

# Comparison of dietary fibre, $\beta$ -glucan, resistant and non-resistant starch and *in vitro* digestibility of commercial extruded dry food for adult dogs

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**ABSTRACT.** This study aimed to compare the carbohydrate, fatty acids and digestion values of 29 commercial extruded dry-type dog foods produced for large breed adult dogs. In the study, total dietary fibre (TDF) values of dog foods differed significantly among commercial brands and ranged from 19.02 to 38.09% dry matter (DM) ( $P < 0.05$ ). The average soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) contents of dog foods were 6.27 and 20.09% DM, respectively. The average values of non-resistant starch and  $\beta$ -glucan in dog foods were 30.90 and 0.52% DM, respectively. The average linoleic acid,  $\alpha$ -linolenic acid (ALA) and eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) levels in dog foods were 2.21, 0.03 and 0.11% in DM, respectively. The total starch value was positively correlated with the gas production value ( $r = 0.516$ ) of dog food ( $P < 0.05$ ). As a result, it has been determined that the resistant starch and  $\beta$ -glucan levels differ in the dog food. EPA + DHA, linoleic and ALA acids were below international standard values in some dog food samples. Although *in vitro* digestibility of dog food was adversely affected by the increase in IDF and palmitic acid contents; *in vitro* digestibility of acid contents in dog food was positively affected by the increase in soluble dietary fibre and stearic acids.

## Introduction

The carbohydrate substances in dog foods can be categorized as digestible (non-resistant starch and disaccharides), absorbable (monosaccharides and sugar alcohols) and fermentable (oligosaccharides, resistant starch and dietary fibre) carbohydrates (NRC, 2006). Carbohydrate content in dog foods is: 46–74% of dry matter (DM) in dry-type, 58–72% DM in semi-moist, and 18–52% DM for wet-type dog foods (Case et al., 2011). Dietary fibre comprises cell wall polysaccharides, non-cellulose polysaccharides and structural non-polysaccharides (lignin) (Englyst, 1989). Resistant starch, oligosac-

charides, non-starch polysaccharides and fermentable fibre substances are fermented by enzymes and microorganisms in dog large intestine. Fibre is a complex and diverse group of compounds that are not easily defined or determined (Fahey et al., 1990). Dietary fibre consists of two crucial fractions (soluble dietary fibre (SDF) and insoluble dietary fibre (IDF)), and the ratio of these two fractions changes the property of dietary fibre. The soluble undigested fibres (or insoluble arabinose, xylose, mannose, galactose and uronic acids) can be fermented in the colon, thereby producing short-chain fatty acids (SCFA) (acetate, propionate and butyrate) at the greatest rate and branched-chain fatty acids at the

lowest rate (Cho and Dreher, 2002; Jaworski et al., 2015). Soluble dietary fibre can form a viscous gel after contact with water in the digestive tract. Insoluble dietary fibre does not develop a gel form in contact with water but can retain water in its structural matrix and produce an increase in stool mass that accelerates intestinal transit (Cho and Dreher, 2002).

Starch, the storage polysaccharide of plants, is stored in intracellular crystalline bodies or starch granules in plants. According to the shape and crystalline structure of these granules it can be classified as one of three types, depending on the density and orientation of amylopectin helices of the starch molecules: A – densely packed in an orthogonal pattern, B – less densely packed in a hexagonal pattern or C – containing both patterns. Cereal starch granules are predominantly A-type and are easily degraded by  $\alpha$ -amylase and hydrolysed in the gastrointestinal tract. B-type starches of tubers (potato) and C-type legume starches are more resistant to enzymatic hydrolysis (Englyst et al., 1992). While most starches are from plants, glycogen is a storage form of starch created by animals. Starch granule structure also affects the enzymatic/fermentative digestion of starch in the digestive cannula (Kara et al., 2019). The organism enzymes in the small intestine (enzymatic digestion) or microbial fermentation (fermentative digestion) in the large intestine also affect the rate of digestion. Peixoto et al. (2018) identified that 1.46% resistant starch (R-S) in dog food was positively affected by dog colonic fermentation. Protein and other nutrient matters may also increase the fermentation rate in the large intestine, except for starch substances that are not digested in the small intestine (NRC, 2006; Case et al., 2011).

The fat levels in dog foods range from 5 to 40% (Glodde et al., 2018). Dogs fed food with ideal protein levels can tolerate high levels of fat (FEDIAF, 2020). The fat/oils in dog foods are of animal, vegetable or both origins (Kara, 2020a). Fatty acids such as linoleic acid, arachidonic acid,  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in dogs and cats are seen as precursors of leukotrienes, prostaglandins and thromboxane. They affect the protection of biological membranes, nervous system and vision. Although the mechanism cannot be fully explained, polyunsaturated fatty acids (PUFAs) also have cellular protective properties against injuries and epithelial degeneration (Walters et al., 2010). Fatty acids in dog foods (essential fatty acids) are balanced with fish or vegetable oils (Kara, 2020a).

This study hypothesizes that the levels of dietary fibre, starch and fatty acid in premium and quality dog foods, which are produced from different carbohydrate and fat feedstuffs, will differ. At the same time, our other hypothesis is to understand whether the different levels of dietary fibre, starch and fatty acids can meet the dog needs for gut health and general health. The present study was aimed to determine the relations between nutrient components and *in vitro* digestion (organic matter and gas production) of different class dog foods. Besides, it was aimed to determine the levels of dietary fibre (soluble and insoluble),  $\beta$ -glucan, starch (resistant and non-resistant) and essential fatty acids of dry-type dog foods produced for the adult animals.

## Material and methods

### Dog foods

Commercial extruded dry-type dog foods have been produced for large breed adult (>18 months of age) dogs. Dog foods were of premium quality and were purchased from a Distributor Company for dog foods (Istanbul, Turkey). In total, 29 different brand dog foods were analysed. Foods were kept in the appropriate warehouse conditions until the sales stage. Carbohydrate and fat/oil ingredients of commercial premium dog foods are given in Table 1.

Commercial dry dog foods were classified on the basis of the definition given by producers as: high quality (n = 6), premium (n = 6), high-premium (n = 6), super-premium (n = 6) and ultra-premium (n = 5).

Analyses were performed in parallel. Mean values were given according to the mean of repetitions.

### Dietary fibre analyses

Dietary fibre contents of dog foods were analysed using a Megazyme assay (cat. no. K-TDFR-100A/K-TDFR-200A 04/17; Megazyme, Wicklow, Ireland). The samples of dog foods were incubated with a MES-TRIS buffer solution (pH 8.2) and a heat-stable  $\alpha$ -amylase (50  $\mu$ l) at 100 °C for 30 min. The bottles were cooled to 60 °C, and then 100  $\mu$ l of protease solution was added to each sample. The bottles were incubated in a shaking water bath at 60 °C for 30 min. After 30 min, fluid acidity values in bottles were adjusted to pH 4.1–4.5 with 5 ml of 0.561 N HCl. Then, bottles were incubated with 200  $\mu$ l of amylo-glucosidase at 60 °C for 30 min.

