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Comparison of fermentative digestion levels of processed different starch sources by Labrador Retrievers at different ages

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Abstract: Extruded commercial dog foods contain high levels of carbohydrates. The limited starch digestive capacity of dogs can change with age. The effectiveness of the extrusion (heat-steam pressure) process applied to raw/by-product feedstuffs (different starch sources in terms of starch digestion) may also differ. Therefore, in this study we determined the effects of age and the heat-steam pressure process on the *in vitro* digestion of different starch sources in dogs. The *in vitro* digestion was done in faecal inoculums from Labrador Retrievers of different ages (puppy; six months, mature; two years, and geriatric; eight years). The substrates (barley, corn, wheat, rice, oat and potato flours) were studied for *in vitro* digestion after both extrusion processes (processed; 2.4 bar and 134° C for 14 min) and a non-extrusion (unprocessed). The extrusion process generally increased the *in vitro* total gas production and true organic matter disappearance (T-OMd) (at 6–48 h) of barley, corn, wheat, rice, and oat flours ($P < 0.05$). The extrusion process increased T-OMd of potato flour at 6 h ($P = 0.005$), but did not change at 12–48 h ($P > 0.05$). The T-OMd at 6–12 h of barley flour by faecal inoculums of ≥ two-year-old dogs was higher than that of six-month-old dogs. The T-OMd and gas production of starch sources cumulatively increased with incubation time ($P < 0.05$). The molarities of acetic acid, butyric acid and toal volatile fatty acids in the fermentation fluids of barley, rice and wheat flours increased with the extrusion process or faecal inoculums of two- and eight-year-old dogs ($P < 0.05$). As a result, the extrusion process positively affected digestion of starch sources for medium to large breed dogs at ≥ six months of age. We advise that food meant for medium-size breed dogs that are six months and older should be made with more potato, oat and wheat flours rather than other sources.

Keywords: *in vitro* true digestibility; *in vitro* gas production; carbohydrate; dog; puppy; geriatric

Dogs are close to being omnivorous in contrast to cats, which are obligate carnivores, and thus, dog food contains more carbohydrates than feline diets. Companion animal diets may contain up to 50% starch, which is derived mainly from cereal grains. The starch in cereal grains is organised in concentric layers of semi-crystalline or amorphous regions in the endosperm. Starch is a non-structural plant storage polysaccharide (α-glucan), and it is the main carbohydrate in cereal grains (Bauch-Knudsen 1997). It is composed of amylose (a linear glucose chain with α-1,4-glucosidic linkages) and amylopectin (a branched glucose polymer containing both α-1,4- and α-1,6-linkages) (NRC 2006).

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The structural features and components of starch substances are associated with the starch granule (Tran et al. 2008). The National Research Council (NRC 2006) has stated that oat grain (40%), barley grain (50–52%), wheat grain (58–62%), corn grain (63–73%), rice grain (81%) and dried potatoes (65%) include high dietary starch. The α-amylases in the duodenal cavity of the dog are major enzymes involved in degrading the α-1,4-bonds of amylose, amylopectin and maltodextrins of the starch molecule (Colonna et al. 1992). The activity of this enzyme is higher in dogs than in cats and canine amylase activity is more sensitive to the dietary levels of total starch (TS), which are composed of non-resistant starch (N-RS) and resistant starch (RS). The N-RS is completely hydrolysed in the small intestine and its rate of digestion is influenced by the source (Kienzle 1993; van der Meulen et al. 1997). The extrusion process in dog foods causes swelling and rupture of the granules, modification of the crystalline spectra, an increase in cold water solubility, reduction in viscosity of the starch and (partial to complete) release of amylose and amylopectin (Tran et al. 2008). The organic matter disappearance was higher for extruded potato starch and cereal grains than for their native counterparts with the exception of wheat flour, as determined by Murray et al. (2001). These findings were similar to those of Bednar et al. (2001). The results of previous researchers demonstrated that *in vitro* microbial fermentation of starches is affected by differences in starch source, fraction and process (Bednar et al. 2001; Murray et al. 2001). Moreover, amylase activity (7.1–1623 IU/g) in puppies (four to ten months of age) is lower than that of a two-year-old dog (4665 IU/g), demonstrating an age-related ability of dogs to digest starch (NRC 2006). However, there is not enough information concerning dogs of different ages. Even in the "Nutrient Requirements of Dogs and Cats" of The National Academies Press (NRC 2006) or in publications of other international scientific committees, such as "TheAssociation of American Feed Control Officials" and "The Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs" (AAFCO 2008; FEDIAF 2013), information is lacking concerning digestion proportions and digestion end-products for different types of carbohydrate-rich feedstuffs which are used in commercially extruded dry foods or home-made foods.

The *in vitro* gas production technique was first carried out using dog faecal inoculums by Sunvold et al. (1995a). This *in vitro* technique is a modification an *in vitro* method for humans (Titgemeyer et al. 1991) and was first used to determine the digestion of fibrous compounds in the human digestive tract. This technique is different from the one used for herbivores (i.e. ruminants and rabbits). Recently, the *in vitro* digestion values and digestion products of feedstuffs used in dog foods have been studied using the aforementioned technique of dog faecal inoculums and dog gut ingredients (Bednar et al. 2001; Cutrignelli et al. 2009; Bosch et al. 2013). The *in vitro* gas production studies based on dog faecal inoculums have been focused on feedstuffs including high carbohydrates. Sunvold et al. (1995b) stated that the results of *in vitro* digestion studies, which were performed using faecal inoculums of an English Pointer (no age given), were similar to and reflected the results of *in vivo* digestion study. Bednar et al. (2001) showed that the microbial fermentation described in an *in vitro* digestion study carried out with dogs (mixed breeds and no ages given) mirrored those of an *in vivo* study in terms of starch and fibre degradability. Moreover, our preliminary study showed that heat-steam pressure processing of starch sources positively influenced *in vitro* digestion indicators and also that *in vitro* starch digestion of puppy dogs was lower than that of older dogs (Kara et al. 2016).

We hypothesised that the application of the extrusion process to different starch feedstuffs would have different effects on digestibility values in puppies, mature or geriatric dogs. In this case, an answer to the "Which starch source can we use at different ages?" question was sought. Thus, the aim of the present study was to determine and compare *in vitro* total gas production and *in vitro* true organic matter disappearance after incubation times of varying durations. Further, we wished to determine the volatile fatty acid composition of the digestive fluids of extruded and non-extruded cereal and potato flours using inoculums prepared from the faeces of dogs of different ages.

MATERIAL AND METHODS

The animal care procedures for the experiment and other processes were conducted under research protocol 14/021-2014 and were approved by the Local Ethics Committee for Animal Experiments of Erciyes University, Kayseri-Turkey.

