https://doi.org/10.17221/139/2019-VETMED

Determination of the *in vitro* digestibility and nutrient content of commercial premium extruded foods with different types of protein content for adult dogs

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Citation: Kara K (2020): Determination of the *in vitro* digestibility and nutrient content of commercial premium extruded foods with different types of protein content for adult dogs. Vet Med-Czech 65, 233–249.

Abstract: The purpose of this study was to compare the in vitro digestibility levels and chemical compositions of commercial extruded dry-type adult dog foods with different types of protein contents [fish meat (F-dog foods) (n = 7), lamb meat (L-dog foods) (n = 9), or poultry meat (P-dog foods) (n = 8)]. The in vitro digestion values of premium commercial dog foods were examined at three stages: gastric digestion, small intestine digestion and large intestine digestion/fermentation. The metabolisable energy (ME), crude protein (CP), starch, diethyl ether extract (EE) and ash contents and the in vitro cumulative gas production values of all the premium dog foods differed significantly among the commercial brands in the same category (F-, L- or P-dog foods) ($P < 0.05$). The crude fibre (CF) and the CP/1 000 kcal ME values of the F- and P-dog foods demonstrated a significant difference among the commercial brands ($P < 0.05$). The organic matter disappearance (OMd) values of the L-dog foods showed a significant difference among the commercial brands ($P < 0.05$); but the OMd values of the F- and P-dog foods did not differ among the commercial brands ($P > 0.05$). The average values of the OMd for the F-dog foods were more rapid than the average for the L- and P-dog foods, in the evaluation of all the foods ($P = 0.001$). Besides, the price of the L-dog foods was positively correlated with the OMd and CP of the L-dog foods; however, it was negatively correlated with the NFE (nitrogen free extract) and CHO (total carbohydrates) of the L-dog foods ($P < 0.05$). The CP values of the L-dog foods were positively correlated with the OMd values ($P < 0.05$). Although price is an important determinant of food quality in the L-dog foods, it is not in the F- and P-dog foods. In the general evaluation of all the dog foods, there was no correlation among the food price and the digestibility and the nutrient content for all of the premium dog foods. The present study indicated that the energy, nutrient matter and digestibility of premium dog foods changed with the change in the variety and the amount of the feedstuffs. The digestibility of the dog foods with the fish meat were higher than those of the other dog foods. The amount of protein that an adult dog will receive with 1 000 kcal of DM (dry matter) consumption of premium dog foods with fish meat and chicken meat, varied among the brands. This point showed the need to pay attention to the food consumption amount of the dogs and the energy-protein balance in their diets, especially dog foods with fish meat and chicken meat.

Keywords: carnivore; chemical composition; dog food quality; dog food type; purchase

Supported by the Research Fund of Erciyes University (Project No.: TSA-2019-9607).

https://doi.org/10.17221/139/2019-VETMED

Dogs are closer to being omnivorous animals than cats, which are obligate carnivores due to the anatomy of their gastrointestinal tract and digestive enzyme activities. Thus, dog diets may contain more carbohydrates and less protein than feline diets (Case et al. 2011). The recommended minimum maintenance protein requirement for adult dogs based on the maintenance energy requirements (MER) of 110 kcal/kg$^{0.75}$ and 95 kcal/kg$^{0.75}$ are 18% and 21% in dry matter (DM), respectively (FEDIAF 2018). These levels mean that high-quality protein is more than 16% (about 2 g/kg body weight/day) of the dietary energy. The protein needs of dogs increase during the growth period, the end of pregnancy and in lactation periods, and they reach 25–30% of the diet energy (Buffington et al. 2004). According to the NRC National Research Council – NRC (2006), it has been reported that dog diets with a density of 4 000 kcal ME/kg (dry matter basis, DM) containing high quality protein should contain a minimum of 180 g/kg protein (basis DM). According to the Association of American Feed Control Officials – AAFCO (2015), an adult dog food provides 18% of the ME in the diet for the maintenance from the protein and provides 22.5% of that for the growth and reproduction period from the protein.

Dry-type dog food is more preferred by pet owners due to the ease of stocking and ease of presentation to the dogs (Daumas et al. 2014). Some terms used in dog and cat food labels can be decisive in the purchase of food by the animal owner. These are commercial brands called generic, high-quality (premium, super- and ultra-premium) and economic brands as the most common, and also basic-nutrition brands, natural, holistic, organic and grain-free (Heinze 2016). Besides, to supply the optimum requirements of their dogs, dog owners prefer to keep food for their pet's long-term health (Daumas et al. 2014). The sale of premium dog food all over the world tends to increase. The share of premium foods in the total pet food sales ($12.9 billion) for the US in 2001 was 44% ($5.7 billion). This rate, for 2015, reached 61% ($14.5 billion) in premium pet food in the total pet food sales ($23.7 billion) (Heinze 2016). Pet owners are more interested in premium food, though the price is higher than that of economic brands. According to the NRC (2006), basic-nutrition brands and economic brands meet the energy, protein and other essential nutrient requirements of the dogs. However, the expression "premium" for commercial dog foods is supposed to be used for foods which include fat, protein and fish oil and other essential nutrients higher than the minimum requirements and have excellent digestibility.

Nevertheless, the formulations and nutrient contents of commercially available company-produced dog foods are highly variable. Presumably, the digestion of these dog foods will also be variable (Hervera et al. 2007). Depending on the raw material contents of the commercial foods and the pre-treatment applied to the foods (extruded, dry or watery-canned type), the fermentation levels of these foods in the digestive tract will also vary widely.

Pet foods include four main nutrient groups: carbohydrates, protein, fat, and minerals (Case et al. 2011). Premium dog foods may include high qualities and quantities of these substances. The dry-type premium dog food sector is usually marketed as highlighting the different protein sources (lamb meat, fish meat, chicken meat, etc.) (Buffington et al. 2004). Generally, different protein sources in premium dog food have used various carbohydrate sources (lamb meat and rice, fish meat and potato, etc.). Besides, the fat and fibre substances of dog foods are the other important components of the dog food (Earle et al. 1998; Carciofi et al. 2006). However, protein sources are the most important cost-forming element in premium dog foods. Commercial premium dog foods are produced with different extruder conditions and they include different protein sources with different carbohydrate sources that may be of or have varied digestibility, energy value, protein and other nutrient compositions (Crane et al. 2010; Rokey et al. 2010).

Premium dog food classified according to the protein source appears to be more realistic to determine the protein content and digestibility. Some previous researchers studied the nutritional values of commercial dog foods according to their economic type (standard, premium, etc.) (Carciofi et al. 2006) or all dog foods in general (not further classified) (Earle et al. 1998; Urrego et al. 2017). Today, there is not enough up-to-date information on the *in vitro* digestion levels of premium dog food classified by the protein sources in literature and international scientific committees [AAFCO and FEDIAF (The European Pet Food Industry)], especially in the NRC (2006). The hypothesis of the present study is that the nutrient

https://doi.org/10.17221/139/2019-VETMED

content and digestibility of premium dry-type dog foods containing different animal protein sources will be different. In this study, we aimed to determine the nutrient content of 24 different extruded premium dry-type dog foods, prepared for adult dogs, based on fish meat, lamb meat or poultry meat as a source of animal protein, and the *in vitro* digestion levels using three-stage digestion techniques. Moreover, we also aimed to compare the nutrient composition and digestion indicators with the sale price in the dog food market.

MATERIAL AND METHODS

The premium dog foods

The commercial extruded dry-type premium dog foods in the present study were produced for adults (> 2 years of age) of large breed dogs (such as German Shepherds, Labrador Retrievers, Golden Retrievers and Belgium Malinois). All the extruded foods were purchased from a Distributor Company for dog foods (Istanbul, Turkey). It has been reported that the foods have been kept in the appropriate warehouse conditions until the time of the sale. The commercial premium dog foods were categorised according to the animal protein included (fish meat/by-products (F-dog food), lamb meat/by-products (L-dog food) and poultry meat/by-products (P-dog food) (Table 1).