**Table 1.** Carbohydrate and fat sources of examined dog foods

Dog food number	Carbohydrate sources*	Fat/oil sources*
1	Oats (whole grain), potato flour, oatmeal, apple pulp, peas, quinoa pods, dried seaweed, chicory root powder	Canola oil, flaxseed
2	Potato flour, pea, tomato, apple, peapod, carrot, pumpkin, clover flour, banana, raspberries, lentil, broccoli, spinach, alfalfa sprouts, dried chicory root powder	Canola oil, flaxseed, coconut oil
3	Potato flour, peas, potato starch, sweet potato, tapioca, spinach, broccoli, apple, carrot, banana, broccoli, pumpkin, seaweed, yucca	Chicken fat, canola oil, coconut oil, salmon oil, seaweed
4	Potato flour (50%), dried sugar beet pulp, dried apple (1%), chicory powder (1%), brewer's yeast (with 0.05% glucans)	Poultry fat, fish oil
5	Rice (whole grain) (20%), rice flour, rice bran, rice protein, sugar beet pulp (reduced sugar), chicory powder (1%), yeast (dehydrated, containing 0.05% glucans)	Fish oil (1.5%), poultry oil, flaxseed, olive oil (0.1%)
6	Rice (14%), maize, sorghum, barley, dried beet pulp (2.6%), fructo-oligosaccharides (0.26%)	Animal fat, fish oil
7	Barley (whole grain), maize (whole grain), sorghum (whole grain), rice (whole grain) (7%), wheat (whole grain), wheat bran, sugar beet pulp (sugar removed), peas (dried), yeast (dried, 0.5% mannan-oligosaccharides, 0.1% $\beta$ -glucans)	Poultry fat, linseed (2.5%), salmon oil (0.5%)
8	Rice (whole grain) (min. 20.5%), maize (whole grain), barley (whole grain), tomato pulp, peas, carrot, apple, sugar beet pulp, brown rice, dried brewer's yeast, yeast	Poultry fat, flaxseed, fish oil (min. 0.4%)
9	Brown rice (whole grain), barley (whole grain), oats (whole grain), sugar beet pulp, apple, pea	Linseed, animal fat
10	Rice (whole grain) (10%), maize (whole grain), soybean meal, rice flour, maize gluten meal	Linseed, animal fat, vegetable oil
11	Wheat, maize, wheat bran, rice, dried carrot, sugar beet pulp, dried peas, dried brewer's yeast	Linseed, animal fat
12	Wheat, maize, wheat bran, dried sugar beet	Linseed, animal fat
13	Wheat (whole grain), maize (whole grain), sugar beet, carrot, wheat bran, maize gluten, rice, dried yeast	Linseed, animal fat, fish oil
14	Maize (whole grain) (20%), rice (4%), vegetable derivatives (beet pulp, 1.1%), maize gluten meal (chicory root 1.1%)	Beef fat
15	Barley (whole grain), maize (whole grain), millet (whole grain), rice, potato flour (4%), wheat flour, wheat (whole grain), sugar beet pulp (sugar-free), yeast (0.1% mannan-oligosaccharides, 0.06% $\beta$ -glucans), peas, chicory (dried)	Fish oil
16	Wheat, barley (whole grain), maize (whole grain), wheat flour, sugar beet pulp, pea, yeast (0.1% mannan-oligosaccharides, 0.06% $\beta$ -glucans), peas, chicory	Poultry oil, fish oil
17	Rice (16%), barley (whole grain), sorghum (whole grain), maize (whole grain), wheat (whole grain), wheat flour, sugar beet pulp (sugar removed), wheat bran, yeast (0.1% mannan-oligosaccharides, 0.06% $\beta$ -glucans), chicory	Poultry oil, fish oil
18	Rice, maize (whole grain), sugar beet pulp, pea, carob, Brewer's yeast, dried sugar beet, mannan-oligosaccharides psyllium, seaweed, blueberry powder	Salmon oil (6%), anchovy oil (2%)
19	Rice (16%), maize (whole grain), sugar beet pulp, peas, peas, brewer's yeast, carob, dried sugar beet, prebiotic mannan-oligosaccharides, seaweed, blueberry powder, psyllium	Refined chicken fat, anchovy oil (2%)
20	Wheat (whole grain), maize (whole grain), wheat gluten meal, rice (whole grain), sugar beet pulp, propylene glycol, malt flour	Fish oil, animal fat
21	Cereals, carrots, sugar beet pulp, peas	0.2% sunflower oil, 0.25% fish oil
22	Maize (whole grain), wheat (whole grain), sugar beet pulp, carrot, peas	0.2% sunflower oil, 0.25% fish oil
23	Peas, dried clover, tomato, banana, alfalfa, yeast, fructo-oligosaccharides (0.2%), mannan-oligosaccharides (0.2%), chicory, apple, pomegranate, pumpkin, tomato	Fish oil, chicken fat, flaxseed oil (0.4%)
24	Spelled (10%), oats (10%), beet pulp, pea husk, carrot, alfalfa, inulin, fructo-oligosaccharides, blueberry (0.5%), apple, dried pomegranate, dried sweet orange, psyllium (0.3%), dried brewer's yeast	Animal fat, herring-salmon oil
25	Potato flour (20%), maize (whole grain), sugar beet pulp, apple pomace, brewer's yeast, seaweed (0.5%), chicory root, fructo-oligosaccharides (0.012%), yucca schidigera extract (0.01%)	Chicken fat (8%), fish oil, salmon oil (1%)
26	Maize (whole grain), rice (20%), wheat (whole grain), wheat germ, sugar beet pulp, dried yeast, yucca	Animal fat
27	Wheat (whole grain), maize germ, sugar beet pulp, chicory root powder-inulin (0.63% fructo-oligosaccharides)	Chicken fat, beef fat, maize oil, flaxseed
28	Rice (32%), dried apple, brewer's yeast, mannan-oligosaccharides (180 mg/kg), herbs and fruits (rosemary, cloves, citrus, turmeric, 180 mg/kg), fructo-oligosaccharides (120 mg/kg), yucca schidigera	Salmon oil (4%), herring oil, chicken fat, evening primrose oil (1%)
29	Rice (38%), dried apple, mannan-oligosaccharides (150 mg/kg), herbs and fruits (rosemary, cloves, citrus fruits, turmeric, 150 mg/kg), fructo-oligosaccharides, yucca schidigera, inulin	Chicken fat, salmon oil (2%)

\* ingredients listed in decreasing order of inclusion

### Analyses of total dietary fibre

Four times, 95% ethanol pre-heated to 60 °C for the volume of residue plus solutions was added to the bottles. The residue plus solutions in the bottles were precipitated at room temperature for 60 min. The bottles' residue was filtered twice on a dietary fibre filtration unit (porosity #2; Velp Scientifica, Usmate Velate, Italy) with 15 ml of 78% ethanol, 95% ethanol and acetone in a crucible. The crucible containing residue was dried overnight at 105 °C in an oven and then weighed. The organic residue in the crucible was burned in a carbon oven for 5 h at 550 °C and then weighed. The total dietary fibre (TDF) content (as % DM) was calculated using dried residue and ash residue.

### Analyses of insoluble dietary fibre

The bottles' residue was filtrated in a crucible using a dietary fibre filtration unit (porosity #2; Velp Scientifica, Usmate Velate, Italy). The crucible was washed twice with 10 ml distilled water pre-heated to 70 °C. The crucible was washed twice with 10 ml of 95% ethanol and 10 ml of acetone. And then crucible was dried overnight at 105 °C in an oven and then weighed. The organic residue in the crucible was burned in a carbon oven for 5 h at 550 °C and then weighed. The insoluble dietary fibre (IDF) content (as % DM) was calculated using dried residue and ash residue.

### Analyses of soluble dietary fibre

Soluble dietary fibre content (as % DM) in the sample was calculated by taking the difference between the TDF content and the IDF content.