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The starch sources. In the present study, non-structural carbohydrate sources, which are used in commercial dog food, such as barley, corn, wheat, rice, oat and potato flours were used. The feedstuff was purchased from a company in Kayseri, Turkey. The samples of feedstuff were "processed feedstuffs", i.e. extruded at 134 °C and 2.1 bar pressure for 14 minutes (processing time for 4 min + drying time for 10 min) using an autoclave (SM-23B, Elektro-Mag, Turkey) (Serrano 1997) and cooled under laboratory conditions. The processed flours hardened after cooling, and then they were milled to pass through a 1-mm sieve (IKA Werke, Staufen im Breisgau, Germany). The *in vitro* digestion technique was performed for "unprocessed feedstuff" (non-extruded or without heat and pressure) and "processed feedstuff" (or extruded feedstuffs) samples for the aforementioned feedstuffs.

The inoculum of dog faeces. The faecal samples used as an inoculum in the current study were obtained from two six-month-old, two two-year-old and two eight-year-old male Labrador Retriever dogs, which were kept in individual cages (2.50 × 2.50 × 1.25 m) at a kennel facility. The dogs were fed with commercial dry-type extruded dog food for four weeks before the faeces were collected. The puppies were fed with a commercial dry-type extruded dog food containing approximately 30% of crude protein (CP), 17% of ether extract (EE), 7% of ash and 2.5% of crude fibre (CF) on a dry matter (DM) basis. The mature dogs were fed a commercial extruded dog food containing approximately 25% crude protein, 15% ether extract, 8% ash and 3% crude fibre on a dry matter basis. The geriatric dogs were fed with approximately 18% crude protein, 10% ether extract, 8% ash and 5% crude fibre on a dry matter basis. The daily amount (g) given to the dogs was calculated individually each week according to live weight changes. The total daily amount of food was given as two meals for the puppies and one meal for the adult dogs. The daily metabolisable energy consumption of the dogs was calculated using the following formulas proposed for puppies (1) and adult dogs (2) by the National Research Council (NRC 2006):

$$ME = 130 \times BW^{0.75} \times 3.2 \times [e^{(-0.87 \times p)} - 0.1] \quad (1)$$

where:

ME – metabolisable energy (kcal/day);

p – BW_a/BW_m;

BW_a – actual body weight at time of evaluation (kg);

BW_m – expected mature body weight (kg);

e – base of natural log @ 2.718.

$$ME = 105 \times BW^{0.75} \quad (2)$$

where:

ME – metabolisable energy (kcal/day);

BW – body weight (kg).

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). Faecal samples were collected soon after defecation, were transferred into a thermos containing water at 39 °C and then transported to the laboratory. The samples were diluted at a 1 : 10 ratio with 0.9% sterile serum physiologic solution (Polifleks, Polifarma, Turkey) using a laboratory type blender (Waring Products Division, Torrington C.T., USA). 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 inoculum.

Chemical analyses of feedstuffs. Association of American Feed Control Officials (AOAC 1990) methods were used to determine dry matter, ash, crude protein, ether extract, and crude fibre composition in the different starch sources in the form of flours. Nitrogen-free extract was calculated according to the formulation:

$$NFE = DM - (EE + CP + ash + CF) \quad (3)$$

where:

NFE – nitrogen-free extract (%);

DM – dry matter (%);

EE – ether extract (%);

CP – crude protein (%);

CF – crude fibre (%).

Total starch, resistance starch and non-resistance starch contents of feedstuffs were analysed using Megazyme assay procedures (Bray Business Park, Bray, Co. Wicklow, Ireland).

For determination of starches, the 100 ± 5 mg samples of feedstuff were incubated in a shaking water bath with 4.0 ml of pancreatic α-amylase

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(10 mg/ml) containing amyloglucosidase (3 IU/ml) for 16 h at 37 °C, during which time the non-resistant starch was solubilised and hydrolysed to D-glucose. The reaction was terminated by addition of an equal volume of ethanol and the resistance starch was recovered as a pellet upon centrifugation. This was then washed twice with 4.0 ml of ethanol (99% and 50% v/v, respectively), and then centrifuged at 3000 rpm for 10 min. Free liquid was removed by decantation. At the end of this procedure, hydrolysis and solubilisation of non-resistance starch was completed.

For measurement of resistance starch, 2 ml of 2.0 M KOH were added to each tube to re-suspend the pellets (and dissolve the resistance starch) by stirring for 20 min in a cold water bath over a magnetic stirrer. This solution was neutralised with 8 ml of 1.2 M sodium acetate buffer (pH 3.8) and the starch was quantitatively hydrolysed to glucose with 0.1 ml of amyloglucosidase. The D-glucose was measured with glucose oxidase/peroxidase reagent and this was a measure of the resistance starch content of the sample.

For measurement of non-resistant starch, the supernatant solutions obtained on centrifugation of the initial incubation were combined with the supernatants obtained from the subsequent two 50% ethanol washes and the volume was adjusted to 100 ml with 100 mM sodium acetate buffer (pH 4.5) in a volumetric flask. The 0.1 ml aliquots of this solution (in duplicate) were incubated with 10 µl of dilute amyloglucosidase solution (300 IU/ml) in 100 M sodium malate buffer (pH 6.0) for 20 min at 50 °C. Then, 3.0 ml of glucose oxidase/peroxidase reagent was added, and the tubes were incubated for another 20 min at 50 °C. The absorbance of liquid was measured at 510 nm against a reagent blank, and the content of non-resistant starch was calculated. The total starch was calculated as:

$$TS = RS + N\text{-}RS \quad (4)$$

where:

TS – total starch;

RS – resistance starch;

N-RS – non-resistance starch.

The *in vitro* digestion technique. As inoculum, fresh faecal samples were used. In the *in vitro* digestion technique, the starch sources (substrates) were incubated with faecal inoculum and fermentation medium, which contained solution A, solution B, trace mineral solution, water-soluble vitamins, folate:biotin solution, riboflavin solution, hemin solution, short-chain fatty acids, resazurine, yeast extract, trypticase, Na_2CO_3 and cysteine $HCl*H_2O$ (Table 1) (Sunvold et al. 1995a; Bosch et al. 2008). The 310 ± 10 mg dry matter of feedstuff samples were incubated with 30 ml of fermentation medium mixture and 1 ml of fresh faecal inoculum in a 100-ml aerobic glass fermenter (Model Fortuna, Haberle Labortechnik, Germany). The *in vitro* gas production was carried out separately for both extruded and non-extruded feedstuffs and faecal inoculums of dogs at three different ages (six months, two years and eight years). The initial volumes of the

