by the reduction of intestinal pH. These findings can be considered in the use of prebiotics in clinical nutrition.
Key words: Ammonia. Blood urea. Extrusion. Intestinal health.

Resumo

Neste trabalho, objetivou-se avaliar os efeitos de uma mistura de mannan-oligossacarídeos (MOS) e frutoligossacarídeos (FOS), na proporção de 1:1 adicionados a uma dieta para cães, antes ou após o processo de extrusão, sobre os coeficientes de digestibilidade aparente das dietas, energia metabolizável,

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metabolismo do nitrogênio (curva de uréia sérica pós-prandial, balanço orgânico de nitrogênio, concentração fecal de amônia e excreção de uréia urinária) e parâmetros fermentativos fecais (pH fecal e concentrações de ácidos graxos de cadeia curta). Foram utilizados 18 cães adultos da raça Beagle, distribuídos ao acaso em três tratamentos, sendo uma ração controle mais duas dietas em que a mistura dos prebióticos foram adicionados antes ou após o processo de extrusão das rações. Não houve diferença na digestibilidade, energia metabolizável, teor de amônia fecal e de ácidos graxos de cadeia curta (acético, propiônico e butírico) entre as dietas controle e com adição dos prebióticos. Entretanto, os cães suplementados com prebiótico, antes ou após a extrusão, apresentaram menor pH fecal ($p < 0,05$) em relação à dieta controle e foi observado ainda redução na concentração pós-prandial de uréia sérica ($p < 0,01$) nos animais que receberam prebióticos, observados pelas áreas abaixo da curva de uréia e do seu incremento. O processo de extrusão não afetou os efeitos biológicos destes prebióticos sobre os parâmetros avaliados nos cães. Possíveis mecanismos implicados no efeito dos prebióticos sobre a redução nas concentrações de uréia sérica são a diminuição na formação e absorção intestinal de amônia, favorecidas pela redução no pH intestinal. Estes achados podem ser considerados no uso de prebióticos em nutrição clínica.
Palavras-chave: Amônia. Extrusão. Saúde intestinal. Uréia sérica.

Introduction

Prebiotics are nutritional substances that nourish a select group of microorganisms that live in the intestine, favoring their multiplication. In general, prebiotics are used to increase beneficial bacteria, to the detriment of pathogenic bacteria. The term prebiotic was defined by Gibson and Roberfroid in 1995 as non-digestible nutritional ingredients that positively affect the host by selectively stimulating the growth and activity of one or more species of beneficial intestinal bacteria, thereby improving the health of the host (FREITAS et al., 2014). Among the prebiotics used in commercial dog food, the fructooligosaccharides (FOS) and mannan-oligosaccharides (MOS) are the most common, and have shown promising results in regards to the intestinal health of dogs.

FOS are composed of a fructose unit with β (2-1)-glycosidic bonds and one terminal D-glucose unit. These compounds are fermented by the beneficial microbiota present in the large intestine, resulting in the production of short-chain fatty acids (SCFA) and lactate, which, in addition to being an energy source for the host, reduce the pH in the intestine and control the proliferation of pathogenic microorganisms (ROBERFROID et al., 2010). MOS are isolated from the intestinal cell wall of yeast, such as *Saccharomyces cerevisiae*, and have lower intestinal fermentability compared to FOS (ZENTEK et al., 2002). These compounds have the ability to reduce pathogenic bacteria in the intestine and modulate the immunological system. MOS also regulate the growth of intestinal pathogenic bacteria by being present on certain epithelial cell sites in the intestinal mucosa, preventing the colonization of pathogenic bacteria on these sites (SWANSON et al., 2002b).

High-quality dog food has a high concentration of protein, since animals consuming this food have a considerable physiological need for amino acids. However, a high-protein diet may favor the formation of undesirable compounds during intestinal fermentation. In general, preventing carbohydrates from being the energy source in the large intestine leads to microbial proteolysis, and, in turn, the release of substances toxic to the host, such as ammonia and biogenic amines. In this regard, prebiotics may reduce the concentration of ammonia in the intestine by increasing fermentation, resulting in higher amounts of nitrogen being converted into bacterial proteins."

In addition, the use of prebiotics can reduce proteolysis and the production of putrefactive intestinal compounds, increasing the number of beneficial bacteria and the production of SCFA, with a decrease in the luminal pH (PINNA; BIAGI, 2014).