### Analyses of total, resistant and non-resistant starch

The total starch content was determined according to the analysis procedure of samples containing resistant starch (R-S). Total starch (T-S) contents of dog foods were analysed using Megazyme assay (cat. no. K-TSTA-100A; Megazyme, Wicklow, Ireland). The R-S and non-resistant (solubilized) starch (NR-S) contents of dog foods were analysed using Megazyme assay (cat. no. K-RSTAR 05/19; Megazyme, Wicklow, Ireland).

### Analyses of $\beta$ -glucan assay procedure

Mixed-linkage  $\beta$ -glucan contents of dog foods were analysed using Megazyme assay procedure (McCleary method) (cat. no. K-BGLU 08/18; Megazyme, Wicklow, Ireland).  $\beta$ -glucan contents of dog foods were calculated as % DM.

### Determination of fatty acid compositions in dog foods

The fat/oils samples were methylated with the modified (Kara, 2020b) three-step procedure of Wang et al. (2015). The methylated fatty acids were analysed according to the chromatograph application conditions and the method of Kara (2020b) for a gas chromatography. Polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA), medium-chain fatty acids (MCFA) (fatty acids with chains containing from 6 to 12 atoms of C), long-chain fatty acids (LCFA) (fatty acids with chains containing from 14 to 20 atoms of C) and very-long-chain fatty acids (VLCFA) (fatty acids with chains containing above 20 atoms of C) were detected.

### *In vitro* digestion technique

The faecal samples used as an inoculum in the current study were obtained from two two-year-old male Labrador Retrievers. The dogs were fed commercial dry-type extruded dog food for four weeks before the faeces were collected. They were fed a commercial extruded dog food containing approximately 25% crude protein, 15% diethyl ether extract, 8% ash and 3% crude fibre on DM basis. The faecal samples were selected with a score ranging from 2.0 to 2.5 according to the Waltham Stool Scoring System (Waltham Centre for Pet Nutrition, Leicestershire, UK). The *in vitro* digestion of extruded commercial dog foods was carried out in three stages (Hervera et al., 2007; Kara, 2020a).

**I. Stage (*in vitro* gastric digestion).** The  $310 \pm 10$  mg DM of dog food were mixed with 10 ml of phosphate buffer (0.1 M, pH 6) into an anaerobic glass fermenter with a 100 ml volume (Model Fortuna, Häberle Labortechnik, Lonsee, Germany). Five ml of 0.2 M HCl were added to this mixture and the pH value was adjusted to pH 2.0 (with 1 M HCl and 1 M NaOH). Then 1 ml of a freshly prepared pepsin solution was added, containing 10 mg of pepsin. One ml of a chloramphenicol solution (0.5 g in 100 ml ethanol) was added to the mixture and then the clips of the *in vitro* fermenters were closed. The fermenters were incubated at  $39.0 \pm 0.2$  °C for 2 h in a thermostatic water bath (Hervera et al., 2007).

**II. Stage (*in vitro* small intestine digestion).** After the gastric digestion, the glass fermenters were cooled and 5 ml of the phosphate buffer (0.2 M, pH 6.8) and 2.5 ml of 0.6 M NaOH were added. The pH value was adjusted to 6.8 (with 1 M HCl and 1 M NaOH). Then 1 ml of the freshly prepared pancreatin solution containing 50 mg of the powdered pancreatin was added to each glass fermenter.

After closing with clips, the glass fermenters were incubated for 4 h at  $39.0 \pm 0.2$  °C in a thermostatic water bath (Hervera et al., 2007).

**III. Stage (*in vitro* large intestine digestion/fermentation).** After the *in vitro* small intestine digestion, the pre-digested dog foods (substrates) and digestion fluids were incubated with the faecal inoculum (1 ml) and fermentation medium (30 ml), which contained solution A, solution B, trace mineral solution, water-soluble vitamins, folate:biotin solution, riboflavin solution, hemin solution, short-chain fatty acids, resazurin, yeast extract, trypticase,  $\text{Na}_2\text{CO}_3$  and cysteine  $\text{HCl} \cdot \text{H}_2\text{O}$  (Sunvold et al., 1995; Bosch et al., 2008). The initial volumes of the fermenters were recorded, and the fermenters were incubated in a water bath with a thermostat set up at  $39.0 \pm 0.2$  °C for up to 48 h. In addition, six blank fermenters (no template = medium mixture plus the faecal inoculum) were used to calculate the total gas production.

The *in vitro* digestion and fermentation were performed with four replicates per dog food sample. In the *in vitro* large intestine fermentation, the total cumulative gas volume was recorded from the fermenter's calibrated scale at 24 h. For the *in vitro* true-organic matter disappearance (OMd) determination of dog foods, the *in vitro* fermenter incubation was stopped at 24 h. The *in vitro* Omd was determined by filtering the fermentation residues using a vacuum unit (Kara et al., 2019).

### Statistical analyses

The experimental data were first subjected to Levene's test to detect the variance homogeneity. The distribution of controlled samples was examined and was consistent with normal values. The multivariate analyses were implemented for homogeneous variances by General Linear Model procedures to test treatment differences. The one-way variance analysis was conducted to TDF, IDF, SDF,  $\beta$ -glucan, R-S, NR-S and T-S contents tested in dry-type adult dog foods. Data were analysed by the following statistical model:

$$Y_{ij} = \mu_{ij} + S_i + e_p$$

where:  $Y_{ij}$  – general mean for each feature investigated,  $\mu$  – mean of commercial dog food for each feature researched,  $S_i$  –  $i^{\text{th}}$  effect of different commercials on the observed features, and  $e_i$  – standard error value.

The means were separated by Tukey's multiple range test at  $P < 0.05$ . Linear relations among nutrient contents, and *in vitro* digestion values were determined using Pearson's correlation. Correlations among food quality and nutritional parameters were analysed using Spearman's correlation (SPSS 17.0 software, IBM Corp., Armonk, NY, USA).

## Results

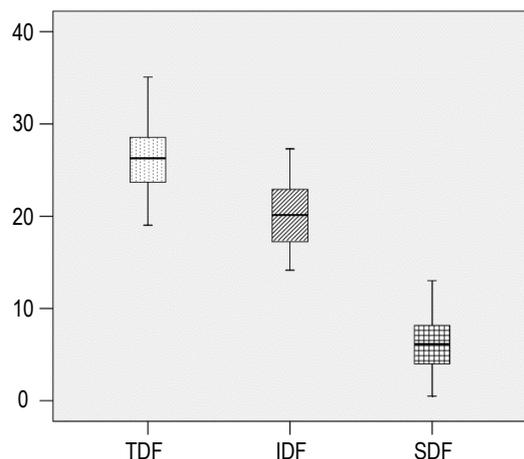
The carbohydrate and fat/oil ingredients of dry-type dog foods produced for large adult dogs are given in Table 1.