Table 1. Composition of *in vitro* fermentation medium

Component	Amount
Solution A[a] (ml/l)	330.0
Solution B[b] (ml/l)	330.0
Trace mineral solution[c] (ml/l)	10.0
Water-soluble vitamins[d] (ml/l)	20.0
Folate: biotin solution[e] (ml/l)	5.0
Riboflavin solution[f] (ml/l)	5.0
Hemin solution[g] (ml/l)	2.5
Short-chain fatty acids[h] (ml/l)	0.4
Resazurine[i] (ml/l)	1.0
Distilled H_2O (ml/l)	296.0
Yeast extract (g/l)	0.5
Trypticase (g/l)	0.5
Na_2CO_3 (g/l)	4.0
Cysteine $HCl*H_2O$ (g/l)	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 tetraacetic 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; $Na_2MoO_4*2H_2O$, 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, isovalerate, isobutyrate and DL alpha-methylbutyrate, 250 ml/l

[i]Composition: 1 g resazurine/l distilled water

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fermenters were recorded and incubated in a water bath with a thermostat (Yapar Stainless Steel, Turkey) at 39.0 ± 0.2 °C up to 48 hours. The *in vitro* gas production was performed with six replicates (fermenter) per feedstuff sample. In addition, six blank fermenters (no template = medium mixture plus faecal inoculum) were used to calculate the total gas production.

Determination of cumulative gas production. The total cumulative gas volume of the incubated samples was recorded from the calibrated scale on the syringe at 6, 12, 24 and 48 hours.

Determination of *in vitro* true organic matter disappearance and volatile fatty acid values. For the *in vitro* true organic matter disappearance (T-OMd) and determination of feedstuffs, incubation of the *in vitro* fermenter was stopped at 6, 12, 24 and 48 hours. The *in vitro* fermenters were stopped at 12 and 24 h, and then the volatile fatty acid concentrations in the fermentation fluids were analysed.

The *in vitro* T-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; the residual was burned at 550 °C. *In vitro* T-OMd was calculated as:

$$\text{T-OMd} = 1 - [(\text{OM residue} - \text{OM blank}) / \text{initial OM})] \times 100 \quad (5)$$

where:
T-OMd – true organic matter disappearance;
OM – organic matter.

Fermentation of the approximately 10 ml of digestive fluid in the fermenter was halted at 12 and 24 h and samples were collected in Falcon tubes. The digestive fluid was filtered through four layers of muslin; 2 ml of the sample were mixed with 0.5 ml of 25% (w/v) meta-phosphoric acid and kept frozen (–20 °C) in microcentrifuge tubes. The frozen samples were thawed at 4 °C and centrifuged at 15 000 *g* for 15 min using a microcentrifuge (Gyrozen 1524, Daejeon, Republic of Korea). Then, the supernatants were filtered using a filter apparatus with a 0.22-mm pore diameter (Millex Filter Unit, Merck Millipore Ltd, Ireland), and filtrates were transferred into vials (Chromacol, USA). Analysis of the volatile fatty acid was carried out using a gas chromatograph (TRACE^{TM} 1300, Thermo Scientific, USA), which was equipped with a flame ionization detector with an auto sampler (AI 1310, Thermo Scientific, USA) and polyethylene glycol columns (length: 60 m, i.d: 0.25 mm × 0.25 µm, film thickness: 0.25 µm) (TG-WAXMS, Thermo Scientific, USA). The carrier gas was helium at a constant flow rate of 1.5 ml/min. The injection volume was 0.5 µl. The samples were injected via split mode. The injection port temperature was 280 °C. The oven temperature was programmed to increase from 160 °C to 180 °C at a rate of 20 °C/min. The airflow was 350 ml/min, and hydrogen flow was 35 ml/min. The temperature of the flame ionization detector was 300 °C. Oven run time was 10 min. The concentrations of volatile fatty acid as mmol/l were identified using the Xcalibur^{TM} software program (Thermo Fisher Scientific, USA). The percentage of volatile fatty acid [acetic (A), butyric (B), and propionic (P) acids], and A/P and (A + B)/P ratios were determined (Ersahince and Kara 2017).

Statistical Analysis. The experimental data were first subjected to Levene's test to detect the homogeneity of variance. Multivariate analyses were implemented for homogeneous variances using general linear model procedures to test treatment differences. Data for *in vitro* dog digestion were analysed using a randomised complete design with each process (extruded or non-extruded) × three different inoculums (six months, two years and eight-year-old dogs).

The data were analysed using the following statistical model:

$$Y_{ijk} = \mu + E_i + D_j + ED_{ij} + e_{ijk} \quad (6)$$

where:
Y_{ijk} – the dependent variable;
μ – overall mean;
E_i – effect of i – process on the observed parameters;
D – effect of j – inoculums on the observed parameters;
ED_{ij} – interaction between the i – process and j – inoculums;
e_{ijk} – the standard error term.

The means were separated by Tukey's multiple range test at $P < 0.05$. Because extruded processing × inoculum interactions were significant for the parameters, data were then analysed among extruded processing and inoculum using one-way

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Table 2. Chemical compositions of different starch sources used in the study

	DM	Ash	EE	CP	CF	RS	N-RS	TS	NFE	NFE-TS
Barley flour	95.2	2.0	1.1	12.7	1.5	0.0	74.7	74.8	82.5	7.7
Corn flour	95.5	0.5	1.4	9.2	0.6	0.2	45.1	45.4	88.2	42.8
Oat flour	96.6	1.2	1.2	9.3	1.2	0.2	58.4	58.7	86.9	28.2
Potato flour	96.0	3.0	0.2	10.5	0.9	1.3	54.4	55.7	85.2	29.4
Rice flour	94.8	0.9	0.2	8.5	0.2	3.6	63.8	67.5	90.1	22.6
Wheat flour	95.6	0.6	0.8	9.8	0.5	0.4	73.3	73.7	88.3	14.5

CF = crude fibre; CP = crude protein as % in DM; DM = dry matter as % in feedstuff; EE = ether extract as % in DM; NFE = nitrogen-free extract as % in DM; NFE-TS = nitrogen-free extract – total starch as % in DM; N-RS = non-resistance starch as % in DM; RS = resistance starch as % in DM; TS = total starch as % in DM

variance analyses. Orthogonal polynomial contrasts were used to examine the linear and quadratic effects of increasing times of incubation.

Analyses were performed using SPSS 17.0 software (IBM Corp., USA).

RESULTS

Chemical compositions of starch sources

The total starch, resistant starch, non-resistant starch , nitrogen-free extract and other nutrient compositions of starch sources used in the study are given in Table 2.

In vitro total gas production

The *in vitro* total gas production of barley flour up to 48 h was positively affected by heat-pressure processing, except for the first 6 hours. Gas levels produced by barley flour with the eight-years-old dog's inoculum at 12 h and after 12 h of incubation were higher than those of the six-month-old dogs and two-year-old dogs (Table 3).