The degradation of proteins in the colon starts with their hydrolysis into smaller peptides and amino acids by bacterial proteases, which are more active at a neutral to alkaline pH. In the proximal colon, the pH is more acidic due to the production of SCFA during carbohydrate fermentation. In the distal region of the colon, there are fewer carbohydrates, and therefore the pH increases and protein fermentation becomes more efficient. Ammonia is synthesized by bacteria via amino acid deamination and by the hydrolysis of endogenously produced urea. The latter reaction is catalyzed by bacterial ureases (BLACHIER et al., 2007). Due to bacterial degradation and the recycling of endogenous nitrogen, the colon epithelium is constantly subjected to ammonia, which can interfere with the intermediate metabolism and morphology of the intestinal epithelial cells, increase DNA synthesis, and affect cellular viability; high concentrations of this metabolite in the colon lumen results in adverse effects on intestinal health (SMITH; MACFARLANE, 1997; HUSSEIN et al., 1998).

In humans, 3.5-4.0 g ammonia is released every day in the intestine (LIN; VISEK, 1991), resulting in luminal concentrations as high as 60 mmol kg^{-1} luminal content (MACFARLANE; CUMMINGS, 1986). The produced ammonia is subsequently absorbed into the bloodstream and converted into urea, which is less toxic to the organism, and is later eliminated via the feces or urine (GUYTON; HALL, 2006). A stable, slightly acidic pH in the intestine reduces the activity of intestinal deaminases and urease-producing bacteria, resulting in a reduction in intestinal ammonia production (VINCE; BURRIDGE, 1980).

Due to the close association between intestinal pH, ammonia production, and urea concentration, prebiotics can modify nitrogen metabolism, with potential long-term benefits for animal health and clinical nutrition, especially in the context of pathologies that are characterized by urea or ammonia accumulation in the organism. To investigate the potential beneficial effects of prebiotics in dogs, we analyzed the effects of the association between FOS and MOS, added before or after extrusion, on fermentative intestinal parameters, nutrient digestibility, and nitrogen metabolism.

Material and Methods

The study was conducted at the Centre for the Study of Companion Animal Nutrition of the Animal Science Department in the Federal University of Lavras, located in Lavras, MG, Brazil. The study was approved by the Bioethics Committee on the Use of Animals at the same institution (protocol no. 057/2012).

Animals and diets

We used eighteen adult (4-7 years old) Beagles (male and female) that were healthy, vaccinated, and wormed, with an average weight of 14.0 ± 1.2 kg. The nutritional diets were formulated according to the recommendations of the Association of American Feed Control Officials (AAFCO, 2010) for adult dogs. The control treatment was a commercial diet consisting of the following ingredients: poultry visceral meal, dehydrated egg, blood plasma powder, fish meal, broken rice, ground whole corn, wheat bran, linseed, poultry offal fat, sugar beet pulp, DL-methionine, vitamin C, flavor agent, antifungal additive, antioxidant additive, vitamin mineral premix, and sodium chloride. This diet contained the following vitamin and element concentrations per kilogram of product: 20,000 UI vitamin A; 2,000 UI vitamin D_3; 48 mg vitamin E; 4 mg vitamin B1; 8 mg vitamin B2; 32 mcg vitamin B12; 4.8 mg vitamin K3; 56 mg niacin; 16 mg pantothenic acid; 800 mg choline; 0.20 mg selenium; 15 mg copper; 150 mg zinc; 30 mg manganese; and 1.5 mg iodine.

The chemical composition observed in the control diet (dry matter) was as follows: 33.1% crude protein; 19.2% acid ether extract; 1.3% crude fiber; 11% mineral matter; 28.7% nitrogen-free extractives (calculated); and 4,908.2 kcal kg^{-1} gross energy.

For the remaining treatments, a mixture (1:1) of MOS (yeast cell wall extract, Bio-MOS, Alltech Tools do Brasil) and FOS (inulin 90%, Beneo Oraft SA, Belgium) prebiotics was added to that formulation at a concentration of 1% dry matter. The prebiotics were added before or after extrusion in order to determine if changes in their structures, as a result of the extrusion process, affected the analyzed parameters. For the addition of the prebiotics before extrusion, the mixture was added into the mixer together with the other ingredients of the control diet, extruded, and covered with poultry offal fat and flavor agent. For the addition after extrusion, the prebiotics powder was weighed and homogenized. Subsequently, the prebiotics mixture was incorporated (horizontal mixer for 2 min at room temperature) into the previously extruded formulation immediately after the addition of the poultry offal fat and flavor agent.