Chemical analyses of the premium dry type dog foods

In the study, different premium type dog foods were analysed to determine the DM, ash content, CP, EE, and CF composition using the Association of American Feed Control Officials (AOAC 1990) methods. The nitrogen-free extract was calculated according to the formulation:

$$\text{NFE (\%)} = \text{DM\%} - (\text{EE\%} + \text{CP\%} + \text{ash\%} + \text{CF\%}) \quad (1)$$

where:
NFE – nitrogen-free extract;
DM – dry matter;
EE – diethyl ether extract;
CP – crude protein;
CF – crude fibre.

The total starch contents of the foods were analysed using Megazyme assay (Megazyme, cat. No. K-TSTA-100A) procedures (Bray Business Park, Bray, Co. Wicklow, Ireland) (Hall 2015). Thermostable α-amylase hydrolyses starch into soluble, branched and unbranched maltodextrins. The resistant starch in the sample is pre-dissolved by stirring the sample with cold 1.7 NaOH, followed by neutralisation with a sodium acetate buffer and hydrolysis with α-amylase. Amyloglucosidase (AMG) quantitatively hydrolyses maltodextrins to D-glucose. D-Glucose is oxidised to D-gluconate with the release of equimolar amounts of hydrogen peroxide (H_2O_2) which is quantitatively measured in a colorimetric reaction employing peroxidase and the production of a quinoneimine dye:

$$\text{Starch (\%)} = \Delta A \times F \times EV \times D/W \times 0.90 \quad (2)$$

where:
ΔA – absorbance of the sample solution read against the reagent blank;
F – factor to convert the absorbance values to mg glucose;
EV – sample extraction volume;
D – further dilution of the sample solution;
W – sample weight in mg.

The total carbohydrates (CHO) were calculated using the following formula:

$$\text{CHO (\%)} = \text{CF (\%)} + \text{NFE (\%)} \text{ (as DM)} \quad (3)$$

where:
CHO – carbohydrates;
CF – crude fibre;
NFE – nitrogen-free extract.

The metabolic energy (ME) levels of the extruded dry type dog foods were calculated according to the NRC (2006), using the following 4-step-calculation formula:

I. Calculate the gross energy (GE): GE (kcal) = (5.7 × CP%) + (9.4 × EE%) + [4.1 × (NFE% + CF%)]
II. Calculate the energy digestibility (%): energy digestibility (%) = 91.2 – (1.43 × CF%) (4)
III. Calculate the digestible energy: kcal DE = (kcal GE × energy digestibility)/100
IV. Calculate the metabolisable energy: ME (kcal) = kcal DE – (1.04 × CP%)

https://doi.org/10.17221/139/2019-VETMED

Table 1. The important components declared in the label information of the premium dog foods used in the study

Group	Food code	Price	Protein sources in dog foods	Other nutrient sources in dog foods		
				starch sources	fat sources	fibre sources
Dog foods high in fish meat	1	H	salmon (32%), poultry, beef meat	oats, oat meal, potato	canola oil	apple pulp
	2	M	fish meat (20%: 5% salmon, 5% cod, 5% sardine, 5% trout)	oats	fish oil, poultry oil	rice bran, sugar beet pulp, peas
	3	M	tuna (16%), soybean meal, chicken and turkey meal, corn gluten meal	rice, corn flour, rice flour	animal oil	rice brain
	4	M	fish meal, poultry meat meal	barley, corn, wheat flour, wheat, potato flour	animal oil	sugar beet pulp, peas
	5	L	salmon protein (32%), anchovy flour (5%)	rice, corn	salmon oil, anchovy oil	sugar beet pulp, peas
	6	H	herring meat (25%), dried tuna (16%), dried salmon meat	peas	chicken oil, flax seed oil	dehydrated alfalfa, tomato
	7	M	salmon meat (35%), herring fish meat (10%)	rice	chicken oil, salmon oil	apple pulp
Dog foods high in poultry meat	1	H	turkey meat, dried whole egg	potato	canola oil	tomato, apple, carrot, pumpkin, clover, peas
	2	H	chicken, turkey, salmon, trout, dried egg	potato, tapioca	chicken oil, canola oil, coconut oil, salmon oil	spinach, broccoli, apple, carrot, peas
	3	M	duck meat flour (20%), potato protein	potato flour	fish oil, poultry oil	dried radish grated, dried apple
	4	M	chicken, turkey, lamb, dried egg	rice, corn, sorghum, barley	animal oil	sugar beet pulp
	5	H	chicken, dried chicken, chicken flour	rice, barley, oats, peas	animal oil	sugar beet pulp, flax seed
	6	M	poultry meat meal (>15%), meat meal, hydrolysed meat meal, fish meal	wheat, barley, corn, wheat flour	animal oil	sugar beet pulp, peas
	7	L	poultry meat and meat and its by-products	grains	sunflower oil, fish oil, animal oil	carrot, sugar beet pulp, peas
	8	L	chicken meat (60%), chicken liver	potatoes flour, corn	poultry oil, fish oil, salmon oil	sugar beet pulp, apple pulp
Dog foods high in lamb meat	1	M	lamb meat, poultry meal	rice, wheat, corn	chicken oil	wheat bran, sugar beet pulp
	2	H	lamb meat, dried egg	rice, wheat, corn	chicken oil	sugar beet pulp, tomato pulp, dried peas
	3	L	lamb meat, animal protein, corn gluten, dried yeast	wheat, corn, rice	animal oil, flax seed	wheat bran, carrot, sugar beet pulp, dried peas
	4	M	lamb meat (>5%), poultry meat meal, fish meal, hydrolysed protein	rice, barley, corn, wheat	animal oil	sugar beet pulp, wheat bran
	5	L	dried lamb protein (24%), hydrolysed lamb protein (17%), anchovy flour	rice, corn	chicken oil, anchovy oil	sugar beet pulp, peas
	6	M	lamb meat(dried), dried eggs, herring	wheat, oat	chicken oil, herring-salmon oil	sugar beet pulp, carrot
	7	M	lamb meat 13%, wheat embryo	rice, corn, wheat	animal oil	sugar beet pulp
	8	M	lamb meat, poultry meat, meat-bone meal, reindeer meat, hydrolysed chicken, pork, corn embryo meal	wheat, corn	animal oil	sugar beet pulp
	9	M	lamb meat (38%)	rice	chicken oil, salmon oil	tomato pulp

*Price of one kg: was over $8 for the high (H), was between $4 and $8 for the medium (M); and was less than $4 for the low (L) price

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The *in vitro* digestion technique

The *in vitro* digestion of the extruded commercial dog foods was carried out at three stages: gastric digestion, small intestine digestion and large intestine digestion/fermentation. The *in vitro* digestion and fermentation were performed with four replicates per dog food sample (i.e., the F-dog foods, L-dog foods and P-dog foods).

The faeces inoculum for large intestine digestion/fermentation

The faecal samples used as an inoculum in the current study were obtained from two 2-year-old Labrador Retriever males. The dogs were fed with a commercial dry-type extruded dog food for four weeks before the faeces were collected. The food contained approximately 25% CP, 15% EE, 8% ash, and 3% CF in DM basis.

The total daily amount of the food was given as two meals for the puppies and one meal for the adult dogs. 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 faecal samples were diluted at a 1 : 10 ratio with a 0.9% sterile physiologic solution (Polifleks, Polifarma, Turkey) using a laboratory type blender (Waring Products Division, Torrington, CT, USA). The diluted faecal inoculums were filtered through four layers of cheesecloth under constant CO_2 gas (anaerobically) and used in the *in vitro* digestion technique as the faeces inoculum.