Table 3. *In vitro* cumulative total gas production and true organic matter disappearance of barley flour after *in vitro* incubations of varying duration in dogs

		Incubation times for barley flour							
		in vitro cumulative total gas production (ml/0.3 g DM)				*in vitro* true-organic matter disappearance (%)			
		6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Non-extruded	6 months	0.0	10.5	44.6^{B}	83.6^{B}	23.5^{C}	24.2^{B}	52.5	55.4
	2 years	0.4	21.7	65.6^{A}	86.9^{B}	22.6^{C}	34.6^{B}	50.9	53.9
	8 years	0.9	26.0	77.2^{A}	82.9^{B}	25.0^{C}	31.3^{B}	46.1	47.0
Extruded	6 months	0.4	54.7	75.1^{A}	80.0^{B}	43.1^{B}	57.6^{A}	58.7	65.7
	2 years	0.0	54.0	69.2^{A}	78.4^{B}	53.4^{AB}	60.6^{A}	63.9	68.8
	8 years	0.4	62.0	80.0^{A}	109.3^{A}	56.2^{A}	63.9^{A}	63.5	66.7
Inoculum	6 months	0.2	32.6^{c}	59.9^{b}	81.8^{b}	33.3^{b}	40.9^{b}	57.0	59.1
	2 years	0.2	37.9^{b}	67.4^{b}	80.3^{b}	38.0^{a}	47.6^{a}	58.9	59.8
	8 years	0.7	44.0^{a}	78.6^{a}	96.1^{a}	40.6^{a}	47.6^{a}	54.8	56.9
Processing	non-extruded	0.4	19.4	62.5	78.9	23.7	30.0	51.8	50.1
	extruded	0.3	56.9	74.8	93.3	50.9	60.7	62.0	67.1
SEM		0.50	4.15	3.04	3.57	1.84	2.93	4.20	2.39
P-value	processing	0.732	< 0.001	0.003	0.003	< 0.001	< 0.001	0.025	0.012
	inoculum	0.571	0.047	0.002	0.008	0.020	0.039	0.653	0.541
	processing × inoculum	0.604	0.394	0.006	0.050	0.033	0.029	0.313	0.201

DM = dry matter; SEM = standard error of the mean

Extrusion processing: 134 °C and 2.1 bar pressure for 14 minutes (4 minutes holding time + 10 minutes drying time)

^{a,b}Differences among means indicated with different superscripts in same column for "inoculum" are statistically significant

$^{A–C}$Differences among means indicated with different superscripts in the same column for "processing × inoculum" interaction are statistically significant

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The *in vitro* total gas produced by corn flour with two- and eight-year-old dog inoculums were generally higher than that of the six-month-old dog's inoculum. In addition, *in vitro* total gas production of corn flour was positively affected by the extrusion process. The heat pressure processing in corn flour was five to seven times higher than the *in vitro* gas production at 12 h (Table 4).

Heat pressure processing increased the *in vitro* total gas production after incubations of 12–48 h of wheat flour. The *in vitro* gas production values of wheat flour (for both extruded and non-extruded flours) at 48 h were similar for different inoculums of faeces. For incubation times of 12–24 h, *in vitro* gas production in wheat flour in which the extrusion process was performed with faecal inoculums from two- and eight-year-old dogs were higher than those with inoculums from six-month-old dogs (Table 5).

The levels of *in vitro* total gas production of rice flour with different inoculums were as follows: eight years > two years > six months at 12 h (16.30–49.43 ml/0.3 g DM) and 24 h (65.93–2.97 ml/0.3 g DM) incubations (Table 6).

The *in vitro* total gas produced by oat flour increased with extrusion process, but the values for extruded oat flour after 12–24 h incubations were similar for faecal inoculums of dogs of different ages.

Potato flour had not produced gas after 6 h of incubation. The *in vitro* gas production of potato flour at 24 h was affected by the heat pressure process, age and heat pressure*age interaction. The *in vitro* gas production of potato flour did not change with inoculums from dogs of different ages or the heat pressure process for 24 hours. The volumes of gas produced by potato flour for faecal inoculums of geriatric dogs at 48 h were higher than those of faecal inoculums of the two-year-old dogs.

Notably, the levels of total gas produced by barley, corn, wheat, rice, oat and potato flours were higher with increasing processing incubation times (for both extruded and non-extruded flours or different faecal inoculums) (Tables 3–8).

Generally, the extrusion process increased the *in vitro* total gas production of barley, corn, wheat, rice and oat flours (Tables 3–7).

Table 4. *In vitro* cumulative total gas production and true organic matter disappearance of corn flour after *in vitro* incubations of varying duration in dogs

		Incubation times for corn flour							
		in vitro cumulative total gas production (ml/0.3 g DM)				*in vitro* true-organic matter disappearance (%)			
		6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Non-extruded	6 months	0.9	1.3^{C}	8.2^{D}	53.7^{B}	20.9^{B}	22.6^{D}	24.1^{C}	61.6^{AB}
	2 years	0.9	3.2^{C}	29.0^{C}	71.9^{B}	25.6^{B}	33.5^{DC}	43.2^{B}	63.5^{AB}
	8 years	0.4	4.4^{C}	22.1^{C}	87.4^{A}	22.3^{B}	26.7^{D}	36.6^{BC}	50.5^{B}
Extruded	6 months	0.8	2.1^{C}	56.4^{B}	84.9^{A}	31.6^{AB}	46.6^{BC}	61.9^{A}	63.8^{AB}
	2 years	0.0	22.0^{B}	75.3^{A}	79.6^{A}	47.7^{A}	63.0^{A}	66.6^{A}	80.7^{A}
	8 years	0.0	42.1^{A}	81.5^{A}	105.4^{A}	44.6^{A}	59.9^{AB}	63.9^{A}	68.5^{AB}
Inoculum	6 months	0.8^{a}	1.7^{c}	32.3^{b}	69.3^{b}	26.3^{b}	34.6^{b}	43.0^{b}	62.7
	2 years	0.4^{ab}	12.6^{b}	52.1^{a}	75.7^{ab}	36.6^{a}	46.7^{a}	53.6^{a}	72.1
	8 years	0.2^{b}	23.2^{a}	51.8^{a}	96.4^{a}	33.4^{ab}	44.8^{a}	51.6^{a}	59.5
Processing	non-extruded	0.7	3.0	19.8	71.0	22.9	27.6	34.7	58.5
	extruded	0.2	22.1	71.1	90.0	41.3	56.5	64.1	71.0
SEM		0.17	2.84	3.61	7.50	2.95	2.74	2.74	4.14
P-value	processing	0.018	< 0.001	< 0.001	0.021	< 0.001	< 0.001	< 0.001	0.010
	inoculum	0.022	0.001	0.004	0.029	0.032	0.009	0.018	0.053
	processing × inoculum	0.131	0.002	0.224	0.359	0.002	< 0.001	< 0.001	0.029