The experimental diets were produced using a single screw extruder (model X235, Wenger Manufacturing, Kansas, U.S.A.). The temperature conditions during food production were as follows: 90ºC (conditioner), 130°C (extruder), and 110°C (dryer). The extruded formulation remained in the dryer for 35 min. The formulations were offered to the dogs once a day (08:30), with a sufficient amount being provided to meet the metabolizable energy needs (MEN) of the animals, based on the equation, $MEN = 130 \times body\ weight^{0.75}$, as recommended by the National Research Council (NRC, 2006). Water was provided *ad libitum*.

Digestibility assay

CTTAD and ME of the utilized food were determined using an *in vivo* method, based on the total feces and urine collection procedure (AAFCO, 2010). For this purpose, the animals were placed in individual metabolic cages (70 cm height × 85 cm length × 70 cm width), where they remained for 10 days, with five days of adaptation to the cages and five days for the total feces and urine collection. All feces were collected, weighed, and frozen at -15°C; the urine was collected in a plastic cup containing 1 mL sulfuric acid and kept in a freezer at -15°C. The feces were classified according to the score proposed by Carciofi et al. (2008), and given grades between 0 and 5: 0 = liquid feces; 1 = loose and shapeless feces; 2 = loose feces that take the shape of the collection receptacle; 3 = loose, well-shaped, moist feces that leave a mark at the bottom of the collection recipient; 4 = well-shaped and consistent feces that do not leave a mark at the bottom of the collection recipient; and 5 = well-shaped, stiff, dry feces. Values between 3 and 4 are considered normal.

At the end of the collection process, feces and urine were thawed and homogenized (one sample per animal). Subsequently, the feces samples were dried in an incubator with forced ventilation (55°C for 72 hours). Feces and food samples were then ground through a 1.0 mm sieve, and analyzed for dry matter (DM) content, crude protein (CP), crude fiber (CF), mineral matter (MM), and gross energy (GE), according to Silva and Queiroz (2002), and ether extract after acid hydrolysis (EEAH), determined by an automated extraction system (Ankom Hydrolysis System, Ankon Technology model HCL I, New York, U.S.A.). The nitrogen-free extractives (NFE) were estimated using the following equation: 100 - (% moisture + % CP + % CF + % EEAH + % MM).

Based on the laboratory results, we determined the coefficient total ttact apparent digestibility coefficient CTTAD and ME of the diets, using the total collection method.

Ammonia, faecal pH, Short-Chain Fatty Acid production

After the digestibility assay, all dogs were kept for an additional five days for the daily collection of fresh feces (collected immediately after defecation), in order to determine the SCFA, pH, and ammonia nitrogen present in the feces. To measure fecal pH, 1 g fresh feces was diluted in 5 mL milliQ water, and the pH was measured using a digital pH meter with a precision of 0.01 pH (model DM-22, Digimed Analítica Ltda, São Paulo, Brazil). To determine the SCFA (acetic, propionic, and butyric) concentration, 10 g fresh feces was quickly homogenized and mixed with 30 mL 16% formic acid solution (1:3 w/v). This mixture was kept refrigerated to five °C for three days before being centrifuged three times at 4,500 x *g* for 15 min, which the supernatant was set aside and the sediment was discarded. The samples were stored in a freezer (-15°C) and subsequently sent to the State University of Maringá-PR, Department of Animal Science's Laboratory of Food Analysis and Animal Nutrition to be analyzed, according to ERWIN et al. (1961).

The SCFA (acetic, propionic, and butyric) concentration in the feces was determined using gas chromatography (Trace GC Ultra Chromatograph, Thermo Scientific, U.S.A.) with automatic injection, a flame ionization detector at 240°C, and a fused silica 0.20 µm capillary column (100 m length, 0.25 mm inner diameter; Restek 2560). Hydrogen was used as the carrier gas (1.2 mL min^{-1} flow), nitrogen as the auxiliary gas (35 mL min^{-1} flow), and synthetic air and hydrogen as combustibles at 35 and 350 mL min^{-1} flows, respectively. The initial temperature of the column was set to 65°C (maintained for 5 min), then increased to 170°C at a rate of 16°C min^{-1}, and maintained for 7 min before reaching a final temperature of 235°C. The sample (1 µL) was pipetted into a vial coupled to the chromatograph's automatic injector. The sample concentration was calculated by means of comparison, with the peaks formed using a standard solution containing the acids (Sigma Aldrich, São Paulo, Brazil).