I. Stage (in vitro gastric digestion). 310 ± 10 mg DM of dog food were mixed with 10 ml of a phosphate buffer (0.1 M, pH 6) into an anaerobic glass fermenter with a 100 ml volume (Model Fortuna, Haberle Labortechnik, Germany). 5 ml 0.2 M of HCl was added to this mixture and the pH value was adjusted to pH 2.0 (with 1 M of HCl and 1 M of NaOH). Then 1 ml of a freshly prepared pepsin solution was added, containing 10 mg of pepsin. 1 ml of a chloramphenicol solution (0.5 g in 100 ml ethanol) was added to the mixture and then we closed the clips of the *in vitro* fermenters. The fermenters were incubated at 39.0 ± 0.2 °C for 2 h in a thermostatically 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 NaOH 0.6 M 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 them with clips, they were incubated for 4 h at 39.0 ± 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),

Table 2. The composition of the *in vitro* fermentation medium

Component	Amount
ml/l	
Solution A[a]	330.0
Solution B[b]	330.0
Trace mineral solution[c]	10.0
Water-soluble vitamins[d]	20.0
Folate: biotin solution[e]	5.0
Riboflavin solution[f]	5.0
Hemin solution[g]	2.5
Short chain fatty acids[h]	0.4
Resazurin[i]	1.0
Distilled H_2O	296.0
g/l	
Yeast extract	0.5
Trypticase	0.5
Na_2CO_3	4.0
Cysteine $HCl*H_2O$	0.5

[a]Composition (g/l): NaCl, 5.4; KH_2PO4, 2.7; $CaCl_2*H_2O$, 0.16; $MgCl_2*6H_2O$, 0.12; $MnCl_2*4H_2O$, 0.06; $CoCl_2*6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4; [b]Composition: K_2HPO_4, 2.7 g/l; [c]Composition (mg/l): ethylene diamine tetra acetic acid (disodium salt), 500; $FeSO_4*7H_2O$, 200; $ZnSO_4*7H_2O$, 10; $MnCl_2*4H_2O$, 3; H_3PO_4, 30; $CoCl_2*6H_2O$, 20; $CuCl_2*2H_2O$, 1; $NiCl_2*6H_2O$, 2; $Na_2MoO_2*2H_2O$, 3; [d]Composition (mg/l): thiamine-HCl, 100; d-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*- aminobenzoic acid, 5; vitamin B_{12}, 0.25; [e]Composition (mg/l): folic acid, 10; d-biotin, 2; NH_4HCO_3, 100; [f]Composition: riboflavin, 10 mg/l in 5 mmol/l of HEPES; [g]Hemin: Hemin 500 mg/l of 10 mmol/l NaOH; [h]Composition: *n*-valerate, iso-valerate, iso-butyrate and DL alpha-methyl butyrate, 250 ml/l; [i]Composition: 1 g resazurin/l distilled water

https://doi.org/10.17221/139/2019-VETMED

which contained solution A, solution B, a trace mineral solution, water-soluble vitamins, a folate-biotin solution, a riboflavin solution, a hemin solution, short-chain fatty acids, resazurin, a yeast extract, trypticase, Na_2CO_3 and cysteine HCl*H_2O (Table 2) (Sunvold et al. 1995a; Bosch et al. 2008). The initial volumes of the fermenters were recorded, and incubated in a water bath with a thermostat at 39.0 ± 0.2 °C 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.

Determination of the cumulative gas production

In the *in vitro* large intestine fermentation, the total cumulative gas volume was recorded from the calibrated scale on the fermenter at 6, 12, 18, and 24 hours.

Determination of the in vitro true-organic matter disappearance

For the *in vitro* true-organic matter disappearance (OMd), the incubation of the *in vitro* fermenter was stopped at 6, 12, 18 and 24 h. The *in vitro* OMd was determined by filtering the fermentation residues using a vacuum unit (Velp Dietary Fibre Analyzer, Italy) on pre-weighed glass crucibles (Velp, porosity #2, Italy), which were dried at 105 °C and burned the residual off at 550 °C. The i*n vitro* OMd was calculated as 1 – [(OM residue – OM blank)/initial OM)] × 100 (Kara et al. 2019).

Statistically analyses

The experimental data were first subjected to a Levene's test to detect the variance of the homogeneity. Multivariate analyses were implemented for the homogeneous variances by General Linear Model procedures to test the treatment differences. A one-way variance analysis was conducted for the chemical compositions and the *in vitro* digestion values of the premium type adult dog foods were tested.

The data were analysed by the following statistical model:

$$Y_{ij} = \mu_{ij} + S_i + e_i \qquad (5)$$

where:

Y_{ij} – the general mean for each parameter investi gated;

μ_{ij} – the mean of the commercial dog food for each researched parameter;

S_i – the *i*th effect of the different commercial dog food on the observed parameters;

e_i – the standard error value.

The means were separated by Tukey's multiple range test at P < 0.05. The linear relationships among the studied parameters were determined using Pearson's correlation through the SPSS v17.0 software (IBM Corp., Armonk, NY, USA).

RESULTS

The nutrient matter contents and digestion levels of the F-dog foods

The OMd values of the F-dog foods from the different brands were similar (P > 0.05). The *in vitro*

Table 3. The *in vitro* OMd and cumulative gas production values of the F-dog foods

F-dog foods	OMd (%)	*In vitro* cumulative gas production (ml/g DM)			
		6 h	12 h	18 h	24 h
1	86.5	71.5	105.1^{a}	150.5^{a}	158.0^{a}
2	89.2	28.2	84.5^{ab}	104.5^{abc}	119.4^{ab}
3	87.4	34.6	91.0^{a}	124.6^{ab}	136.5^{ab}
4	88.5	31.9	57.1^{bc}	76.3^{bcd}	80.4^{bc}
5	87.1	13.3	33.0^{cd}	51.8^{cd}	57.7^{c}
6	85.5	13.9	26.0^{cd}	41.5^{d}	47.3^{c}
7	91.3	10.5	19.5^{d}	40.5^{d}	47.1^{c}
Average	87.9	29.1	59.4	84.2	92.3
Minimum	83.7	8.9	19.0	29.8	29.8
Maximum	92.9	104.9	120.0	158.1	161.2
SD	2.7	24.5	40.4	42.9	45.1
SEM	0.7	6.5	8.9	11.4	12.0
P-value	0.499	0.103	< 0.001	0.001	0.001

$^{a-d}$Differences among means demonstrated using different superscripts at same column for were statistically important OMd = i*n vitro* organic matter disappearance as % in DM; SD = standard deviation of the mean; SEM = standard error of the mean

https://doi.org/10.17221/139/2019-VETMED

Table 4. The nutritional values of the commercial F-dog foods

Dog food with fish	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal
1	23.8^{ab}	8.2	7.2^{b}	4.6^{ab}	58.8^{b}	63.2^{b}	29.0^{ab}	$3\,754.4^{b}$	63.4^{abc}
2	24.7^{ab}	11.2	5.8^{f}	4.0^{ab}	56.1^{b}	59.9^{b}	27.4^{b}	$3\,955.5^{ab}$	62.5^{abc}
3	19.2^{c}	10.7	4.7^{g}	2.7^{b}	64.7^{a}	67.2^{a}	31.1^{ab}	$4\,051.6^{a}$	47.4^{c}
4	20.3^{c}	6.8	6.0^{e}	3.7^{ab}	65.5^{a}	69.0^{a}	29.5^{ab}	$3\,773.8^{b}$	54.0^{bc}
5	27.7^{ab}	10.4	7.0^{c}	5.5^{a}	50.9^{c}	56.2^{c}	34.1^{a}	$3\,773.7^{b}$	73.3^{ab}
6	30.5^{a}	10.5	9.6^{a}	5.7^{a}	47.5^{c}	53.1^{c}	31.7^{ab}	$3\,762.7^{b}$	81.2^{a}
7	29.0^{a}	11.3	6.6^{d}	5.3^{a}	48.9^{c}	54.1^{c}	28.1^{b}	$3\,835.0^{ab}$	75.8^{a}
Average	25.1	9.9	6.7	4.5	56.1	60.4	30.1	3 843.8	65.4
Minimum	19.2	6.8	4.7	2.3	44.5	50.5	27.0	3 704.4	46.4
Maximum	31.0	13.2	9.7	6.2	67.4	70.5	34.2	4 121.8	82.5
SD	4.3	1.8	1.4	1.1	7.4	6.4	2.3	119.1	12.2
SEM	1.2	0.5	0.3	0.3	1.9	1.8	0.6	31.8	3.2
P-value	0.004	0.058	< 0.001	0.011	0.002	0.002	0.013	0.008	0.003

$^{a–g}$Differences among means demonstrated using different superscripts at same column for were statistically important

CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; SD = standard deviation of the mean; SEM = standard error of the mean; starch = total starch as % in DM

cumulative gas production at the fermentative incubation of the F-dog foods increased from 6 h to 24 h of fermentative incubation (Table 3). The CP, ash, CF, NFE, starch, ME and CP/1 000 kcal DM values of the F-dog foods differed according to the brands of the F-dog foods ($P < 0.05$). The EE values of the F-dog foods did not differ much among the different premium commercial brands ($P = 0.058$) (Table 4).