The TDF, IDF, SDF,  $\beta$ -glucan, R-S, NR-S and T-S contents (% DM) of dog foods are given in Table 2 and Figures 1 and 2. The TDF values of dog foods differed significantly among commercial brands ( $P < 0.05$ ). The average TDF value of 29 different dry-type dog foods produced for the large

**Table 2.** Total dietary fibre (TDF), insoluble dietary fibre (IDF), soluble dietary fibre (SDF),  $\beta$ -glucan, resistant starch (R-S), non-resistant (soluble) starch (NR-S) and total starch content (T-S) in dog food, % dry matter (DM)

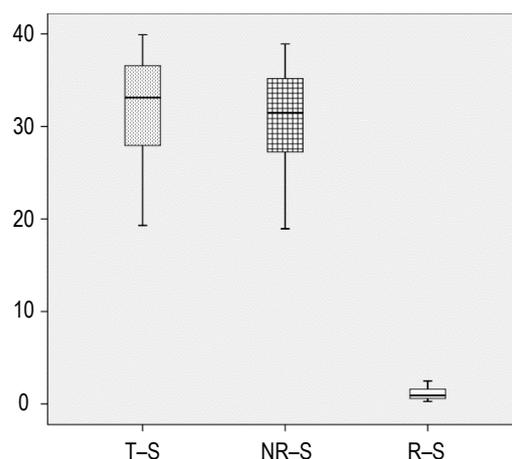
Dog food number	TDF	IDF	SDF	$\beta$ -glucan	R-S	NR-S	T-S
1	26.46 <sup>abcd</sup>	16.99 <sup>f</sup>	9.46 <sup>b</sup>	2.12 <sup>a</sup>	0.78 <sup>abcd</sup>	31.11 <sup>ab</sup>	31.90 <sup>ab</sup>
2	30.93 <sup>abc</sup>	24.50 <sup>abc</sup>	6.41 <sup>bc</sup>	0.06 <sup>d</sup>	0.74 <sup>abcd</sup>	26.47 <sup>b</sup>	27.21 <sup>ab</sup>
3	25.25 <sup>abcd</sup>	21.95 <sup>abodef</sup>	3.28 <sup>c</sup>	0.03 <sup>d</sup>	0.41 <sup>d</sup>	24.64 <sup>b</sup>	25.06 <sup>b</sup>
4	30.16 <sup>abcd</sup>	23.77 <sup>abcd</sup>	6.39 <sup>bc</sup>	0.05 <sup>d</sup>	1.58 <sup>abcd</sup>	36.75 <sup>a</sup>	38.33 <sup>a</sup>
5	25.22 <sup>abcd</sup>	14.66 <sup>f</sup>	10.48 <sup>ab</sup>	0.15 <sup>c</sup>	0.90 <sup>abcd</sup>	38.45 <sup>a</sup>	39.35 <sup>a</sup>
6	19.61 <sup>d</sup>	18.22 <sup>abodef</sup>	1.36 <sup>d</sup>	0.37 <sup>c</sup>	0.70 <sup>abcd</sup>	36.60 <sup>a</sup>	37.30 <sup>a</sup>
7	22.66 <sup>bcd</sup>	19.71 <sup>abodef</sup>	2.96 <sup>c</sup>	0.29 <sup>c</sup>	0.84 <sup>abcd</sup>	27.52 <sup>ab</sup>	28.36 <sup>ab</sup>
8	28.81 <sup>abcd</sup>	22.64 <sup>abode</sup>	6.14 <sup>bc</sup>	0.50 <sup>c</sup>	1.16 <sup>abcd</sup>	35.00 <sup>a</sup>	36.16 <sup>a</sup>
9	26.82 <sup>abcd</sup>	18.69 <sup>abodef</sup>	8.15 <sup>b</sup>	1.87 <sup>a</sup>	0.74 <sup>abcd</sup>	35.41 <sup>a</sup>	36.15 <sup>a</sup>
10	23.77 <sup>abcd</sup>	18.40 <sup>abodef</sup>	5.41 <sup>bc</sup>	0.01 <sup>d</sup>	1.77 <sup>abcd</sup>	36.19 <sup>a</sup>	37.97 <sup>a</sup>
11	28.72 <sup>abcd</sup>	25.22 <sup>ab</sup>	3.52 <sup>c</sup>	0.82 <sup>c</sup>	0.55 <sup>cd</sup>	24.77 <sup>b</sup>	25.33 <sup>b</sup>
12	26.91 <sup>abcd</sup>	26.39 <sup>a</sup>	0.51 <sup>d</sup>	0.75 <sup>c</sup>	0.31 <sup>d</sup>	21.06 <sup>b</sup>	21.37 <sup>b</sup>
13	24.30 <sup>abcd</sup>	22.55 <sup>abode</sup>	1.75 <sup>d</sup>	0.81 <sup>c</sup>	0.71 <sup>abcd</sup>	27.13 <sup>ab</sup>	27.85 <sup>ab</sup>
14	24.85 <sup>abcd</sup>	20.82 <sup>abodef</sup>	4.01 <sup>c</sup>	0.45 <sup>c</sup>	1.60 <sup>abcd</sup>	30.23 <sup>ab</sup>	31.84 <sup>ab</sup>
15	31.95 <sup>ab</sup>	16.76 <sup>f</sup>	15.15 <sup>a</sup>	1.34 <sup>ab</sup>	1.49 <sup>abcd</sup>	28.39 <sup>ab</sup>	29.88 <sup>ab</sup>
16	24.79 <sup>abcd</sup>	18.63 <sup>abodef</sup>	6.18 <sup>bc</sup>	1.61 <sup>ab</sup>	2.15 <sup>a</sup>	31.01 <sup>ab</sup>	33.17 <sup>ab</sup>
17	23.36 <sup>abcd</sup>	18.84 <sup>abodef</sup>	4.48 <sup>bc</sup>	1.51 <sup>ab</sup>	2.05 <sup>abc</sup>	34.33 <sup>a</sup>	36.38 <sup>a</sup>
18	27.14 <sup>abcd</sup>	19.30 <sup>abodef</sup>	7.79 <sup>b</sup>	0.04 <sup>d</sup>	0.61 <sup>bcd</sup>	30.11 <sup>ab</sup>	30.72 <sup>ab</sup>
19	25.02 <sup>abcd</sup>	15.52 <sup>f</sup>	9.46 <sup>b</sup>	0.07 <sup>d</sup>	0.83 <sup>abcd</sup>	35.67 <sup>a</sup>	36.49 <sup>a</sup>
20	25.28 <sup>abcd</sup>	20.62 <sup>abodef</sup>	4.66 <sup>bc</sup>	0.07 <sup>d</sup>	1.26 <sup>abcd</sup>	27.08 <sup>ab</sup>	28.34 <sup>ab</sup>
21	23.74 <sup>abcd</sup>	18.92 <sup>abodef</sup>	4.81 <sup>bc</sup>	0.26 <sup>c</sup>	2.08 <sup>ab</sup>	33.24 <sup>ab</sup>	35.32 <sup>a</sup>
22	23.48 <sup>abcd</sup>	19.39 <sup>abodef</sup>	4.11 <sup>bc</sup>	0.27 <sup>c</sup>	1.47 <sup>abcd</sup>	32.87 <sup>ab</sup>	34.35 <sup>a</sup>
23	28.48 <sup>abcd</sup>	24.81 <sup>abc</sup>	3.69 <sup>d</sup>	0.02 <sup>d</sup>	0.44 <sup>d</sup>	20.60 <sup>b</sup>	21.03 <sup>b</sup>
24	30.85 <sup>abc</sup>	23.72 <sup>abcd</sup>	7.15 <sup>b</sup>	0.55 <sup>c</sup>	0.33 <sup>d</sup>	22.87 <sup>b</sup>	23.20 <sup>b</sup>
25	33.53 <sup>a</sup>	23.09 <sup>abode</sup>	10.48 <sup>ab</sup>	0.30 <sup>c</sup>	1.60 <sup>abcd</sup>	29.85 <sup>ab</sup>	31.45 <sup>ab</sup>
26	28.17 <sup>abcd</sup>	21.00 <sup>abodef</sup>	7.20 <sup>b</sup>	0.26 <sup>c</sup>	0.72 <sup>abcd</sup>	35.85 <sup>a</sup>	36.57 <sup>a</sup>
27	21.02 <sup>cd</sup>	16.52 <sup>f</sup>	4.53 <sup>bc</sup>	0.44 <sup>c</sup>	1.31 <sup>abcd</sup>	34.74 <sup>a</sup>	36.05 <sup>a</sup>
28	27.60 <sup>abcd</sup>	14.58 <sup>f</sup>	12.97 <sup>a</sup>	0.09 <sup>d</sup>	1.10 <sup>abcd</sup>	32.95 <sup>ab</sup>	34.05 <sup>a</sup>
29	25.93 <sup>abcd</sup>	16.63 <sup>f</sup>	9.28 <sup>b</sup>	0.03 <sup>d</sup>	1.40 <sup>abcd</sup>	35.35 <sup>a</sup>	36.75 <sup>a</sup>
Average	26.37	20.09	6.27	0.52	1.09	30.90	32.00
SD	3.71	3.46	3.39	0.60	0.59	5.05	5.34
SEM	0.48	0.45	0.44	0.08	0.08	0.66	0.70
Minimum	19.02	14.14	0.50	0.01	0.28	18.95	19.30
Maximum	38.09	27.30	15.19	2.19	2.50	38.92	39.91
P-value	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SD – standard deviation of means, SEM – standard error of means; a-f – values with different superscripts in the same column are significantly different for each nutrient content