DM = dry matter; SEM = standard error of the mean

Extrusion processing: 134 °C and 2.1 bar pressure for 14 minutes (4 minutes holding time + 10 minutes drying time)

$^{a–c}$Differences among means indicated with different superscripts in the same column for "inoculum" are statistically significant

$^{A–D}$Differences among means indicated with different superscripts in the same column for "processing × inoculum" interaction are statistically significant

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Table 5. *In vitro* cumulative total gas production and true organic matter disappearance of wheat flour after *in vitro* incubations of varying duration in dogs

		Incubation times for wheat flour							
		in vitro cumulative total gas production (ml/0.3 g DM)				*in vitro* true-organic matter disappearance (%)			
		6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Non-extruded	6 months	0.8	2.4^{C}	6.9^{C}	61.2^{B}	21.7^{B}	26.0^{B}	31.1^{B}	76.5^{A}
	2 years	0.9	5.0^{C}	11.8^{C}	39.8^{B}	20.5^{B}	28.0^{B}	35.5^{B}	77.5^{A}
	8 years	0.4	2.9^{C}	9.3^{C}	53.6^{B}	23.2^{B}	26.2^{B}	33.6^{B}	60.6^{B}
Extruded	6 months	0.0	16.3^{B}	72.9^{B}	107.8^{A}	34.6^{B}	66.0^{A}	70.2^{A}	76.8^{A}
	2 years	0.4	69.3^{A}	97.6^{A}	118.1^{A}	58.7^{A}	69.8^{A}	71.2^{A}	77.4^{A}
	8 years	0.4	75.2^{A}	97.1^{A}	116.0^{A}	54.5^{A}	67.1^{A}	70.4^{A}	79.3^{A}
Inoculum	6 months	0.4	9.4^{b}	39.9^{b}	84.5	28.2^{b}	46.0	50.6	76.7
	2 years	0.7	37.1^{a}	54.7^{a}	78.9	39.6^{a}	48.9	53.4	77.5
	8 years	0.4	39.1^{a}	53.2^{a}	84.8	38.9^{a}	46.7	52.0	69.9
Processing	non-extruded	0.7	3.4	9.3	51.5	21.8	26.7	33.4	71.5
	extruded	0.3	53.6	89.2	114.0	49.3	64.3	70.6	77.9
SEM		0.31	3.98	3.09	7.65	2.01	2.13	2.49	2.34
P-value	processing	0.143	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.016
	inoculum	0.611	0.001	0.006	0.703	0.007	0.140	0.280	0.134
	processing × inoculum	0.461	0.001	0.023	0.196	0.007	< 0.001	< 0.001	0.011

DM = dry matter; SEM = standard error of the mean
Extrusion processing: 134 °C and 2.1 bar pressure for 14 minutes (4 minutes holding time + 10 minutes drying time)
^{a,b}Differences among means indicated with different superscripts in the same column for "inoculum" are statistically significant
$^{A–C}$Differences among means indicated with different superscripts in the same column for "processing × inoculum" interaction are statistically significant

Table 6. *In vitro* cumulative total gas production and true organic matter disappearance of rice flour after *in vitro* incubations of varying duration in dogs

		Incubation times for rice flour							
		in vitro cumulative total gas production (ml/0.3 g DM)				*in vitro* true-organic matter disappearance (%)			
		6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Non-extruded	6 months	0.4	16.8^{D}	57.9^{C}	113.5	14.7^{C}	21.9^{B}	53.8^{BC}	58.4^{B}
	2 years	0.9	22.2^{CD}	73.6^{BC}	108.8	15.7^{C}	25.2^{B}	58.4^{BC}	68.1^{B}
	8 years	0.5	37.6^{BC}	98.4^{AB}	130.5	17.5^{C}	26.1^{B}	49.1^{C}	71.7^{B}
Extruded	6 months	0.4	16.7^{D}	73.9^{BC}	107.8	39.3^{B}	68.0^{A}	72.1^{AB}	86.8^{A}
	2 years	0.4	45.5^{AB}	92.3^{AB}	98.2	67.5^{A}	76.4^{A}	85.5^{A}	87.4^{A}
	8 years	0.4	61.2^{A}	107.4^{A}	125.4	64.4^{A}	74.7^{A}	85.7^{A}	86.3^{A}
Inoculum	6 months	0.4	16.3^{c}	65.9^{c}	110.7	27.0^{b}	44.9	62.9	72.6
	2 years	0.7	33.8^{b}	82.9^{b}	103.5	41.6^{a}	50.8	71.9	77.8
	8 years	0.5	49.4^{a}	102.9^{a}	127.9	40.9^{a}	50.4	67.7	78.7
Processing	non-extruded	0.6	25.5	76.6	117.6	16.0	24.4	53.8	66.1
	extruded	0.4	41.1	91.2	110.5	57.1	73.0	81.3	86.6
SEM		0.41	3.28	5.42	8.73	1.62	2.26	3.31	2.41
P-value	processing	0.610	0.007	0.017	0.356	< 0.001	< 0.001	< 0.001	< 0.001
	inoculum	0.788	< 0.001	< 0.001	0.074	< 0.001	0.071	0.090	0.090
	processing × inoculum	0.804	0.002	0.018	0.942	< 0.001	< 0.001	0.026	0.041

DM = dry matter; SEM = standard error of the mean
Extrusion processing: 134 °C and 2.1 bar pressure for 14 minutes (4 minutes holding time + 10 minutes drying time)
^{a,b}Differences among means indicated with different superscripts in the same column for "inoculum" are statistically significant
$^{A–D}$Differences among means indicated with different superscripts in the same column for "processing × inoculum" interaction are statistically significant

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Table 7. *In vitro* cumulative total gas production and true organic matter disappearance of oat flour after *in vitro* incubations of varying duration in dogs