The *in vitro* cumulative gas production values of the F-dog foods were negatively correlated with the CP values, ash and CP/1 000 kcal values of the F-dog foods ($P < 0.05$). The *in vitro* cumu-

Table 5. The Pearson correlations among the chemical and *in vitro* digestion values of the F-dog foods

F-dog foods	GP12	GP18	GP24	OMd	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal	Price
GP6	0.792**	0.744**	0.692**	−0.398	−0.505*	−0.492	−0.151	−0.338	0.526**	0.546*	−0.330	−0.060	−0.460	0.678**
GP12	1	0.960**	0.953**	−0.160	−0.718**	−0.316	−0.506*	−0.653*	0.704**	0.697**	−0.376	0.358	−0.719**	0.540*
GP18	–	1	0.993**	−0.038	−0.664**	−0.295	−0.456*	−0.560*	0.639**	0.637*	−0.299	0.284	−0.659*	0.612*
GP24	–	–	1	−0.007	−0.635*	−0.222	−0.472*	−0.558*	0.602**	0.596*	−0.299	0.337	−0.639*	0.584*
OMd	–	–	–	1	0.141	0.198	−0.335	0.061	−0.151	−0.164	−0.263	0.087	0.106	−0.471
CP	–	–	–	–	1	0.458	0.733**	0.872**	−0.973**	−0.969**	0.194	−0.439	0.992**	−0.084
EE	–	–	–	–	–	1	0.006	0.274	−0.608**	−0.651*	0.053	0.480*	0.361	−0.315
Ash	–	–	–	–	–	–	1	0.762**	−0.668**	−0.637*	0.267	−0.678*	0.729**	0.314
CF	–	–	–	–	–	–	–	1	−0.889**	−0.852**	0.252	−0.711*	0.910**	−0.067
NFE	–	–	–	–	–	–	–	–	1	0.997**	−0.204	0.354	−0.955**	0.129
CHO	–	–	–	–	–	–	–	–	–	1	−0.193	0.287	−0.942**	0.148
Starch	–	–	–	–	–	–	–	–	–	–	1	−0.190	0.219	−0.225
ME	–	–	–	–	–	–	–	–	–	–	–	1	−0.547*	−0.280
CP/1 000 kcal	–	–	–	–	–	–	–	–	–	–	–	–	1	−0.037

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; Starch = total starch as % in DM

https://doi.org/10.17221/139/2019-VETMED

lative gas production values for the fermentative incubation of the F-dog foods were positively correlated with the NFE and CHO contents and the price of the F-dog foods ($P < 0.05$). The EE contents of the F-dog foods were positively correlated with the ME and negatively correlated with the NFE and CHO contents of the F-dog foods ($P < 0.01$). The CF contents of the F-dog foods were negatively correlated with the NFE, CHO and ME contents of F-dog foods ($P < 0.05$) (Table 5).

The nutrient contents and digestion levels of the L-dog foods

The OMd values of the L-dog foods were different among the brands ($P < 0.05$). The *in vitro* cumulative gas production (ml/g DM) of the L-dog foods were different among the brands and increased up to 24 h ($P < 0.01$) (Table 6). The CP values of the L-dog foods ranged widely ($P < 0.01$). The EE, ash, NFE, CHO, starch and values of L-dog foods were different among the dog foods ($P < 0.05$) (Table 7).

The *in vitro* cumulative gas production values of the L-dog foods were negatively correlated with the EE, CP, starch and ME values of the L-dog foods ($P < 0.05$). The *in vitro* cumulative gas production values of the L-dog foods were positively correlated with the CHO content of the L-dog foods ($P < 0.05$). The OMd of the L-dog foods were positively correlated with the price of the L-dog foods ($P < 0.05$). The CP contents of the L-dog foods were positively correlated with the EE, ash and CP/1 000 kcal contents and the prices of the L-dog foods ($P < 0.05$). The EE contents of the L-dog foods were positively correlated with the ME and CP/1 000 kcal of the L-dog foods. The CP, EE and CP/1 000 kcal DM values of the L-dog foods were negatively correlated with the NFE and CHO contents of the L-dog foods ($P < 0.05$). The CF contents of the L-dog foods were positively correlated with the NFE and ME contents of the L-dog foods ($P < 0.05$) (Table 8).

The nutrient contents and digestion levels of the P-dog foods

The OMd and gas production values of the P-dog foods at 6 h of the fermentative incubation were not

Table 6. The *in vitro* organic matter disappearance and cumulative gas production values of the commercial L-dog foods

L-dog foods	OMd (%)	*In vitro* cumulative gas production (ml/g DM)			
		6 h	12 h	18 h	24 h
1	84.0^{ab}	5.8^{b}	26.0^{c}	49.8^{c}	55.7^{c}
2	87.9^{a}	7.3^{b}	81.0^{b}	119.6^{abc}	124.6^{abc}
3	83.0^{ab}	14.8^{b}	28.5^{c}	48.5^{c}	55.6^{c}
4	88.5^{a}	25.8^{b}	84.5^{b}	134.3^{ab}	136.8^{ab}
5	84.1^{ab}	16.5^{b}	66.5^{bc}	87.7^{bc}	96.3^{bc}
6	81.7^{ab}	36.0^{b}	48.5^{bc}	81.5^{bc}	82.5^{bc}
7	83.2^{ab}	123.4^{a}	154.0^{a}	182.6^{a}	184.3^{a}
8	76.3^{c}	27.1^{b}	90.5^{b}	132.3^{ab}	133.7^{ab}
9	77.9^{c}	49.0^{b}	76.5^{b}	106.8^{abc}	111.2^{abc}
Average	83.0	34.0	72.8	104.8	109.0
Minimum	72.2	2.9	24.0	45.8	51.3
Maximum	90.8	151.6	168.0	200.0	203.5
SD	4.3	36.7	38.3	44.4	42.7
SEM	1.0	8.6	9.0	10.4	10.1
P-value	0.013	0.001	< 0.001	0.002	0.002

$^{a–c}$Differences among means demonstrated using different superscripts at same column for were statistically important
OMd = i*n vitro* organic matter disappearance as % in DM; SD = **s**tandard deviation of the mean; SEM = standard error of the mean

Table 7. The nutritional values of the commercial L-dog foods

L-dog foods	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal ME
1	26.3^{ab}	9.5^{bc}	7.3^{c}	3.3	57.6^{a}	60.7^{a}	30.6^{ab}	3 958.0^{a}	66.6
2	23.5^{ab}	10.9^{b}	7.9^{b}	4.5	56.6^{a}	60.8^{a}	32.4^{a}	3 893.5^{ab}	60.6
3	24.5^{ab}	10.9^{b}	8.6^{a}	4.5	55.7^{ab}	59.9^{a}	32.4^{a}	3 893.7^{ab}	63.2
4	24.0^{ab}	7.2^{d}	6.6^{d}	5.8	57.3^{a}	62.8^{a}	30.2^{ab}	3 595.4^{b}	66.9
5	22.4^{b}	10.2^{b}	6.6^{d}	3.7	59.9^{a}	63.4^{a}	30.7^{ab}	3 931.6^{a}	57.2
6	32.0^{a}	14.5^{a}	8.8^{a}	5.6	42.3^{b}	47.7^{b}	30.4^{ab}	3 958.4^{a}	80.8
7	22.8^{ab}	7.6^{d}	8.0^{b}	4.7	60.3^{a}	64.7^{a}	27.6^{b}	3 714.6^{ab}	61.3
8	22.2^{b}	8.6^{cd}	7.8^{b}	5.3	58.6^{a}	63.7^{a}	29.9^{ab}	3 699.0^{ab}	60.1
9	26.7^{ab}	9.8^{bc}	7.9^{b}	5.6	52.5^{ab}	57.8^{ab}	28.9^{ab}	3 739.3^{ab}	71.4
Average	24.9	9.9	7.7	4.8	55.6	60.2	30.4	3 820.4	65.3
Minimum	19.1	7.1	6.4	3.2	41.0	47.1	26.9	3 500.7	48.6
Maximum	32.0	14.6	8.8	6.7	64.0	67.3	33.5	4 021.1	82.1
SD	3.4	2.1	0.7	1.0	5.8	5.5	1.6	143.2	8.4
SEM	0.8	0.5	0.1	0.2	1.3	1.3	0.3	33.7	1.9
P-value	0.046	< 0.001	< 0.001	0.164	0.012	0.006	0.047	0.011	0.097