**Figure 1.** Dietary fibre content (% in DM) in commercial dry-type foods for large adult breed dogs

DM – dry matter, TDF – total dietary fibre, IDF – insoluble dietary fibre, SDF – soluble dietary fibre



**Figure 2.** Starch content (% in DM) in commercial dry-type foods for large adult breed dogs

DM – dry matter, T-S – total starch, NR-S – non-resistance starch, R-S – resistance starch

adult breeds was 26.37% ( $P < 0.05$ ). The lowest TDF (19.61%) value was observed in the sample no. 6 of dog food, and this food contained sugar beet pulp (2.6%) as a source of dietary fibre (due to the label). It was found that contents of IDF in the researched dog foods varied significantly among trademarks and ranged from 14.14 to 27.30% ( $P < 0.05$ ). The average IDF value of dog foods was found to be 20.09% DM. Among the dry-type extruded dog foods, the highest IDF value was found in the sample no. 12, whereas the lowest in the sample no. 28 ( $P < 0.05$ ). Comparing the 29 different commercial dry-type dog foods in terms of SDF content, it was determined that they contained on average 6.27% SDF and that there was a very wide range between the maximum (15.19%) and minimum (0.50%) values of SDF. The  $\beta$ -glucan contents of dry-type dog foods were 0.52% DM on average, and this value ranged from 0.01 (minimum) to 2.19% (maximum). It has been determined that there is a significant

difference in  $\beta$ -glucan value among quality and premium-type commercial food produced for adult large breed dogs ( $P < 0.05$ ). The average total starch content of dry-type dog foods was 32% DM, and there was a significant difference (21.03–39.35%) among the dog foods ( $P < 0.001$ ). Non-resistant starch contents in dog foods were higher than R-S values. Non-resistant starch value was found to be 30.9% (on average) and ranged from a minimum of 21.06% to a maximum of 38.45% ( $P < 0.001$ ). In the present study, the R-S content was found to vary with a minimum of 0.33% and a maximum of 2.16% in the dry-type dog foods ( $P < 0.001$ ).

Pearson's correlations between the dietary fibre and starch substances of dog foods and the *in vitro* Omd and gas production values of dog foods are given in Table 3. In the study, the TDF value of the extruded dry-type dog foods was positively moderate correlated with the IDF and SDF values of the dog foods; negatively low correlated with NR-S, T-S and

**Table 3.** Pearson's correlations between the dietary fibre and starch substances and the *in vitro* true-organic matter disappearance (OMd) and gas production (GP) values in dog foods

	IDF	SDF	$\beta$ -glucan	R-S	NR-S	T-S	<i>in vitro</i> Omd	<i>in vitro</i> GP
TDF	0.393**	0.448**	0.045	-0.145	-0.307*	-0.307*	0.154	-0.323*
IDF	1	-0.481**	-0.116	-0.275*	-0.658*	-0.652**	-0.066	-0.289*
SDF		1	0.186	0.160	0.315*	0.316*	0.290*	-0.115
$\beta$ -glucan			1	0.262*	0.009	0.027	0.186	0.221*
R-S				1	0.429**	0.519**	0.001	0.200
NR-S					1	0.995**	-0.067	0.519**
T-S						1	-0.064	0.516**
<i>in vitro</i> Omd							1	-0.110

TDF – total dietary fibre as %, IDF – insoluble dietary fibre as %, SDF – soluble dietary fibre as %, R-S – resistant starch as %, NR-S – non-resistant starch as %, T-S – total starch as %, *in vitro* Omd – *in vitro* true-organic matter disappearance of 0.3 g dog food dry matter (DM) at 30 h incubation (*in vitro* gastric digestion + small intestine digestion + large intestine fermentation), *in vitro* GP – *in vitro* total gas production of 0.3 g dog food DM at 30 h incubation (see *in vitro* Omd); \*\*, \* – correlation is significant at the 0.01 level, \* – correlation is significant at the 0.05 level

GP values. The IDF value of dog food was negatively correlated with SDF (moderate), R-S (low), NR-S (high), T-S (high) and GP (low). The SDF value of the extruded dog foods was positively low correlated with NR-S, T-S, OMD values and food quality ( $P < 0.05$ ). The  $\beta$ -glucan value of dog foods was positively low correlated with the R-S and GP values of foods. The R-S value of dog foods was positively moderate correlated with NR-S and T-S values of foods ( $P < 0.001$ ). The NR-S value of dog foods was positively correlated with T-S (high) and GP (moderate) ( $P < 0.001$ ). The T-S value was positively correlated with GP value (moderate) of dog food ( $P < 0.05$ ).

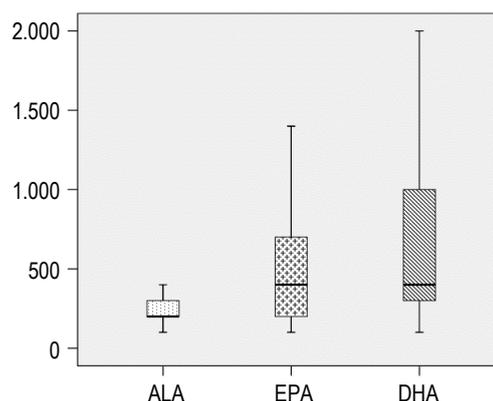
Percentages of fatty acids in total fatty acids (g/100 g DM) of different commercial dog foods are given in Table 4 and Figure 3. The average oleic acid (C18:1;  $\omega$ -9) level in DM of dry-type dog food was 4.18%, the linoleic acid (C18:2;  $\omega$ -6) level was 2.21% and the ALA (C18:3;  $\omega$ -3) level was 0.03%. The arachidonic acid (C20:4;  $\omega$ -6) level was 0.04% and the EPA + DHA (C20:5,  $\omega$ -3; C22:6,  $\omega$ -3) level was 0.11%. The total fatty acids were 4.84% MUFA, 2.52% PUFA, 0.17%  $\omega$ -3 fatty acids, 2.35%  $\omega$ -6 fatty acids, 4.36%  $\omega$ -9 fatty acids, 0.08% MCFA, 10.90% LCFA and 0.19% VLCFA. The  $\omega$ -6/ $\omega$ -3 ratio of dog foods was 19.34.