The amount of ammonia nitrogen in the feces was determined using the Kjeldahl method, excluding the digestion step. The extracts prepared for the measurement of SCFA were used for the referred assay. The extracts were thawed at room temperature and then 2 mL aliquots were transferred to assay tubes, diluted in 13 mL distilled water, and subjected to distillation in a nitrogen distiller (Tecnal T-036/1, Piracicaba, Brazil). Distillation was performed with 5 mL potassium hydroxide (KOH 2 mol L^{-1}) and the nitrogen was collected in an Erlenmeyer flask containing 10 mL solution (boric acid 0.97 N). Subsequently, a titration with HCL (0.005 N) was carried out.

Postprandial response tests

On the last assay day (day 16), all dogs were subjected to a procedure to determine the curve of postprandial serum urea. In the afternoon of the previous day, the dogs were aseptically trichotomized and a heparinized catheter (22 GA × 1.16 inches, BD Angiocath, Becton Dickinson, U.S.A.) was introduced into the cephalic vein. The animals then fasted for 12 hours, but were allowed to drink water *ad libitum*. The first measurement was performed in the morning with the animals still in fasting. Food was then offered to the dogs for 15 min, and the subsequent measurements were carried out at 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, and 720 min after feeding, with time zero being the point right after total food ingestion.

For each measurement, approximately 1.5 mL blood was collected. Blood was immediately transferred to glass assay tubes without anticoagulant and centrifuged at 804, 9 x *g* for 5 min, for serum separation. The serum was then transferred to propylene microtubes using a pipette and stored at -15ºC until analysis. The concentration of serum urea was determined using the commercial kit Uréia-PP (Analisa®, Belo Horizonte-MG), applying the enzymatic colorimetric method. The absorbance at wavelength 600 nm was measured in an ultraviolet-visible spectrophotometry (UV-Vis; Shimadzu apparatus model 1601PC). Urea concentration in the urine was determined using the same methodology used to determine serum urea. The concentration of urea in the urine was calculated by multiplying the observed value by a factor of 50.

Statistical analysis

The experimental data were subjected to an analysis of variance (ANOVA), and tested for normal distribution using the Kolmogorov-Smirnov test, and for equality of variances using the Levene test. When the ANOVA values were significant, the averages were compared using the Tukey's test with a 5% probability. In order to evaluate the responses of postprandial serum urea, a repetitive measurements was utilized, where the effects of period (collection time), treatment, and their interactions were evaluated. The averages were also compared using the Tukey's test at a 5% significance level. Statistical analyses were performed using the GSM function of the SAS software version 9.2 (SCHLOTZHAUER; LITTELL, 1997). The values observed for the serum urea curve at all collection times were transformed to the area under the curve (AUC), and subsequently included in the statistical analysis with the other variables. The statistical software ORIGIN PRO 9.0 32-bit (OriginLab Data analysis and graphing software, Massachusetts, U.S.A.) was used to calculate the AUC.

Results and Discussion

The apparent coefficient of digestibility (ACD) and the apparent metabolizable energy (AME) were similar between the control diet and the diets supplemented with prebiotics P>0.05) (Table 1). The animals consumed, on average, 118.9 ± 1.93 kcal $(kg^{0.75}\ day)^{-1}$; this value was similar in all treatments as well.

In a study evaluating the addition of prebiotics (up to 0.5% DM), Strickling et al. (2000) also found differences in the CTTDA and the ME in dogs. However, in another study with dogs, Zentek et al. (2002) observed that the addition of 1 g MOS kg^{-1} body weight day^{-1} resulted in a decrease of the CTTDA of the CP, EEAH, and DM. It is worth mentioning that in this previous study the MOS amount used corresponded to approximately 2.5% MOS kg^{-1} MS in the diet. Even though prebiotics appear to affect nutrient digestibility, the noted changes are small, which is likely due to the low percentage (0.2% to 1.0%) of these compounds in the food. Improved nutrient digestibility is notthe reason prebiotics are included in pet food, with the main reasons being: proper regulation of the intestinal microbiota; supply of an energetic substrate for the colonocytes (butyrate); and reduction in the formation of intestinal compounds resulting from the fermentation of indoles, phenols, ammonia, amines, and other compounds (HUSSEIN et al., 1998).

Table 1. Body weight, nutrient intake, coefficient of total tract apparent digestibility (CTTAD) of nutrients and metabolizable energy (ME kcal g)$^{-1}$ of experimental diets.