$^{a–d}$Differences among means demonstrated using different superscripts at same column for were statistically important
CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; SD = standard deviation of the mean; SEM = standard error of the mean; starch = total starch as % in DM

Table 8. The Pearson correlations among the chemical and *in vitro* digestion values of the L-dog foods

L–dog foods	GP12	GP18	GP24	OMd	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal	Price
GP6	0.813**	0.702**	0.701**	–0.138	–0.145	–0.312	0.187	0.231	0.117	0.170	–0.740**	–0.395	–0.051	–0.176
GP12	1	0.951**	0.956**	–0.008	–0.451*	–0.529*	–0.106	0.268	0.364	0.444*	–0.594**	–0.585**	–0.315	–0.008
GP18	–	1	0.997**	0.006	–0.437*	–0.525*	–0.124	0.344	0.328	0.418*	–0.472*	–0.637**	–0.286	0.099
GP24	–	–	1	0.010	–0.479*	–0.544*	–0.152	0.318	0.369	0.458*	–0.468*	–0.633**	–0.332	0.083
OMd	–	–	–	1	–0.107	–0.086	–0.365	–0.179	0.159	0.137	0.219	0.076	–0.129	0.353*
CP	–	–	–	–	1	0.660**	0.446*	0.257	–0.919**	–0.947**	0.055	0.306	0.962**	0.339*
EE	–	–	–	–	–	1	0.615**	0.030	–0.766**	–0.821**	0.413	0.672**	0.497*	0.246
Ash	–	–	–	–	–	–	1	0.174	–0.550*	–0.561*	0.102	0.296	0.374	0.067
CF	–	–	–	–	–	–	–	1	–0.512*	–0.371	–0.151	–0.718**	0.473*	–0.152
NFE	–	–	–	–	–	–	–	–	1	0.988**	–0.129	–0.176	–0.912**	–0.337*
CHO	–	–	–	–	–	–	–	–	–	1	–0.168	–0.320*	–0.901**	–0.340*
Starch	–	–	–	–	–	–	–	–	–	–	1	0.395	–0.051	0.087
ME	–	–	–	–	–	–	–	–	–	–	–	1	0.036	0.128
CP/1 000 kcal	–	–	–	–	–	–	–	–	–	–	–	–	–	0.332*

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level
CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; starch = total starch as % in DM

different among the commercial brands ($P > 0.05$). The *in vitro* cumulative gas production (from 12 h to 24 h incubation times) for the fermentative incubation of the P-dog foods changed among the commercial brands ($P < 0.05$) (Table 9). The CP, EE, ash, CF, NFE, CHO, ME and CP/1 000 kcal DM

https://doi.org/10.17221/139/2019-VETMED

Table 9. The *in vitro* OMd and cumulative gas production values of the commercial P-dog foods

P-dog foods	OMd (%)	*In vitro* cumulative gas production (ml/g DM)			
		6 h	12 h	18 h	24 h
1	87.1	4.0	74.0[b]	108.1[abc]	121.2[ab]
2	88.0	4.4	31.5[c]	51.5[bc]	61.8[ab]
3	82.9	3.9	37.7[c]	62.3[abc]	78.9[ab]
4	82.2	17.8	92.9[a]	136.3[a]	136.8[a]
5	83.7	3.0	35.9[c]	48.3[bc]	57.5[ab]
6	83.5	10.5	70.5[b]	111.7[ab]	114.8[ab]
7	85.4	8.8	60.7[b]	98.8[abc]	102.5[ab]
8	82.6	1.7	24.9[c]	36.5[c]	42.6[b]
Average	84.4	6.7	53.5	81.7	89.5
Minimum	79.0	1.5	24.8	36.3	42.6
Maximum	88.8	26.3	96.5	143.3	154.6
SD	2.8	6.2	23.8	37.9	36.3
SEM	0.7	1.5	5.9	9.4	9.0
P-value	0.330	0.113	< 0.001	0.005	0.015

[a–c]Differences among means demonstrated using different superscripts at same column for were statistically important
OMd = i*n vitro* organic matter disappearance as % in DM; SD = **s**tandard deviation of the mean; SEM = standard error of the mean

values of the P-dog foods were different among the premium P-dog foods *(P* < 0.05). The starch values of the P-dog foods were not different among the brands of the P-dog foods (*P* > 0.05) (Table 10).

The *in vitro* cumulative gas production values for the fermentative incubation of the P-dog foods were negatively correlated with the CF and EE contents of the P-dog foods; and positively cor-

Table 10. The nutritional values of the commercial P-dog foods

P-dog foods	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal
1	22.9[bc]	10.8[abc]	8.5[b]	5.1[bc]	56.3[bc]	61.1[b]	30.9	3 830.2[ab]	59.9[bcd]
2	30.8[a]	12.3[a]	9.7[a]	3.9[cd]	49.1[d]	52.8[c]	29.5	4 035.8[a]	76.3[a]
3	27.9[ab]	9.3[cd]	6.5[d]	4.2[cd]	54.6[bc]	58.6[b]	28.0	3 866.7[ab]	72.2[ab]
4	27.7[ab]	10.0[bc]	7.4[c]	2.7[d]	56.8[bc]	59.4[b]	30.5	4 047.9[a]	68.6[abc]
5	23.1[bc]	11.6[ab]	5.7[f]	4.2[cd]	57.1[bc]	61.0[ab]	29.9	3 955.2[a]	58.4[cd]
6	20.8[c]	8.1[d]	6.0[e]	7.0[a]	57.3[ab]	63.9[a]	31.2	3 507.8[c]	59.3[bcd]
7	22.1[bc]	9.4[cd]	8.3[b]	2.8[d]	62.9[a]	65.5[ab]	28.4	3 989.5[a]	55.3[d]
8	23.6[bc]	10.8[abc]	8.5[b]	7.0[ab]	51.8[cd]	58.4[b]	27.8	3 633.3[bc]	65.2[abcd]
Average	24.9	10.3	7.6	4.6	55.7	60.1	29.5	3 858.3[ab]	64.4
Minimum	21.6	7.8	5.7	2.4	47.2	50.5	26.8	3 443.2	54.1
Maximum	33.6	12.3	9.7	7.6	63.2	66.3	32.7	4 094.8	82.0
SD	3.5	1.4	1.3	1.6	4.7	5.1	1.9	194.6	7.5
SEM	0.8	0.3	0.3	0.4	1.2	1.2	0.4	48.6	1.8
P-value	0.002	< 0.001	< 0.001	< 0.001	0.001	0.001	0.615	< 0.001	0.002

[a–d]Differences among means demonstrated using different superscripts at same column for were statistically important
CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; SD = standard deviation of the mean; SEM = standard error of the mean; starch = total starch as % in DM

https://doi.org/10.17221/139/2019-VETMED

related with the NFE, CHO and starch contents of the P-dog foods ($P < 0.05$). The CP content of the P-dog foods was positively correlated with the EE, ME and CP/1 000 kcal values of the P-dog foods ($P < 0.01$); and negatively correlated with the NFE and CHO content of the P-dog foods ($P < 0.01$). The EE content of the P-dog foods was positively correlated with the ME, and negatively correlated with the NFE and CHO contents of the P-dog foods ($P < 0.01$). The CF contents of the P-dog foods were negatively correlated with the ME values of the P-dog foods ($P < 0.05$). The NFE and CHO contents of the P-dog foods were negatively correlated with the CP/1 000 kcal DM values of the P-dog foods ($P < 0.05$) (Table 11).