Pearson correlations between fatty acids percentages and the dog food quality, *in vitro* OMD and *in vitro* GP values are presented in Table 5.

**Table 4.** Content of fatty acids in different commercial dog foods, g/100 g DM

Fatty acids	Mean	SD	SEM	Minimum	Maximum
C18:1	4.18	0.83	0.10	2.43	6.10
C18:2	2.21	0.69	0.09	0.47	3.57
C18:3	0.03	0.01	0.001	0.01	0.05
C20:4	0.04	0.05	0.01	0.00	0.27
C20:5	0.05	0.05	0.01	0.01	0.23
C22:6	0.06	0.07	0.01	0.00	0.34
$\omega$ -3	0.17	0.13	0.01	0.04	0.62
$\omega$ -6	2.35	0.64	0.08	0.81	3.68
$\omega$ -9	4.36	0.87	0.11	2.63	6.44
$\omega$ -6/ $\omega$ -3	19.34	9.85	1.29	4.34	40.10
MUFA	4.84	1.01	0.13	2.92	7.29
PUFA	2.52	0.70	0.09	0.86	3.87
MCFA	0.08	0.17	0.02	0.01	1.04
LCFA	10.90	1.91	0.25	5.91	15.52
VLCFA	0.19	0.12	0.02	0.06	0.52

C18:1 – oleic acid, C18:2 – linoleic acid, C18:3 –  $\alpha$ -linolenic acid, C20:4 – arachidonic acid, C20:5 – eicosapentaenoic acid, C22:6 – docosahexaenoic acid,  $\omega$ -3 – total omega 3 fatty acids,  $\omega$ -6 – total omega 6 fatty acids,  $\omega$ -9 – total omega 9 fatty acids,  $\omega$ -6/ $\omega$ -3 – ratio of total omega 6 fatty acids and total omega 3 fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, MCFA – medium-chain fatty acids, LCFA – long-chain fatty acids, VLCFA – very long-chain fatty acids, DM – dry matter, SD – standard deviation of means, SEM – standard error of means



**Figure 3.** The  $\omega$ -3 fatty acid level in commercial dog foods (in mg/kg DM)

DM – dry matter; ALA – C18:3,  $\alpha$ -linolenic acid; EPA – C20:5, eicosapentaenoic acid; DHA – C22:6, docosahexaenoic acid

**Table 5.** Correlations between fatty acids percentages and the dog food quality, *in vitro* true-organic matter disappearance (OMd) and *in vitro* total gas production (GP) values

Fatty acids	Spearman correlation ( <i>r</i> ) dog food quality <sup>#</sup>	Pearson correlation ( <i>r</i> )	
		<i>in vitro</i> OMD	<i>in vitro</i> GP
C14:0	0.264*	-0.033	-0.059
C16:0	0.099	-0.201	-0.244*
C16:1	0.021	0.049	-0.563**
C18:0	0.208	-0.316*	0.145
C18:1	0.152	-0.089	-0.280
C18:2	-0.392**	0.262	-0.201
C18:3( $\alpha$ )	0.460**	-0.131	-0.203
C18:3( $\gamma$ )	0.129	-0.076	0.162
C20:4	0.456**	0.055	-0.208
C20:5	0.278*	0.280	-0.215
C22:6	0.290*	0.328*	-0.166
$\omega$ -3	0.245	0.390**	-0.156
$\omega$ -6	-0.284*	0.254	-0.280
$\omega$ -9	0.182	-0.070	-0.283
$\omega$ -6/ $\omega$ -3	-0.178	-0.378**	0.146
SFA	0.194	-0.210	-0.103
UFA	0.015	0.117	-0.403**
MUFA	0.170	-0.053	-0.341*
PUFA	-0.216	0.314*	-0.287*
MCFA	0.070	0.128	0.121
LCFA	0.090	-0.067	-0.335*
VLCFA	0.252	0.376**	-0.010

C14:0 – myristic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2 – linoleic acid, C18:3 ( $\alpha$ ) –  $\alpha$ -linolenic acid, C18:3 ( $\gamma$ ) –  $\gamma$ -linolenic acid, C20:4 – arachidonic acid, C20:5 – eicosapentaenoic acid, C22:6 – docosahexaenoic acid,  $\omega$ -3 – total omega 3 fatty acids,  $\omega$ -6 – total omega 6 fatty acids,  $\omega$ -9 – total omega 9 fatty acids, LCFA – long chain fatty acids, MCFA – medium-chain fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids, UFA – unsaturated fatty acids, VLCFA – very long-chain fatty acids; SD – standard deviation of means, SEM – standard error of means; \* – correlation is significant at the 0.05 level, \*\* – correlation is significant at the 0.01 level; <sup>#</sup> – commercial dry dog foods were classified basing on the definition given by producers as: high quality (HQ), premium (P), high-premium (HP), super-premium (SP), ultra-premium (UP)

The food quality (from high quality to ultra-premium type foods) was positively correlated with C14:0, C18:3, C20:4, C20:4, C20:5 and C22:6  $\omega$ -3 fatty acids (in dog food DM). On the other hand, the food quality was negatively correlated with C18:2 fatty acids ( $P < 0.05$ ). The OMD levels of dog foods were negatively correlated with C18:0 fatty acids content in dog food; and positively correlated with C22:6, PUFA,  $\omega$ -3 and VLCFA fatty acids ( $P < 0.05$ ). The *in vitro* GP level of dog foods was negatively correlated with C16:0, C16:1, UFA, MUFA, PUFA and LCFA fatty acids ( $P < 0.05$ ).

Carbohydrate components and *in vitro* digestion values in dog food according to quality classes are given in Table 6. In the current study, there was no difference among the IDF, R-S, *in vitro* GP and *in vitro* OMD values of high quality, premium, high-premium, super-premium and ultra-premium quality dog foods ( $P > 0.05$ ); TDF, SDF,  $\beta$ -glucan, NR-S and T-S values were found to differ among the dog food brands ( $P < 0.05$ ).

chicory root or whole grain) were used in the examined dog foods according to the labels. The end products of the TDF fermentation are used by different body tissues as a source of energy. For example, acetate can be used by the liver and muscle, and propionate by the liver to produce glucose (Voet et al., 2016). Butyrate is an obligate fuel for the colonocytes (Hamer et al., 2008) and has a trophic effect on colonic tissues' development (Voet et al., 2016). The rate of fermentation and the amount of each SCFA is dependent on the dietary fibre source (Sunvold et al., 1995; Guevara et al., 2008). The rate of fermentation and the amount of each SCFA, which is produced from TDF in the large intestine, is dependent on the dietary fibre source (Sunvold et al., 1995; Guevara et al., 2008). In the present study, the digestion levels of these dietary fibre sources in the large intestine may also differ. It is declared in the label information that some of the examined dog foods contain a single type of fibre source and some contain multiple fibre sources.