		Prebiotics			
Item	Control	Before extrusion[1]	After extrusion[2]	CV(%)	P
Body weight	14.2	14.8	13.5	15.1	0.5669
Nutrient intake (g Kg$^{0.75}$ day)$^{-1}$					
Dry matter	29.4	29.2	29.5	11.04	0.7163
Organic matter	26.2	25.9	26.3	11.4	0.3515
Ash	3.25	3.29	3.19	11.5	0.3515
Crude protein	9.75	9.20	9.84	11.2	0.8881
Acid-hydrolyzed fat	5.76	5.41	5.57	11.3	0.6767
ME	121.4	121.6	121.3	11.4	0.5726
CTTAD (%)					
Dry matter	80.7	80.9	77.8	3.2	0.0942
Organic matter	87.5	87.8	85.4	2.1	0.0809
Crude protein	81.9	82.5	79.1	3.2	0.0682
Acid-hydrolyzed fat	95.1	94.6	94.6	0.8	0.4707
ME	4.08	4.12	3.97	3.1	0.1447

[1]Inclusion of 1% of the prebiotics mannan-oligosaccharides (MOS) and fructooligosaccharides (FOS) in a ratio of 1:1 added to the control diet before the extrusion process.
[2]Inclusion of 1% of the prebiotics MOS and FOS in a ratio of 1:1 added to the control diet after the extrusion process.
CV- coefficient of variation; P: probability.

The characteristics of the feces, amount of urea excreted in the urine, and intestinal fermentation indicators are presented in Table 2. The fecal pH was lower ($P<0.05$) for the dogs fed diets containing prebiotics than for the dogs fed the control diet. Similar results were noted by Twomey et al. (2003), with an increase in FOS ingestion resulting in a decrease in fecal pH, which was attributed to an increase in lactate concentration. The final products in the fermentation process affect the concentrations of acids in the feces, and, consequently, the fecal pH. Compounds that change the pH of the intestinal lumen are mostly starches, fibers (especially the fermentable ones), amino acids, and, to a lesser extent, fatty acids. Since there is considerable variation in the nutritional composition and digestibility of commercial foods, prebiotics must be used according to the features of the ingested food in order to maintain the intestinal pH in a range that favors the growth of beneficial microorganisms. Little is known about the ideal pH range of the intestinal lumen for most animal species. However, Mcintyre et al. (1993) observed that reducing the intestinal pH to between 6.2 and 6.6 decreased the formation of tumor cells in mice. In this previous study, the authors managed to reduce the fecal and colon pH with the addition of fermentable fibers. In contrast, ingested amino acids that reach the large intestine have been noted to cause an increase in the intestinal pH, and, consequently, the production of undesirable substances (e.g., biogenic amines, phenolic compounds, and ammonia), which, at high concentrations, may damage intestinal epithelial cells, predisposing the organism to diseases (MACFARLANE; MACFARLANE, 1995). The use of additives to regulate intestinal pH seems to be important, especially for protein-rich diet sources, such as pet food In the present study, the protein concentration in the diets was 81.3 g 1000 kcal^{-1} of ME, and the combined use

of the MOS and FOS prebiotics resulted in the intestinal pH being reduced from 7.09 (control) to 6.7 (prebiotics). This reduction in pH could have an important effect on animal health, although further studies should be performed in order to confirm this hypothesis.

Table 2. Faecal characteristics, short chain fatty acids (SCFA, mmol Kg of faecal dry matter)$^{-1}$ and urinary excretion of urea of dogs fed with a control diet or diets containing prebiotics before or after the extrusion process.

		Prebiotics			
Item	Control	Before extrusion[1]	After Extrusion[2]	CV(%)	P
Faecal score[3]	3.83	4.00	4.00	5.97	0.3911
Faecal pH	7.09[a]	6.67[b]	6.74[b]	2.1	0.0004
Ammonia (mg g)$^{-1}$	12.07	13.2	12.1	13.9	0.7056
SCFA					
Acetic acid	276.8	294.9	378.8	29.93	0.1555
Propionic acid	121.8	151.1	191.2	59.83	0.4280
Butyric acid	127.6	200.4	219.7	92.91	0.6701
Total SCFA[4]	526.3	646.4	789.7	47.29	0.5315
Urinary urea	40.2	35.7	40.6	21.22	0.5315