Table 11. The Pearson correlations among the chemical and *in vitro* digestion values of the commercial P-dog foods

P–dog foods	GP12	GP18	GP24	OMd	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal	Price
GP6	0.649**	0.652**	0.586*	–0.276	0.052	–0.425*	–0.123	–0.399*	0.370*	0.249	0.012	0.178	–0.021	–0.025
GP12	1	0.953**	0.928**	–0.017	–0.248	–0.461*	–0.134	–0.331*	0.530*	0.457*	0.470*	0.062	–0.286	0.070
GP18	–	1	0.988**	0.067	–0.225	–0.568*	–0.108	–0.325*	0.530*	0.470*	0.348*	0.002	–0.247	0.004
GP24	–	–	1	0.135	–0.196	–0.558*	–0.105	–0.322	0.505*	0.442*	0.347*	0.007	–0.216	0.063
OMd	–	–	–	1	0.414	0.379	0.483	–0.266	–0.223	–0.271	–0.081	0.226	0.149	0.166
CP	–	–	–	–	1	0.499*	0.381	–0.403	–0.657**	–0.872**	–0.124	0.593*	0.944**	0.299
EE	–	–	–	–	–	1	0.483	–0.203	–0.556*	–0.663**	–0.003	0.502**	0.315	0.338
Ash	–	–	–	–	–	–	1	–0.142	–0.382	–0.457	–0.145	0.290	0.332	–0.386
CF	–	–	–	–	–	–	–	1	–0.354	0.071	0.025	–0.947**	–0.166	–0.225
NFE	–	–	–	–	–	–	–	–	1	0.926**	0.194	0.058	–0.799**	–0.219
CHO	–	–	–	–	–	–	–	–	–	1	0.211	–0.315	–0.905**	–0.314
Starch	–	–	–	–	–	–	–	–	–	–	1	–0.031	–0.286	0.342
ME	–	–	–	–	–	–	–	–	–	–	–	1	0.296	0.326
CP/1 000 kcal	–	–	–	–	–	–	–	–	–	–	–	–	1	0.257

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; starch = total starch as % in DM

Table 12. The average of some of the chemical compositions and *in vitro* cumulative gas production values of the premium adult dog foods with the different protein types

Premium dog foods	EE (%)	Ash (%)	CF (%)	NFE (%)	CHO (%)	Starch (%)	*In vitro* cumulative gas production (ml/g DM)			
							6 h	12 h	18 h	24 h
F-dog foods	9.9	6.7	4.5	56.1	60.4	30.1	27.6[ab]	59.4	84.2	92.3
L-dog foods	9.9	7.7	4.8	55.6	60.2	30.4	34.0[a]	72.8	104.8	109.0
P-dog foods	10.3	7.6	4.6	55.7	60.1	29.5	6.7[b]	53.5	81.7	89.5
Average	10.0	7.4	4.6	55.8	60.2	30.0	23.0	62.5	91.1	97.6
Minimum	6.8	4.7	2.3	41.0	47.1	26.7	1.5	19.0	29.8	29.8
Maximum	14.6	9.7	7.6	67.4	70.5	34.2	151.6	168.0	200.0	203.5
SD	1.8	1.2	1.2	5.7	5.1	2.0	28.7	33.1	42.4	41.5
SEM	0.2	0.1	0.1	0.8	0.7	0.2	4.1	4.7	6.1	5.9
P-value	0.778	0.059	0.842	0.977	0.987	0.437	0.014	0.220	0.225	0.344

[a,b]Differences among means demonstrated using different superscripts at same column for were statistically important

CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; SD = standard deviation of the mean; SEM = standard error of the mean; starch = total starch as % in DM

https://doi.org/10.17221/139/2019-VETMED

Comparison of the premium dog foods containing different protein sources

When the *in vitro* OMd values of the F-dog foods, L-dog foods and P-dog foods were compared, it was found that the OMd values of the F-dog foods were higher than those of the L-dog foods and P-dog foods ($P = 0.001$). The *in vitro* cumulative gas production at 6 hours of the L-dog foods was higher than that of the P-dog foods; and similar to that of the F-dog foods ($P < 0.05$) (Table 12). It has been shown that there are no differences in the premium dog foods with the different animal protein contents in terms of the EE, ash, CF, NFE, CHO, and starch ($P > 0.05$) (Table 12). The average, maximum and minimum values for the OMd, CP, ME and CP/1 000 kcal ME of the F-dog foods, L-dog foods and P-dog foods are given in Figures 1–4.

The *in vitro* cumulative gas production values of the premium type dog foods containing the different protein sources were negatively correlated with the CP, EE, CF and CP/1 000 kcal contents of the foods; and positively correlated with the NFE and CHO contents ($P < 0.01$). The OMd value of the premium type dog foods containing the different protein sources was negatively correlated with the ash and CF contents of the foods ($P < 0.05$). The CP content of the premium type dog foods con-

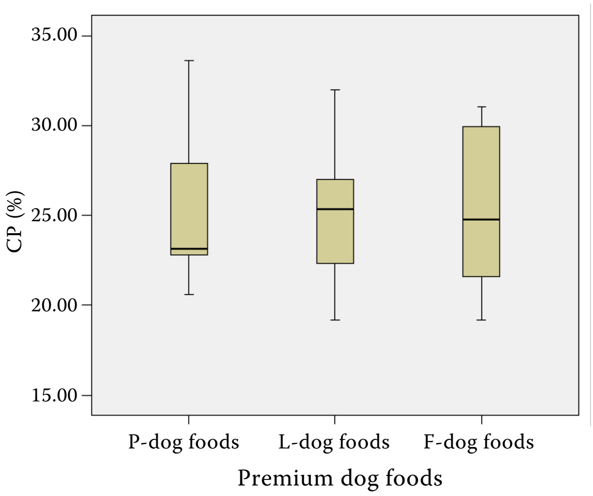

Figure 1. The CP values of the premium adult dog foods including the different animal protein sources
CP = crude protein as % in DM

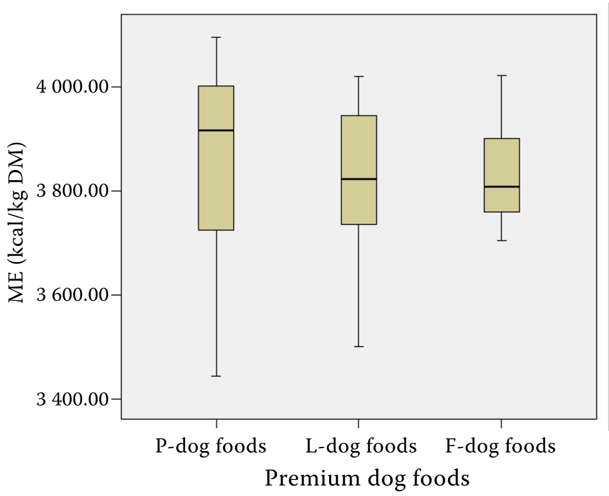

Figure 2. The ME values of the premium adult dog foods including the different animal protein sources
ME = metabolic energy as the amount of kcal/kg in DM

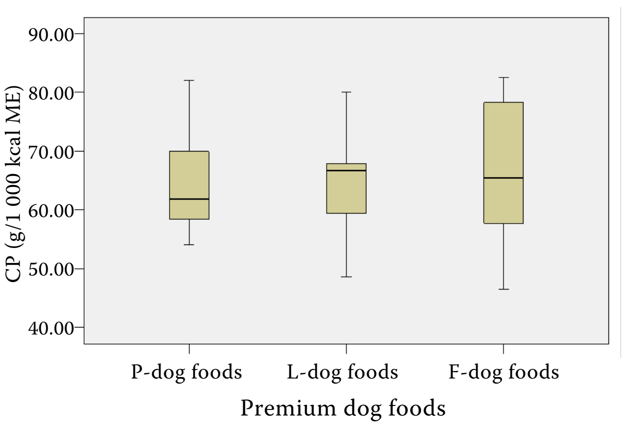

Figure 3. The CP/1 000 kcal ME values of the premium adult dog foods including the different animal protein sources
CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food

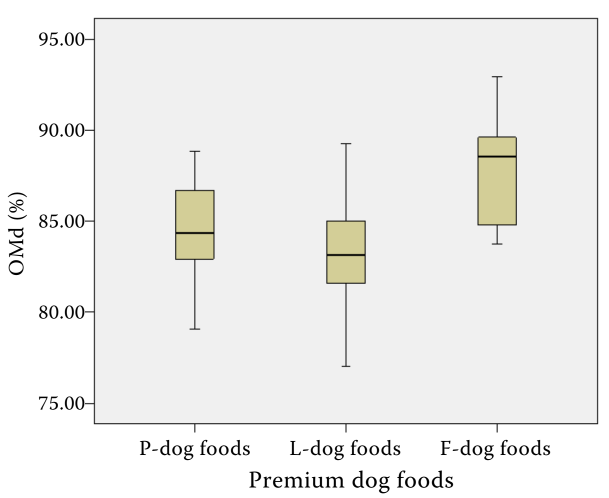

Figure 4. The OMd values of the premium adult dog foods including the different animal protein sources
OMd = *in vitro* organic matter disappearance as % in DM

https://doi.org/10.17221/139/2019-VETMED

Table 13. The Pearson correlations among the *in vitro* digestion parameters and nutritional values of the premium adult dog foods with the different protein types