**Table 6.** Carbohydrate components and *in vitro* digestion values in dog foods according to quality classes

	TDF, %	IDF, %	SDF, %	$\beta$ -glucan, %	R-S, %	NR-S, %	T-S, %	<i>In vitro</i> OMD, %	<i>In vitro</i> GP
Food quality									
HQ	25.93 <sup>ab</sup>	21.28	4.89 <sup>b</sup>	0.40 <sup>b</sup>	0.91	30.08 <sup>b</sup>	31.00 <sup>b</sup>	87.59	102.84
P	22.44 <sup>b</sup>	19.42	3.01 <sup>b</sup>	0.22 <sup>b</sup>	0.98	31.84 <sup>b</sup>	32.82 <sup>b</sup>	85.37	142.08
HP	26.08 <sup>ab</sup>	19.83	6.55 <sup>ab</sup>	1.15 <sup>a</sup>	1.26	30.53 <sup>b</sup>	31.79 <sup>b</sup>	89.18	105.78
SP	28.49 <sup>a</sup>	18.54	9.92 <sup>a</sup>	0.13 <sup>b</sup>	1.31	34.67 <sup>a</sup>	35.98 <sup>a</sup>	87.31	82.13
UP	27.29 <sup>ab</sup>	21.92	5.37 <sup>b</sup>	0.37 <sup>b</sup>	0.81	28.30 <sup>b</sup>	29.11 <sup>b</sup>	85.65	100.63
SD	3.71	3.52	3.39	0.61	0.59	5.05	5.34	3.45	40.67
SEM	0.48	0.47	0.44	0.08	0.07	0.66	0.70	0.49	5.87
P-value	0.047	0.213	<0.001	<0.001	0.173	0.044	0.046	0.118	0.373

TDF – total dietary fibre as %, IDF – insoluble dietary fibre as %, SDF – soluble dietary fibre as %, R-S – resistant starch as %, NR-S – non-resistant starch as %, T-S – total starch as %, *in vitro* OMD – *in vitro* true-organic matter disappearance of 0.3 g dog food dry matter (DM) at 30 h incubation (*in vitro* gastric digestion + small intestine digestion + large intestine fermentation), *in vitro* GP – *in vitro* total gas production of 0.3 g dog food DM at 30 h incubation (see *in vitro* OMD); Food quality classes: HQ – high quality, P – premium, HP – high-premium, SP – super-premium, UP – ultra-premium; SD – standard deviation of means, SEM – standard error of means; <sup>ab</sup> – values with different superscripts in the same column show significant difference for each nutrient content

## Discussion

Total digestible fibre contents in dog food differed significantly between trademarks and ranged from 19.02 to 38.09% DM. The average TDF value was 26.37% DM. This wide range of TDF levels differs fermentation level in the large intestine of dogs, which can change the digestion level of fibre compounds in the food, chyme viscosity and large intestine fermentation (Cho and Dreher, 2002). It is understood that different fibre sources (whole grain, sugar beet pulp, apple pulp, seaweed and

In the present study, depending on the digestibility of TDF in feed materials, which are the source of fibrous compounds, there was a difference in reaching a healthy colonic fermentation (Hamer et al., 2008; Voet et al., 2016).

Insoluble-digestive fibre, which is a non-fermented carbohydrate in the TDF group, contributes directly to faecal bulking and reduces intestinal transit time (NRC, 2006). In dog food IDF contents differed significantly among brands and ranged from a minimum of 14.14% to a maximum of 27.30% in DM, the average value was 20.09% DM. The highest

IDF value for the dry-type extruded dog foods was determined in the sample no. 12. The lowest IDF for the dry-type extruded dog foods was noted in the sample no. 28. In the present study, the highest SDF content (13%) in the sample no. 28 of dog food can be explained by the fact that it contained apple pulp – the most important fibre carbohydrate component. This feed ingredient increases the SDF content and decreases the IDF content in dog food (NRC, 2006).

Carbohydrates, especially absorbed and enzymatically digested, positively affect the *in vitro* feed digestion and *in vitro* GP value (Calabrò et al., 2013; Kara et al., 2019). The contents of IDF in dog foods in the present study were negatively correlated with dog food *in vitro* GP as were expected. In the present study, NR-S and T-S contents in dog foods were positively correlated with *in vitro* GP values of dog foods and were in line with our previous results (Kara et al., 2019). It was expected that easily digested carbohydrates would positively affect *in vitro* GP value (Sunvold et al., 1995; Guevara et al., 2008). In a previous study (Kara et al., 2019), it was determined that the *in vitro* GP levels (92–97 ml/0.3 g DM) of the extruded forms of the feedstuffs with high NR-S and high T-S contents (rice and wheat) at the 24-h incubation in adult dogs (2 years of age) were lower than in raw materials with higher starch content.

The average SDF value in 29 different commercial dry-type dog foods in the present study was 6.27% DM. Dog foods have an extensive SDF value (ranging from 0.50 (minimum) to 15.19% (maximum) in DM), resulting from the difference in the types of IDF and SDF sources in food components and their contents. Dog food samples (6, 12 and 13), which had the lowest SDF values, included whole wheat, whole maize, wheat bran and sugar beet pulp as feedstuffs with high fibrous content. According to the label information, the highest SDF content (>9% DM) was present when apple pulp, oat (whole grain), quinoa, seaweed and chicory root (inulin) feedstuffs were added to the food. It was also observed that the whole grains (such as oats, wheat), sugar beet pulp, tomato pulp, apple pulp, wheat bran, rice bran, quinoa husk, alfalfa meal, pea husk, carrot, pumpkin, seaweed, chicory root and psyllium husk have been widely used as TDF sources in dog foods formulation. Apart from that, beet pulp, quinoa husk, psyllium husk, tomato pulp, seaweed, chicory root, dried pomegranate and dried orange are used in dog foods as soluble fibre sources. Seaweed, which was used in some dog foods, contains a high percentage of SDF, with an average of 24.5% SDF and 21.8% IDF. The SDF/IDF ratio in the TDF content of sea-

weed is greater than the values observed in terrestrial plants (Peñalver et al., 2020). Another important SDF source is sugar beet pulp, containing approximately 20% SDF in DM and mostly consisting of pectin (Klopfenstein, 1990). On the other hand, apple pulp is a balanced source of dietary fibre with 51% TDF, 36% IDF and 15% SDF in DM (Sudha et al., 2007). The average SDF/IDF ratio (6.27/20.09) of dry-type dog foods was 0.31. In the present study, SDF/IDF values of samples no. 5, 15, 19 and 28 were 0.71, 0.90, 0.61 and 0.89, respectively. High SDF/IDF ratios in some dog foods can be connected with the addition of sugar beet pulp, psyllium, seaweed and apple pulp (containing high SDF levels, according to the producer information).

$\beta$ -glucan, a type of fibre that has been widely investigated by scientists in recent years, is a glucose polymer found in the cell walls of grains (oats, barley), certain types of mushrooms (Reishi, shiitake, maitake), yeast and seaweed.  $\beta$ -glucans are structural components of cell walls in many different sources such as bacteria, fungi, algae, yeast and grains (barley, oats and rye). In the present study, the positive correlation between  $\beta$ -glucan and *in vitro* GP may be caused by the soluble and fermentable properties of this carbohydrate (El Khoury et al., 2012). The structure of  $\beta$ -glucans differs according to sources and explains the differences in their physiological functions (Jacob and Pescatore, 2014). In the present study, it has been determined that  $\beta$ -glucans were present at an average rate of 0.52%, and this value ranged from 0.01 (minimum) to 2.19% (maximum) in DM in dry-type extruded commercial dog foods. Besides, there was a significant difference in  $\beta$ -glucan values among the quality-class and premium-class dog foods produced for adult large breed dogs. The dog foods that contained lower than average value (0.52%) of  $\beta$ -glucan and had the lowest  $\beta$ -glucan value (0.015–0.065%) were numbered as samples no. 2, 3, 4, 10, 18, 19, 20, 23 and 29. The information on the labels was that they do not contain feed substances with high  $\beta$ -glucan levels.  $\beta$ -glucan (soluble fibre) and R-S are prebiotic carbohydrates. The fact that the R-S content in dog food was positively correlated with the  $\beta$ -glucan content can arise from using the same carbohydrate sources containing high R-S and high  $\beta$ -glucan (such as oat and barley grains) in dog foods.