[a-b]Means within a row and without common superscripts differ (P<0.05).
[1]Inclusion of 1% of the prebiotics mannan-oligosaccharides (MOS) and fructooligosaccharides (FOS) in a ratio of 1:1 added to the control diet before the extrusion process.
[2]Inclusion of 1% of the prebiotics MOS and FOS in a ratio of 1:1 added to the control diet after the extrusion process. CV: coefficient of variation; P: probability.
[3]Faecal score based on the following scale: 0 = watery liquid, which can be poured; 1 = soft, unformed; 2 = soft, malformed stool, which assumes shape of container; 3 = soft, formed, and moist, which retains shape; 4 = wellformed and consistent stool, which do not adhere to the floor; and 5 = hard, dry pellets, which are small and hard mass.
[4]Total SCFA = total short-chain fatty acids; the sum of acetic acid, butyric acid, and propionic acid.
Numerical data CV (%) and P-value for SCFA were transformed into logarithm to Base10 (P <0.05).

The fecal score of the dogs did not differ between treatments (P>0.05). A fecal score between 3 and 4 is considered ideal, and the scores in present study were always within this range. Propst et al. (2003) supplemented the diet of dogs with FOS, up to 0.9% (oligofructose and inulin), but did not observe any effect on the fecal quality or the coefficient of digestibility of the nutrients, even though the DM decreased after the addition of the prebiotic.

The ammonia content in the excreted feces did not differ among the treatments (P>0.05) (Table 2). Swanson et al. (2002a) found a lower concentration of ammonia in the feces of dogs when their diets were supplemented with 4 g FOS day^{-1}. However, in a previous study (SWANSON et al., 2002b), there was no difference in the ammonia content in the dog feces when the animals received a diet supplemented with half the dose (i.e., 2 g FOS day^{-1}). Because most of the produced ammonia is absorbed by the cells of the intestinal mucosa, and its production and absorption are directly affected by the intestinal pH, its quantification in the feces is not a suitable indicator for the fermentative activity in the intestine, since an increase or decrease in the fecal concentrations of ammonia may be wrongly interpreted.

In the present study, no differences were observed between the treatments for the fecal concentrations of SCFA, despite the fact that the treatments with prebiotics resulted in higher absolute values for

these parameters. A possible explanation for this is the high the coefficient of variation (CV) observed for them. For the statistical analyses, the data were transformed into a logarithmic scale, but even then, the CV remained high and without statistical differences between the treatments. For dogs fed a control diet (without supplementation with prebiotics) or 0.3, 0.6, and 0.9% fructans (oligofructose or inuline) supplemented diets, Propst et al. (2003) observed concentrations of total SCFA of 406.4, 529.9, 538.3, and 568.8 μmol g^{-1} fecal dry matter, respectively."

In the present study, these values were even higher, which was probably a result of the ingestion of the control diet as it contained fiber sources (e.g., wheat bran, root beet pulp, and linseed) that may have contributed to these higher SCFA concentrations. For this reason, a comparison between dog studies must include a critical analysis of the basal diet, since the considerable variation between ingredients used in the different studies hinders any comparison. Swanson et al. (2002a) and Zentek et al. (2002) also did not find any differences in the fecal concentrations of SCFA for adult dogs fed daily with a diet containing 4 g FOS (in a gelatin capsule).

Data on the consumption, absorption, excretion, retention, and balance of nitrogen in the dogs is shown in Table 3. All treatments showed a positive nitrogen balance, which was expected due to the high levels of proteins in the diet. According to Hendriks et al. (1997), cited by the NRC (2006), maintenance of adult dogs requires approximately 210 mg $(kg^{0.75}\ day)^{-1}$ in order to keep the nitrogen balance neutral, which corresponds to the ingestion of 4,000 kcal kg^{-1} containing approximately 80 g kg^{-1} crude protein. In the present study, the diet contained more than 300 g kg^{-1} crude protein, resulting in high nitrogen balance values. The nitrogen excreted in the feces and urine, the retained nitrogen, and the ratio between retained and absorbed nitrogen did not differ between treatments (P>0.05).

Table 3. Consumption and apparent absorption, excretion and retention of nitrogen (expressed in g $Kg^{0.75}$ per day)$^{-1}$ and nitrogen balance (expressed in mg of nitrogen per $kg^{0.75}$ day)$^{-1}$ of dogs fed with a control diet or diets containing prebiotics before or after the extrusion process.