	GP12	GP18	GP24	OMd	CP	EE	Ash	CF	NFE	CHO	Starch	ME	CP/1 000 kcal	Price
GP6	0.756**	0.643**	0.621**	−0.150	−0.211	−0.358*	−0.020	−0.023	0.437**	0.263	−0.332	−0.207	−0.147	0.193
GP12	1	0.949**	0.943**	−0.104	−0.467**	−0.454**	−0.195	−0.289*	0.494**	0.517**	−0.185	−0.159	−0.432**	0.190
GP18	–	1	0.992**	−0.091	−0.435**	−0.459**	−0.163	−0.366*	0.461**	0.487**	−0.114	−0.188	−0.394**	0.219
GP24	–	–	1	−0.058	−0.441**	−0.440**	−0.192	−0.286*	0.470**	0.492**	−0.125	−0.160	−0.409**	0.241
OMd	–	–	–	1	0.063	0.051	−0.308*	−0.345*	0.008	−0.020	0.001	0.131	0.014	0.175
CP	–	–	–	–	1	0.533**	0.528**	0.128	−0.857**	−0.917**	0.041	0.289*	0.964**	0.095
EE	–	–	–	–	–	1	0.298*	0.017	−0.658**	−0.721**	0.149	0.536**	0.388**	−0.026
Ash	–	–	–	–	–	–	1	0.214	−0.505**	−0.508**	0.068	0.004	0.498**	−0.050
CF	–	–	–	–	–	–	–	1	−0.533**	−0.377*	0.055	−0.833**	0.373*	−0.057
NFE	–	–	–	–	–	–	–	–	1	0.981**	−0.083	−0.054	−0.905**	−0.031
CHO	–	–	–	–	–	–	–	–	–	1	−0.080	−0.132	−0.914**	−0.047
Starch	–	–	–	–	–	–	–	–	–	–	1	0.033	0.016	−0.024
ME	–	–	–	–	–	–	–	–	–	–	–	1	−0.051	0.020
CP/1 000 kcal	–	–	–	–	–	–	–	–	–	–	–	–	1	0.099

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

CF = crude fibre as % in DM; CP = crude protein as % in DM; CP/1 000 kcal = crude protein as g taken for 1 000 kcal of food; EE = diethyl ether extract as % in DM; ME = metabolic energy as the amount of kcal/kg in DM; NFE = nitrogen free extract; starch = total starch as % in DM

taining the different protein sources was positively correlated with the EE, ash and CP/1 000 kcal values of the foods ($P < 0.05$). The EE content of the premium type dog foods containing the different protein sources was positively correlated with the ash, ME and CP/1 000 kcal ($P < 0.05$). The CP, CP/1 000 kcal ME, and EE values of the dog foods were negatively correlated with the NFE and CHO contents of the foods ($P < 0.05$). The CF content of the premium type dog foods containing the different protein sources was negatively correlated with the ME values of the dog foods ($P < 0.05$) (Table 13).

DISCUSSION

Commercial pet foods are extruded using temperatures between 80 °C and 200 °C for 10 s to 270 s, with of 10% to 25% moisture levels (Crane et al. 2010). The kibbles, the digestibility of which is increased through the extrusion process, are in an aqueous form when they come out of the extruder device (Rokey et al. 2010). The kibbles are then dried in dryers to reduce the moisture content to less than 6–9% (Crane et al. 2010). Finally, the pet food is coated with fat and/or a palatability enhancer and subsequently packaged. The extrusion process is controlled by several process parameters. The differentials in the temperature, moisture and press conditions in the extruding process of the dog foods can differ among the commercial producers (Crane et al. 2010). Even if two commercial dog foods have the same food ingredients and the same food amount, their nutrient digestion may differ. The effects of the process parameters, i.e., temperature, moisture and mechanical shear, on the dog foods and the dog food ingredients are closely related to the digestibility of the food (Case et al. 2011).

According to the NRC (2006) and FEDIAF (2018), the minimum recommended nutrient and energy values of the food consumed by dogs at different physiological life periods have been reported. In the present study, generally, the average CP contents of the premium type adult dog foods were 25%. The CP values of the F-dog foods, L-dog foods and P-dog foods in the present study findings were at the minimum recommended protein levels for the adult dog-food based on the MER (95 and 110 kcal/kg$^{0.75}$) of FEDIAF (2018). However, some commercial dog foods in the F-dog foods, L-dog foods and P-dog foods categories had an energy content higher than the maintenance protein requirements as recommended by the NRC (2006)

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and FEDIAF (2018). The CP contents at 1 000 kcal of ME of the F-dog foods in the present study were higher than the recommended minimum levels for adult dog diet by FEDIAF (2018). In the current study, the CP content of the premium adult dog foods were parallel to the values of the premium dry-type adult dog foods, as detected by Carciofi et al. (2006) and Hervera et al. (2007). In the previous study, the CP contents in the DM of commercial wet-type foods for adult dogs were higher than those of the present study (Urrego et al. 2017).

Although most of the adult dog foods in the present study contained animal fats (fish oil, poultry oil, salmon oil, anchovy oil or herring oil) as a fat source, a small number of adult dog foods contained vegetable oils (canola oil, sunflower oil, coconut oil or flaxseed oil). It was not clear which animal fat/vegetable oil were contained in the label information of some commercial dog food producers. However, in the present study, the EE contents of the premium type-adult dog foods with fish meat, lamb meat and poultry meat had higher than the minimum recommended EE levels for adult dog-based on the MER of FEDIAF (2018). There was a difference of two times between the minimum and maximum values of the EE levels of the commercial dog food brands in the present study. The differences in the EE content may lead to a change in the food consumption by the dogs. The EE contents of the dog foods in the present study were at the same level with the values stated for commercial dry-type dog foods of healthy adult dogs by Carciofi et al. (2006), but lower than those stated for commercial wet-type foods for healthy adult dogs by Urrego et al. (2017).

The average ME values of the F-dog foods, L-dog foods and P-dog foods in the present study can be in relation with their same average values of the CP, NFE and EE of these dog foods. The ME values of cats and dogs may vary greatly depending on various factors (NRC 1985; NRC 2006). The energy allowances, recommended for the maintenance of adult dogs, differ widely, with 90–200 kcal ME/kg$^{0.75}$. This diversity is not surprising when we consider the variation in adult size between the different breeds, which, with mature body weights range from 1 kg (Chihuahua) to ≥ 90 kg (St. Bernard), is the greatest diversity across mammalian species (Lauten 2006). The amount of energy a particular dog will finally need is significantly influenced by factors such as the age, breed, size, activity, environment, temperament, insulation characteristics of the skin and hair coat, body condition or disease. According to the NRC (2006), the energy density of dog foods ranges from 2 800 kcal/kg to 4 050 kcal/kg ME depending on the processing, ingredients and additives. The energy values given on the commercial product label or pet nutrition book presume an energy density of 4 000 kcal ME/kg of DM. Some dog foods will have energy densities close to this amount. However, many commercial dog food brands in the present study may have DM energy densities considerably greater/lower than the presumed values (AAFCO 2015). When these more energy-dense products are fed to the dog, the dog will require less of the food to meet its caloric requirements. Under these circumstances, the concentrations of the other nutrients in the food should be increased proportionately, so that the dog or cat will receive the needed amount of each nutrient in a smaller amount of food. Therefore, when the ME density of the dog food exceeds 4 000 kcal ME/kg of DM, the nutrient concentrations should be corrected for the caloric content before any valid comparisons to the appropriate nutrient profiles (NRC 2006; AAFCO 2015; FEDIAF 2018) are made.