		Incubation times for oat flour							
		in vitro cumulative total gas production (ml/0.3 g DM)				*in vitro* true-organic matter disappearance (%)			
		6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Non-extruded	6 months	0.9	1.9^{B}	8.0^{B}	67.3	23.6^{C}	30.9^{B}	44.6^{C}	61.6
	2 years	0.5	6.2^{B}	31.3^{B}	67.5	24.4^{BC}	32.5^{B}	46.6^{C}	63.2
	8 years	0.4	6.3^{B}	29.6^{B}	104.9	24.3^{BC}	28.2^{B}	49.6^{BC}	67.9
Extruded	6 months	0.0	55.2^{A}	79.6^{A}	87.8	33.3^{BC}	60.8^{A}	65.4^{AB}	73.4
	2 years	0.5	60.8^{A}	72.5^{A}	87.7	59.2^{A}	64.7^{A}	66.6^{AB}	71.6
	8 years	0.5	62.0^{A}	74.7^{A}	100.3	42.5^{AB}	58.6^{A}	68.0^{A}	73.4
Inoculum	6 months	0.5	28.5	43.8	77.5^{b}	28.4^{b}	45.8	55.0	67.5
	2 years	0.5	33.5	51.9	77.6^{b}	41.8^{a}	48.6	56.6	67.4
	8 years	0.5	34.1	52.1	98.9^{a}	33.5^{ab}	43.4	58.8	70.7
Processing	non-extruded	0.6	4.8	23.0	79.9	24.1	30.5	46.9	64.2
	extruded	0.3	59.3	75.6	89.4	45.0	61.4	66.6	72.8
SEM		0.37	2.76	4.19	6.34	3.29	2.60	3.20	3.41
P-value	processing	0.346	< 0.001	< 0.001	0.117	< 0.001	< 0.001	< 0.001	0.022
	inoculum	0.999	0.164	0.157	0.023	0.019	0.210	0.520	0.586
	processing × inoculum	0.397	0.917	0.021	0.068	0.024	< 0.001	0.005	0.680

DM = dry matter; SEM = standard error of the mean

Extrusion processing: 134 °C and 2.1 bar pressure for 14 minutes (4 minutes holding time + 10 minutes drying time)

[a,b]Differences among means indicated with different superscripts in the same column for "inoculum" are statistically significant

[A–C]Differences among means indicated with different superscripts in the same column for "processing × inoculum" interaction are statistically significant

In vitro true organic matter disappearance

The T-OMd of barley, corn, wheat, rice and oat flours were high for the extruded process 6 h, 12 h, 24 h and 48 h (Tables 3–8). Otherwise, for *in vitro* incubation of potato flour, the extrusion process did not change the T-OMd at 12h, 24h or 48 h; an increased was observed at 6 hours. The T-OMd of barley flour with faecal inoculums of two- and eight-year-old dogs at 6 h and 12 h was higher than that of faecal inoculums obtained from dogs at six months of age (Table 8).

The T-OMd levels of barley, corn, wheat, rice, oat and potato flour accumulatively increased with increasing incubation time. For 12 h and 24 h of incubation, the T-OMd of corn flour with the faecal inoculums of two-year-old dogs was higher than those of other inoculums. In the first 6 h of incubation, the T-OMd values of extruded wheat, rice and oat flours with faecal inoculums of two- and eight-year-old dogs were higher than for the puppy faecal inoculum, but other times for the extrusion process were similar. The T-OMd values of potato flour obtained for inoculums from dogs of different ages were similar at each incubation time.

The organic acid composition of *in vitro* digestive fluid

The molarities of acetic acid, butyric acid and volatile fatty acid in the fermentation fluid of barley and wheat flours at 24 h of incubation increased with the extrusion process or the faecal inoculum of two- and eight-year-old dogs (Table 9). The molarities of these acids in the fermentation fluid for potato flour were decreased by the extrusion process (Table 10).

The molarities of acetic acid, propionic acid, butyric acid and volatile fatty acid in the fermentation fluid of corn flour after 24 h of incubation was not affected by the extrusion process or the identity of the faecal inoculum (Table 9).

The molarities of acetic acid, butyric acid and volatile fatty acid in the fermentation fluid of rice flour after 24 h of incubation increased with the extrusion process. Therefore, the molarity of propionic acid in the fermentation fluid of rice flour was decreased by the extrusion process or the inoculums of mature and geriatric dogs. The molarities of acetic acid in the fermentation fluid of oat flour after 24 h of incubation was increased

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Table 8. *In vitro* cumulative total gas production and true organic matter disappearance of potato flour after *in vitro* incubations of varying duration in dogs

		Incubation times for potato flour							
		in vitro cumulative total gas production (ml/0.3 g DM)				*in vitro* true-organic matter disappearance (%)			
		6 h*	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Non-extruded	6 months	0.0	18.9^{D}	77.0	81.6^{B}	58.4	74.4^{B}	78.8	86.2
	2 years	0.0	59.1^{AB}	80.3	84.9^{B}	66.3	78.3^{AB}	79.5	85.9
	8 years	0.0	70.6^{A}	99.2	112.2^{A}	66.3	79.3^{AB}	79.2	86.9
Extruded	6 months	0.0	32.8^{CD}	85.3	92.7^{AB}	75.2	78.1^{AB}	80.7	84.3
	2 years	0.0	47.3^{ABC}	75.7	83.0^{B}	78.2	81.3^{A}	81.4	84.6
	8 years	0.0	37.3^{BCD}	70.5	91.5^{AB}	77.4	81.4^{A}	81.9	84.4
Inoculum	6 months	0.0	25.8^{b}	81.1	87.2^{b}	66.8	76.3	79.8	85.2
	2 years	0.0	53.2^{a}	77.9	83.9^{b}	72.3	79.8	80.4	85.3
	8 years	0.0	53.9^{a}	84.8	101.8^{a}	71.9	80.4	80.5	85.6
Processing	non-extruded	0.0	49.5	85.5	92.9	63.7	77.3	79.1	86.4
	extruded	0.0	39.1	77.1	89.0	76.9	80.2	81.3	84.4
SEM			4.36	5.78	4.35	3.78	0.97	1.02	1.01
P-value	processing		0.027	0.127	0.320	0.005	0.211	0.146	0.057
	inoculum		0.001	0.531	0.013	0.343	0.312	0.736	0.914
	processing × inoculum		0.005	0.058	0.029	0.728	0.018	0.899	0.851

DM = dry matter; SEM = standard error of the mean
Extrusion processing: 134 °C and 2.1 bar pressure for 14 minutes (4 minutes holding time + 10 minutes drying time)
*Potato flour did not produce gas until 6 h of incubation
[a,b]Differences among means indicated with different superscripts in the same column for "inoculum" are statistically significant
[A–D]Differences among means indicated with different superscripts in the same column for "processing × inoculum" interaction are statistically significant

Table 9. Volatile fatty acid compositions (mM/l) of dog digestive fluid with barley, corn and wheat flours after 24 h of *in vitro* incubation