		Prebiotics			
Item	Control	Before extrusion[1]	After extrusion[2]	CV(%)	P
Nitrogen intake	1.55	1.47	1.57	0.13	<0.0001
Nitrogen excreted faeces	0.22	0.21	0.26	14.05	0.0559
Nitrogen excreted urine	0.178	0.182	0.207	40.39	0.7811
Nitrogen retained	1.15	1.07	1.10	7.81	0.2986
N retained: N absorbed (%)	86.52	85.47	84.07	6.98	0.7783
Nitrogen balance	1.150	1.070	1.100	7.81	0.2986

[1]Inclusion of 1% of the prebiotics mannan-oligosaccharides (MOS) and fructooligosaccharides (FOS) in a ratio of 1:1 added to the control diet before the extrusion process.
[2]Inclusion of 1% of the prebiotics MOS and FOS in a ratio of 1:1 added to the control diet after the extrusion process. CV: coefficient of variation; P: probability.

Nitrogen is present in all organism in the form of proteins, amino acids, nucleic acids, purines, pyrimidines, vitamins, hormones, antibodies, enzymes, urea, ammonia, and many other compounds, being excreted mainly as non-digested protein and microbial protein in the

feces, and as urea in the urine, which is produced in the liver by the catabolism of amino acids and the metabolism of ammonia originating from the intestine.

A portion of the urea produced by the organism enters to the intestine, however, all urea in the intestine is excreted in the feces due to the presence of bacterial urease in the large intestine, which breaks down the urea molecule into ammonia, CO_2, and water. Most of the ammonia is reabsorbed and metabolized in the liver by the enzyme carbamoyl phosphate synthetase, and is then converted into urea again and excreted in the urine (FULLER, 2004). However, the formation and absorption of intestinal ammonia from urea depends on the urease activity and on favorable pH conditions. It is known that approximately 99% of the ammonia produced every day in the large intestine is absorbed by the colonocytes non-ionic diffusion mechanism. Nevertheless, as the intestinal pH decreases, the ability of the urea to diffuse from the intestine into the bloodstream also decreases, and as a result, more ammonia is excreted in the feces (CUMMINGS et al., 1976).

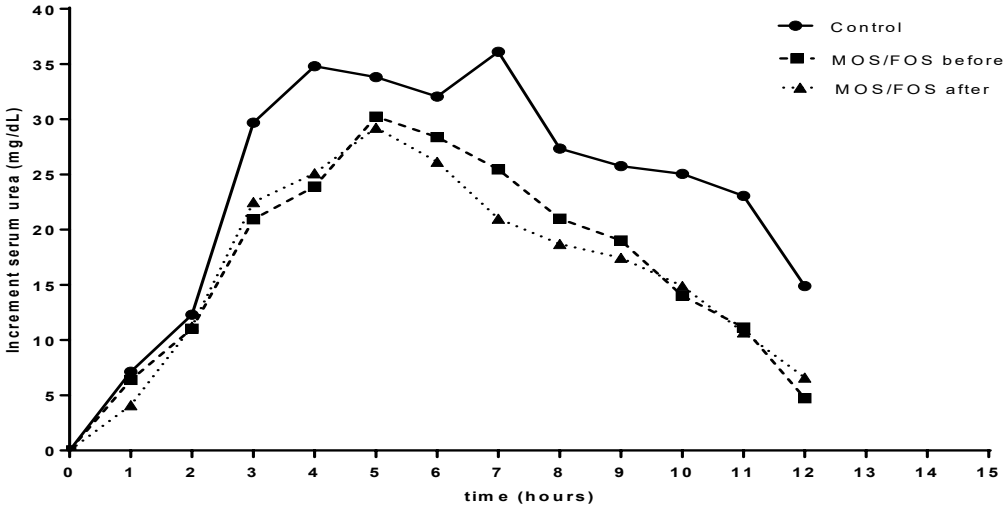
Because ammonia can be used in the organism for the synthesis of either amino acids or urea, it is expected that a higher absorption of this substance in the intestine results in an increased urea concentration in the plasma. In the present study, the concentrations of urea in the serum and in the urine were analyzed together in order to better understand the effect of prebiotics on the formation and absorption of ammonia in the intestine. The noted concentrations of serum urea in dogs during fasting (preprandial) and after feeding (postprandial) for each diet type is shown in Table 4. According to the AUC for urea (Table 4), a lower concentration of serum urea was noted for the dogs that received the diets with prebiotics than for the dogs that received the control diet ($P<0.05$). A higher urea increment (Figure 1) was also noted for the dogs that received the prebiotics.