In the current study, the CHO contents of the dog foods, which include fish, lamb or poultry meats, were at the levels as reported for commercial dry-type dog foods by Case et al. (2011). In the present study, the commercial F-dog foods, L-dog foods and P-dog foods mostly include rice flour (with grain), potato flour (grain-free), and other grains (wheat, corn, oat and barley flours) as the starch sources according to their label information. Kara et al. (2019) demonstrated that the digestion levels of extruded rice, potato, wheat, corn, barley and oat flours in adult dogs were at a high level and changed according to the carbohydrate source. The CHO, starch and NFE values of the dog foods in the present study showed a significant difference among the commercial dog food brands. In addition to the difference in the amount of starch in the dog foods in the study, the OMd and gas production values also may be due to the digestibility of starch. Easily digestible and structural carbohydrate, which are the most important nutrient contribution to the *in vitro* gas volume, change their gas levels according to their differences in the digestion (in the small intestine) and fermentation (in the large intestine) (Sunvold et al. 1995a; Sunvold et al. 1995b; Bosch

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et al. 2008; Calabro et al. 2013). The *in vitro* cumulative gas production of the P-dog foods in the present study was positively correlated with the NFE. The starch values of the dog food can be in relation to the potato and rice flour included in these dog foods (Kara et al. 2019). However, the *in vitro* cumulative gas production of the F-dog foods and L-dog foods did not have a positive correlation to the starch content; it may be related to the feedstuffs (cereal, potato or pea) in the dog food (Englyst et al. 1992). The starch, which is stored in crystalline intracellular bodies or starch granules, divides into three types depending on the density and orientation of the 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) types. Cereal starch granules are predominantly the A-type and are easily degraded by α-amylase and hydrolysed in the small intestine. The B-type starches of tubers (potato) and C-type legume starches are more resistant to enzymatic hydrolysis and are fermented in the large intestine (Englyst et al. 1992; Kara et al. 2019). Heating starch at lower moisture conditions with a higher temperature irreversibly gelatinises the granular structure of the starch and improves its solubilisation (Colonna et al. 1992). In the extrusion technology, which is performed for pet foods, the digestibility of the carbohydrates for pet animals can, thus, be increased (Kara et al. 2019). The starch and NFE on the *in vitro* OMd or cumulative gas production of the studied premium type dog foods that have different effects may be due to inclusion of the different types (A, B, and C) of starch in the premium dog foods. Besides, the digestion of the dog food may be affected by the extrusion processing conditions during the production of the dog food in a factory and other nutrients in the dog food.

The ash contents of premium dog foods in the present study had a very large range in parallel with the results of Urrego et al. (2017). The level of the variations in the inorganic compounds of the dog food may be due to different amounts of animal mineral sources, such as bone meal, chicken meat meal with bone, fish meal or other mineral sources, such as di-calcium phosphate and calcium carbonate ingredients (Case et al. 2011).

Pet foods often include moderately fermentable fibre sources because they tend to increase the production of short-chain fatty acids that are beneficial to the small and large intestine, without decreasing the nutrient digestibility (Silvio et al. 2000). The average CF values of the dog foods were the same for all the dog foods. However, the minimum and maximum CF values of the premium type dry dog foods were very different from each other; it may be due to the different fibre sources and cellulose content of the fibre sources used in the commercial production processing of the dog food (Carciofi et al. 2006; Hervera et al. 2007; Urrego et al. 2017). The average CF values (0.12–0.13 g/100 kcal ME for dog food in DM) of the premium adult dog foods in the current study were lower than the values reported by Buffington et al. (2004) for commercial adult dog food.

In the present study, the CF contents of the F-dog foods, L-dog foods and P-dog foods were negatively correlated with the ME values of the dog foods; and were parallel with the results of Earle et al. (1998). The average CF contents of the F-dog foods, L-dog foods and P-dog foods were negatively correlated to the *in vitro* OMd values of the dog foods; and agreed with the results given for the different dog foods in an earlier study (Earle et al. 1998). In cats and dogs, undigested food remains in the large intestine for up to 12 hours. The dietary fibre increases the faecal volume and faecal weight in dogs, so that the contractions of the proximal column of a dog may be more effective (NRC 2006; Case et al. 2011). In some of the dog foods studied, when the CF content falls < 4%, it may cause problems, such as adversely affecting the faecal quality (hardness, low viscosity) and may cause constipation, intestinal immobility and decrease the prebiotic activity in the large intestine.

The premium commercial dog foods with high fish, lamb or poultry meats in the present study mostly contained sugar beet pulp and other fruit fibres-pomaces (rice bran, wheat brain, tomato pomace, peas, spinach, broccoli, apples and carrots). Fruit fibres and pomaces are by-products of the processing of fruits to a juice or puree that are dried and, to some extent, further processed and ground to a fine particle size (Walter et al. 1985). A general characteristic of the fruit fibres is their high content of pectin and hemicelluloses in relation to the cellulose, accompanied by the low fat and protein contents (Calabro et al. 2013; Godoy et al. 2013). Whole oats and barley grains, as dietary fibre sources, are two cereal grains that are good sources of β-glucan, a water-soluble fibre fraction

https://doi.org/10.17221/139/2019-VETMED

that has plasma lipid- and glycaemic-lowering effects in humans and animals (Godoy et al. 2013).

In the present study, the *in vitro* gas production of the dog foods at 24 h and the OMd values were very different among the brands, which may be related to the feedstuffs' digestibility, extruding process or carbohydrates: protein: fat ratios of the dog foods. In general, the high digestion values of the F-dog foods may be related to the quality of the feedstuffs used. In the study, the *in vitro* OMd and gas production values were found to be very different among the brands of dog foods in each category, although all of them were premium dog foods. These differences may be due to the different types and quantities of the starch source and dietary fibre source used in the foods, as well as the conditions of the applied extrusion process. The high OMd digestion in the premium dog foods examined is ideal for the gut health and defecation. In previous reports, medium (Saluki, German Shepherd, Basset Hound) and small breed (Dachshund, Beagle) dogs were reported to have similar food digestion results (NRC 2006). However, previous researchers have found a decrease in the weight of the gastrointestinal tract with an increase in the body weight of the dog (Kirkwood 1985; Meyer et al. 1993). An empty intestinal tract in small dog breeds can account for 6–7% of the body weight, while it decreases to 3–4% in large and giant breeds (Weber et al. 2003). It was determined that the digestion of the dry-extruded dog food in large-giant dogs was much more efficient than in small breeds, but, in large breeds, a decreased stool score and increased stool water concentration was observed (Weber et al. 2003). As shown in the results of the present study for large breeds, the OMd value was high for all the premium dog foods. This should be taken into consideration when choosing the food. According to the NRC (2006), the typical CF content of dry pet food is 2.5% to 4.5%; but 9% to 10% for a reduced-calorie diet. The CF contents of the F-dog foods, L-dog foods and P-dog foods from the different commercial brands in the present study ranged from 2.3% to 7.6% (average 4.6%).

As a result, the premium type foods of the large adult dogs (for all types) generally have 3 840 kcal/kg ME values and an average of 25% CP, 10% EE, 7.4% ash, 4.6% CF, 60% CHO, 30% starch and 65 g CP/1 000 kcal contents, which were at the recommended minimum values according to the NRC (2006). It was also found that there was no correlation among the food price (in the general evaluation) and the digestibility and nutrient content for the all the premium dog foods. Generally, for all the premium adult dog foods, the gas production was positively correlated with the carbohydrate content; the OMd values were negatively correlated with the ash and fibre contents. However, the prices of the L-dog foods have a positive effect on their *in vitro* OMd and CP values. The present study indicated that the energy, nutrient and digestibility of the premium dog foods changed with the change in the variety and amount of the feedstuffs. The digestibility of the F-dog foods was higher than those of the other dog foods. The amount of protein that an adult dog will receive with 1 000 kcal of DM consumption of premium dog foods with fish meat and chicken meat varied among the brands. This point showed the need to pay attention to the food amount consumed and the energy-protein balance in the dog diets (dog foods with fish meat and chicken meat).

Acknowledgement

I would also like to thank the Editing Office of Erciyes University-Turkey for the English language review of this work.

Conflict of interest

The author declares no conflict of interest.

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Received: October 2, 2019
Accepted: March 21, 2020