		Barley flour				Corn flour				Wheat flour			
		AA	PA	BA	VFA	AA	PA	BA	VFA	AA	PA	BA	VFA
Non-extruded	6 months	48.0^{C}	24.9	8.3^{C}	81.3^{C}	47.8^{B}	24.8^{B}	9.7^{A}	82.4^{B}	47.1^{B}	24.9	8.9^{B}	81.0^{B}
	2 years	51.1^{AB}	25.0	12.4^{B}	88.5^{BC}	49.4^{A}	24.8^{A}	9.4^{A}	83.7^{A}	47.7^{B}	24.8	8.3^{B}	80.9^{B}
	8 years	50.0^{BC}	24.8	11.4^{B}	86.3^{C}	48.2^{A}	24.9^{A}	8.9^{B}	82.2^{B}	47.9^{B}	24.8	9.2^{B}	81.9^{B}
Extruded	6 months	52.6^{A}	24.7	13.1^{AB}	90.5^{AB}	47.8^{B}	24.7^{B}	9.0^{A}	81.6^{C}	50.2^{A}	24.7	12.7^{A}	87.7^{A}
	2 years	51.7^{AB}	24.7	12.9^{B}	89.5^{B}	47.6^{B}	24.7^{B}	8.8^{B}	81.3^{C}	49.9^{A}	24.7	12.6^{A}	87.2^{A}
	8 years	52.8^{A}	24.7	15.1^{A}	92.7^{A}	49.0^{A}	24.7^{B}	10.4^{A}	84.2^{A}	50.7^{A}	24.7	14.2^{A}	89.7^{A}
Inoculum	6 months	50.3^{b}	24.8	10.7^{b}	85.9^{b}	47.8	24.7	9.3	82.0	48.6	24.8	10.8	84.3
	2 years	51.4^{a}	24.8	12.7^{a}	89.0^{a}	48.5	24.8	9.1	82.5	48.8	24.7	10.4	84.0
	8 years	51.4^{a}	24.8	13.3^{a}	89.5^{a}	48.6	24.8	9.7	83.2	49.3	24.7	11.7	85.8
Processing	non-extruded	49.7	24.9	10.7	85.4	48.5	24.8	9.3	82.7	47.5	24.8	8.8	81.3
	extruded	52.3	24.7	13.7	90.9	48.1	24.7	9.4	82.4	50.3	24.7	13.2	88.2
SEM		0.20	0.02	0.21	0.29	0.17	0.01	0.13	0.23	0.23	0.03	0.23	0.46
P-value	processing	< 0.001	0.220	< 0.001	< 0.001	0.236	0.203	0.690	0.347	< 0.001	0.054	< 0.001	< 0.001
	inoculum	0.034	0.372	0.001	0.001	0.064	0.187	0.138	0.064	0.320	0.466	0.053	0.145
	processing × inoculum	0.004	0.340	0.003	< 0.001	0.013	0.023	0.005	0.004	0.001	0.627	0.004	0.665

AA = acetic acid; BA = butyric acid; PA = propionic acid; SEM = standard error of the mean; VFA = total volatile fatty acids (acetic + propionic + butyric acids)
[a,b]Differences among means indicated using different superscripts in the same column for "inoculum" are statistically significant
[A–C]Differences among means indicated using different superscripts in the same column for "processing × inoculum" interaction are statistically significant

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Table 10. Volatile fatty acid compositions (mM/l) of dog digestive fluid with rice flour, oat flour and potato flour after 24 h of *in vitro* incubation

		Rice flour				Oat flour				Potato flour			
		AA	PA	BA	VFA	AA	PA	BA	VFA	AA	PA	BA	VFA
Non-extruded	6 months	50.3^{B}	25.3^{A}	12.9	88.5	51.9^{A}	25.1	12.4	89.5	51.6	24.8	13.6	90.0
	2 years	50.6^{B}	25.0^{A}	13.2	88.8	50.9^{B}	25.0	11.5	87.4	51.4	24.7	12.9	89.1
	8 years	52.2^{B}	24.8^{A}	16.4	93.4	50.6^{B}	25.0	12.1	87.7	53.2	24.8	15.1	93.0
Extruded	6 months	52.1^{B}	24.8^{A}	16.1	93.0	52.4^{A}	24.8	13.4	90.7	50.3	24.8	11.8	86.9
	2 years	51.0^{B}	24.7^{B}	13.3	89.1	50.8^{B}	24.8	12.0	87.6	50.0	24.8	12.2	86.9
	8 years	55.4^{A}	24.7^{B}	19.3	99.5	52.8^{A}	24.8	13.8	91.3	52.5	24.8	13.8	91.1
Inoculum	6 months	51.1^{ba}	25.0^{a}	14.5^{b}	90.8^{b}	52.2^{a}	25.0	12.9	90.1	51.0^{b}	24.8	12.7^{b}	88.4^{b}
	2 years	50.7^{b}	24.8^{b}	13.3^{b}	89.0^{b}	50.8^{b}	24.9	11.7	87.5	50.7^{b}	24.8	12.6^{b}	88.0^{b}
	8 years	53.8^{a}	24.7^{c}	17.9^{a}	96.5^{a}	51.7^{b}	24.9	12.9	89.5	52.8^{a}	24.8	14.5^{a}	92.0^{a}
Processing	non-extruded	51.0	25.0	14.2	90.3	51.2	25.0	12.0	88.2	52.0	24.8	13.9	90.7
	extruded	52.8	24.7	16.3	93.9	52.0	24.8	13.0	89.8	50.9	24.8	12.6	88.3
SEM		0.20	0.01	0.38	0.56	0.20	0.04	0.42	0.60	0.30	0.01	0.21	0.50
P-value	processing	0.001	0.001	0.009	0.004	0.027	0.009	0.140	0.101	0.038	0.201	0.004	0.014
	inoculum	0.001	0.001	0.001	0.001	0.026	0.356	0.243	0.099	0.012	0.101	0.003	0.006
	processing × inoculum	0.023	0.001	0.097	0.057	0.043	0.840	0.751	0.309	0.771	0.607	0.339	0.773

AA = acetic acid; BA = butyric acid; PA = propionic acid; SEM = standard error of the mean; VFA = total volatile fatty acids (acetic + propionic + butyric acids)

[a,b]Differences among means indicated using different superscripts in the same column for "inoculum" are statistically significant

[A–C]Differences among means indicated using different superscripts in the same column for "processing × inoculum" interaction are statistically significant

by the extrusion process, but decreased by the faecal inoculums of the two- and eight-year-old dogs. Potato flour had the highest acetic, butyric and total volatile acid values with the geriatric dog inoculums com pared to those in the other age inoculums (Tables 9 and 10).

DISCUSSION

We observed that the digestion levels of the dogs differed between different starch sources; the extruding process positively affected the digestion of the starch sources (barley flour, corn flour, rice flour and oat flour), which were not pre-treated before extrusion. The digestion level of potato flour, which was pre-treated before the extruding process, did not change much in response to the extruding process performed in the study. In addition, the type of starch source (grain or grain-free) in dog foods could be changed with the advancing age of dogs.