Table 4. Postprandial serum urea (mg dL) $^{-1}$ of dogs fed with a control diet or with inclusion of prebiotic before or after extrusion.

	Prebiotics				
Item	Control	After extrusion[1]	Before extrusion[2]	Mean	S.E.M
AUC urea	709.9[A]	607.3[A]	590.1[A]	635.7	43.5
AUC increment	296.3[A]	213.8[B]	201.3[B]	238.1	12.3
Urea peak	66.5	63.0	61.4	32.8	3.8
Increment peak	41.9[A]	31.4[B]	30.6[B]	34.6	1.3
Time urea peak	5.1	5.1	5.0	5.0	0.4

[1]Inclusion of 1% of the prebiotics mannan-oligosaccharides (MOS) and fructooligosaccharides (FOS) in a ratio of 1:1 added to the control diet before the extrusion process.
[2]Inclusion of 1% of the prebiotics MOS and FOS in a ratio of 1:1 added to the control diet after the extrusion process. S.E.M= standard error of the mean.
[A-B]Means with the same letter capital on the line do not differ by Tukey test ($p> 0.05$).
AUC: Area under the curve urea. AUC increment: Area below the increment curve.

Figure 1. Increments of postprandial serum urea of dogs fed with experimental diets.



We also observed differences in the concentration of serum urea during the postprandial period. At a certain time after food ingestion, there was an increase in the blood concentrations of this metabolite, which is a physiological behavior that is expected after food ingestion. Matsuoka et al. (1990), cited by Zentek et al. (2002), noted that in Beagles, the ingestion of lactulose decreases the absorption of nitrogenous compounds, resulting in a reduced blood concentration of ammonia in the portal circulation. For the present study, we believe that the decrease in the concentrations of serum urea in the animals that received diets supplemented with prebiotics (before or after extrusion) was due to reduced ammonia production (and consequently absorption) in the intestine. Since the ingestion of proteins was the same between treatments, and the digestibility of this protein also did not differ, this hypothesis is more likely to be confirmed, as reflected in the reduction of the fecal pH in animals that consumed the MOS and FOS prebiotics, which by themselves decrease the production and absorption of ammonia in the intestine (CUMMINGS et al., 1976).

A reduction in ammonia production by intestinal bacteria is important to maintain the lower intestinal pH, which favors the establishment of beneficial bacteria to the detriment of bacteria that are potentially pathogenic and those able to ferment protein. It is currently known that a few products of protein fermentation, when in excess, contribute to the development of inflammatory intestinal disturbances and colon cancer. Since the food formulated for dogs and cats have high protein concentrations, prebiotics may be beneficial because of their ability to increase SCFA production and control potentially pathogenic microbiota (SEGAIN, 2000; HAMER et al., 2008).

Figure 1 show the increments of postprandial serum urea in dogs fed the control diet and the diet supplemented with prebiotics, either before or after extrusion. Another beneficial effect of the prebiotics that was noted in the present work was the potential reduction in intestinal absorption, and, consequently, serum concentrations of urea. This finding is important for the field of clinical nutrition, since, in cases of liver failure or portosystemic shunts, one of the goals is to prevent ammonia toxicity, which is achieved through the restriction of protein ingestion and the use of lactulose (HAND, 2010). It is possible that MOS and FOS could also provide similar benefits. For other health issues,

such as chronic kidney failure, one of the goals is to prevent urea accumulation in the organism (HAND, 2010), and prebiotics could also be beneficial in this context.

In the food industry, supplementation with prebiotics is easier when they are added to the food prior to extrusion. However, it is possible that the thermal processing during extrusion (110-130°C) and drying (approximately 110°C) may affect the biological activity of prebiotics. Prebiotics are highly stable, enduring pH levels <3, and temperatures above 140°C (SILVA et al., 2007). In the present study, even though the dog food was subjected to high temperatures during extrusion, it remained at these temperatures only for a few seconds, and therefore it was unlikely that the structure of the prebiotics was damaged. Therefore, we did not observe any effect of processing on the biological effect of the MOS and FOS prebiotics.

Conclusions

The use of prebiotics in a diet does not change the nutrient CTTDA, the ME, the concentrations of fecal ammonia, or the nitrogen balance. However, the ingestion of prebiotics does reduce the concentrations of postprandial serum urea. The proportions of prebiotics utilized in the present study does not change the fecal concentrations of SCFA, but does decrease the fecal pH. The extrusion process does not alter the biological effects of the prebiotics for the evaluated parameters in dogs.

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