Starch granules' structure also affects the enzymatic/fermentative digestion of starch in the digestive cannula (Kara et al., 2019). The level of resistant starch and non-resistant starch should be well adjusted in the diet. The average total starch

value of dry-type dog foods was 32% in DM and showed a significant difference among brands (21.03–39.35%). Non-resistant (soluble) starch contents in dog foods were higher than the R-S contents. Non-resistant starch values were found to be an average of 30.9% and ranged from a minimum of 21.06% to a maximum of 38.45%. In some dry-type dog foods, the total starch contents were above 35%, which was connected with the addition of high amounts of potato flour, rice, maize, sorghum, barley or oats.

Tubers (such as potatoes, sweet potatoes and tapioca) and legumes (such as peas) are commonly used ingredients in dog diets and are known to show some, partial resistance to  $\alpha$ -amylase ingestion. The reason for this resistance is the lack of starch granule pores in tubers and legumes, and the pores and channels that increase the surface area for enzyme adsorption in most cereal starches (Dhital et al., 2017; Martens et al., 2018). However, most legumes also have a protein matrix tightly bound with starch granules that form a physical barrier to enzymatic digestion (Berg et al., 2012; Dhital et al., 2017). Besides, C-type starch found in legumes has a lower swelling capacity than cereals or tubers (Wani et al., 2016) and a higher amylose content (Martens et al., 2018), which contributes to enzymatic resistance. In the present study, the contents of resistant starch in dog foods were found to range from 0.33 to 2.16% DM. According to the label information, dog foods (samples no. 3, 11, 12, 23 and 24) with low R-S ( $\leq 0.5\%$ ) contents contained potato flour (sweet potato, potato starch), pea, some grain flours, oats and wheat as a starch source. In a previous study, it was found that commercial extruded dog and cat foods had low R-S contents and R-S contents even lower than 1% in total starch content (0.703% in cat foods vs 0.945% in dog foods) (Alvarenga and Aldrich, 2020). In the present study, the R-S contents of dry-type extruded dog foods were higher than 1.1% in 14 dog food samples (no. 4, 8, 10, 14, 15, 16, 17, 20, 21, 22, 25, 27, 28 and 29) or over 1.4% in 10 dog food samples (no. 4, 10, 14, 15, 16, 17, 21, 22, 25 and 29). However, low contents of R-S ( $< 1\%$  DM) in other dog foods in the present study may not be sufficient to improve colon health (Peixoto et al., 2018; Alvarenga and Aldrich, 2020). Peixoto et al. (2018) identified that 1.46% R-S in dog food was positively correlated with colonic fermentation, and this R-S content increased butyrate production and improved nutrient absorption. Another beneficial effect of R-S can decrease the glycaemic index of food (Kimura, 2013), which lowers the

rate of insulin release and positively affects health. This can help reduce the obesity and incidence of insulin resistance. Dogs readily develop insulin resistance and hyperglycemia in obesity, but this does not in 100% meet the clinical definition of type 2 diabetes (fasting blood glucose most often remains within normal limits in dogs). In obese dogs, blood insulin levels are higher after feeding to keep glucose under control and peak glucose after glucose challenge may be higher, but generally fasting glucose in obese dogs does not reach the clinical threshold to achieve the definition of diabetes. Thus hyperinsulinemia and impaired glucose tolerance is common in obese dogs, not type 2 diabetes. Having said that, chronic intermittent hyperglycemia does have major negative health impacts in dogs (Fleeman and Rand, 2001; Catchpole et al., 2013).

There was no difference between IDF, R-S, *in vitro* GP and *in vitro* OMD values of high quality, premium, high-premium, super-premium and ultra-premium quality dog foods in the present study. Therefore, there was no difference among the TDF, SDF,  $\beta$ -glucan, NR-S and T-S values among dog foods classes.

Dietary fats in pet foods are both a source of essential nutrients, energy and flavour. There are ways to increase pet food palatability, such as mixing dietary fats with food raw materials or applying it to the surface of dry-pelleted dog food (Case et al., 2011). The fat/oils in dog foods comprise animal, vegetable or both (Kara, 2020a). Fatty acids such as linoleic acid, arachidonic acid, ALA, EPA and DHA in dogs and cats are used as precursors of leukotrienes, prostaglandins and thromboxane. They have activities on blood coagulation (thromboxane), protecting biological membranes (such as skin diseases), the nervous system and vision. Although the mechanism cannot be fully explained, PUFAs also have cellular protective properties against injuries and epithelial degeneration (Walters et al., 2010). Some complete dry dog food labels state that they contain a balanced combination of  $\omega$ -6 and  $\omega$ -3 fatty acids. The ALA can be converted to other  $\omega$ -3 fatty acids, especially EPA and DHA in the dog organism (Beynen, 2020). The average  $\omega$ -3 ALA content in dog foods in the present study was 0.03 g/100 g DM. This ALA content ranged from 0.01 to 0.05 g/100 g DM. It was so determined that the ALA content was below the recommended level in food for dogs at growth and reproduction stages, fed at the maintenance level (FEDIAF, 2020). The EPA (0.01–0.23%) and DHA (0.0%–0.34%) fatty acid levels of the dog foods

varied widely. It was found that EPA and DHA contents were high in dog foods containing fish oil. The EPA+DHA levels of dog foods in the present study were below recommended minimum levels recommended by FEDIAF (2020) and NRC (2006). It was reported that the minimum EPA+DHA requirements of dogs fed at the maintenance level were 0.05 g/100 g DM (NRC, 2006; FEDIAF, 2020). The levels of linoleic acid (C18:2;  $\omega$ -6) and arachidonic acid (C20:4;  $\omega$ -6), among the  $\omega$ -6 fatty acids recommended to be present at a certain level in dog foods with essential properties, were below the level of need in some samples. It was determined that along with the increase in food quality (ultra-premium-quality class), the level of essential  $\omega$ -3 fatty (ALA, DHA, EPA) acids increased. This shows that pet owners can prefer super-premium or ultra-premium foods because of their essential  $\omega$ -3 fatty acids content. However, the decrease in the level of  $\omega$ -6 linoleic acid in dog food associated with the increase in dog food quality in the study is not as positive as it should be. The negative correlation between the palmitic acid (C16:0) and stearic acid (C18:0) levels and the *in vitro* OMD and GP value of dog food is consistent with the previous study results (Yuangklang et al., 2016).

According to the results of this study, the fact that the essential fatty acids in some quality and premium class dog foods are below the required level (NRC, 2006; FEDIAF, 2020) may be due to reasons such as the insufficient addition of  $\omega$ -3 and  $\omega$ -6 fatty acid sources to the food, the oxidation of fatty acids depending on the shelf life, and the insufficiency of antioxidants in the food (Case et al., 2011; Hillestad, 2018).

## Conclusions

It was found that the resistant starch and  $\beta$ -glucan levels in some dog foods differed among commercial dry dog foods. It has been observed that some dog foods are insufficient in essential oils EPA+DHA, linoleic acid and  $\alpha$ -linolenic acid, and that some dog foods contained sufficient levels according to NRC and FEDIAF. Insoluble fibre, palmitic acid and palmitoleic acid in dog food adversely affected *in vitro* gas production. Although *in vitro* digestibility of dog food is adversely affected by the increase in insoluble dietary fibre and palmitic and palmitoleic acids contents; *in vitro* digestibility of dog food is positively affected by the increase in soluble dietary fibre and stearic acid contents.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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