The extrusion technology in feed, as performed for production of commercial dry-type dog food, includes the water absorption of carbohydrates with steam and the use of a short high heat (80–130 °C) treatment. As a result of this process, substantial amounts of starch gelatinise and separate into dextrin subunits. The production of conserved dog food also includes a heat and pressure process and digestion is positively affected (Tran 2008; Tran et al. 2008). The results of the present study demonstrated that a warm pressure process increased *in vitro* gas production, as shown by digestion level after 24 h of incubation. Colonna et al. (1989) stated that heating (> 50 °C) starch in an excess of water and at lower moisture conditions with higher temperature (100 °C to 150 °C) irreversibly gelatinises the granular structure of starch and improves its solubilisation. The gas production volumes of non-extruded barley, corn, wheat and oat flours in faecal inoculums of adult and geriatric dogs were higher than those of puppies and this high gas volume increased even more with the extrusion process. The gas production of barley flour with puppy faecal inoculum up to 24 h of incubation was lower than those of the adult and geriatric dog faecal inoculums. This may be related to the inadequate microorganism count, which helps starch digestion and degrada-

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tion (Suchodolski et al. 2008; Garcia-Mazcorro and Minamoto 2013). The levels of gas production of extruded barley flour were also the same for faecal inoculums of the three dogs of different ages up to 24 h of incubation. This positive effect can be connected to the breakdown into dextrin subunits of starch molecules. Normally, dextrin polysaccharides, which form from monosaccharides connected with alfa links and in alfa configuration, occur during degradation of starch in digestive cannula or during technologies applied to some commercial feedstuffs/food (Case et al. 2011). In the current study, the highest digestion levels were reached by the faecal inoculums of six-month-old dogs from non-extruded feedstuffs of rice, oat and potato flours. Generally, the T-OMd for puppy inoculums of cereal and potato flours were lower than those of more mature dogs and can be related to the age-dependent ability of dogs to digest starch (NRC 2006).

According to the results of the current study, heat pressure treatment significantly increases the *in vitro* T-OMd levels of barley, wheat, corn and rice flours. The *in vitro* T-OMd level achieved for barley (the first 12 h), corn (the first 24 h), wheat (the first 6 h), rice (the first 6 h) and oat (the first 6 h) flours by faecal inoculums of two and eight-year-old dogs were higher than that of the six-month-old puppy. When the total effect of inoculums was evaluated, there was no difference among the T-OMd achieved by inoculations from dogs of different ages with barley (after the first 12 h), corn (after the first 24 h), wheat (after the first 6 h), rice (after the first 6 h) or oat flours (after the first 6 h) with cumulative incubation times.

In plants, starch is stored in crystalline intracellular bodies or starch granules. The shape and crystalline structure of these granules can be 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 pack in a hexagonal pattern) or C (containing both patterns). Cereal starch granules are predominantly the A-type and are easily degraded by α-amylase and hydrolysed in the gastrointestinal tract. The B-type starches of tubers (potato) and C-type legume starches are more resistant to enzymatic hydrolysis (Englyst et al. 1992). The potato flour was the non-extruded starch source with the highest T-DMd (58–86% for six-month-old dogs, 66–86% for two-year-old dogs and 66–87% for eight-year-old dogs) for all inoculums and all incubation times. When potato flour is obtained from potatoes, it is thought that it can pre-digested through the application of heat and technology (Slizewska et al. 2012). This potato flour can be easily used in homemade dog diets, with no need for a heat pressure process because potato starch molecules, comprised of the resistance B-type starches, can be altered during the production of the flour from raw potato (Colonna et al. 1989). The starch sources of the cereal grains used in the study do not reach the high T-OMd values of the potato flour without an extrusion process. Hilton (1990) emphasised that raw potato and corn starch were poorly digested (19 and 47%, respectively) compared to processed potato starch and corn starch (both 84%). The T-OMd of extruded corn flour in the present study increased by approximately 50% at 6 h and 12 h (for all three ages) compared to non-extruded flour, and reached about 81% T-OMd at 48 hours. The highest T-OMd values for non-extruded flours of cereal grains were in barley (range from 46% to 52%) and rice (range from 48% to 58%) flours at 24 h and wheat flour (ranging from 60% to 78%). The highest T-OMd values for the extruded process for six-month-old dog faecal inoculums were barley and potato flours at 6 h, potato and rice flours at 12 h, potato and rice flours at 24 h and rice flour at 48 hours. The highest T-OMd values for the extrusion process for faecal inoculums of two- and eight-year-old dogs were potato flour up to the first 12 h and rice flour after the first 12 hours. Our digestibility values are similar to the findings of Calabro et al. (2013). Some researchers have studied the *in vitro* digestion values of fibrous substances of the English Pointer (five years old) and German Shepherd (three years old) dogs, (Sunvold et al. 1995a; Calabro et al. 2013). In previous *in vitro* digestion studies, there was no information that determined interactions or compared different extruded (or non-extruded) starch sources at cumulative incubation times for the different ages of certain breeds (toy, large or giant breeds). This study provides valuable guidance for starch digestion in medium- or large-breed dogs, especially Labrador Retrievers.

The most important short chain fatty acids formed in the digestive tract of dogs as a result of carbohydrate digestion are butyric, acetic and propionic acids (Swanson et al. 2002). The bu-

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tyric acid in a dog's digestive tract is produced by the *Faecalibacterium* species, as stated by Garcia-Mazcorro et al. (2012). *Eubacterium* and *Roseburia,* which produce butyric acid in the digestive tract of dogs and cats, have been previously mentioned by Handl et al. (2011). In the present study, the concentration of volatile fatty acid after *in vitro* digestion fermentation of starch sources using dog faecal inoculums ranged from 77 mM/l to 99 mM/l at 24 hours. The molarities of organic acid in digestive fluid for barley, wheat, oat and rice flours were increased by the extrusion process and matched the *in vitro* gas production and the T-OMd results of these feedstuffs. Previous researchers have indicated that higher organic acid compositions of digestive fluid have been demonstrated to enhance the degradability and digestibility of feedstuffs (Ersahince and Kara 2017).

In conclusion, the starch sources, which were digested at the highest rate by puppies, originated from non-extruded (unprocessed) rice, potato and oat flours. The extruded wheat flour was the most digested (T-OMd) starch source for both puppies and mature dogs. The extruded potato flour did not change organic matter disappearance due to the previous production process in the factory. In addition, potato flour, which has a high organic matter digestion (> 75% at 6 h), can be added to dog foods (commercial and homemade) for each dogs of all ages.

The extruded oat and rice flours, which exhibit increased organic matter digestion and gas production, can be used in dog foods for canines of all ages. Extruded corn flour can also be digested at a moderate level by puppies and geriatric dogs. Therefore, extruded corn flour may be used in foods for mature dogs.

Extruded barley flour is digested to a moderate level by dogs, similar to *in vitro* observations. The extrusion process in barley flour for ≥ two-year-old dogs can increase organic matter digestion at by 50–100% and can be present in the food for these dogs.

When all the results are considered, the extrusion process positively affects fermentative digestion (fermentation) of starch sources for medium to large breed dogs that are six months of age and older. Consequently, we advise that potato, oat, and wheat flours be included more often than starch from other sources.

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