



## Starch gelatinization implications for nutrient digestibility and fermentation products in the faeces of Beagle dogs

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### ABSTRACT

A combination of raw material particle size and extrusion parameters promotes starch gelatinization (SG). However, the extent of processing, represented by the SG degree, needed for a proper digestibility of dog diets is not completely understood. The effects of processing conditions and SG on the coefficients of total tract apparent digestibility (CTTAD) of nutrients for dogs were evaluated in two studies. First, a formulation with maize as starch source was ground in a hammer mill using screen sieve sizes of 0.5, 1.8 or 2.0 mm and extruded with different specific mechanical energy (SME) applications to obtain treatments with: 99.9% SG (raw material mean geometric diameter [MGD]=224 mm; SME=22 kW-h/t); 76% SG (MGD=283 mm; SME=12 kW-h/t); 63% SG (MGD=312 mm; SME=11 kW-h/t). A fourth treatment used raw material with an MGD= 312 mm, which was pelletized to obtain 26% SG. Twenty-four Beagle dogs were used (six animals per treatment) to determine CTTAD and to assess fermentation products in faeces. The results were evaluated by polynomial contrast according to SG degree ( $P < 0.05$ ). In the second study, data from a scientific publication ( $n = 9$ ) and two databases of commercial extruded diets (UNESP, Jaboticabal, Brazil [ $n = 18$ ] and University of Zaragoza, Zaragoza, Spain [ $n = 33$ ])) were used to construct polynomial regressions between SG degree and CTTAD of nutrients. In Study 1, feed intake was similar ( $P > 0.05$ ) and faeces production was adequate for all dogs. A linear increase in crude protein ( $P < 0.002$ ) and a quadratic increase in starch CTTAD ( $P < 0.001$ ) followed the increase in SG of the diets. No differences were detected for the CTTAD of the other nutrients ( $P > 0.05$ ). Fermentation products in faeces decreased linearly as the SG level of the diets increased ( $P < 0.01$ ). Lactic acid in the faeces of dogs fed the pelletized diet was 6.8 times greater than the observed for 99.9% SG diet ( $P < 0.001$ ). In Study 2, the CTTAD of organic matter ( $R^2$  varying from 0.63 to 0.26, depending on the database), crude protein ( $R^2$  from 0.91 to 0.26), nitrogen-free extract ( $R^2$  from 0.95 to 0.23), and fat ( $R^2$  from 0.84 to 0.46) all increased with increasing SG ( $P < 0.05$ ). The CTTAD of crude protein was the most positively influenced by SG. Overall, the SG degree has an impact on nutrient digestibility, and although a linear response was found for some databases, values from 83% to 87% maximized the CTTAD of nutrients. SG also influences

**Abbreviations:** CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DM, dry matter; GE, gross energy; ME, metabolizable energy; MGD, mean geometric diameter; NFE, nitrogen-free extract; OM, organic matter; SAS, Statistical Analysis System; SG, starch gelatinization; SME, specific mechanical energy.

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fermentative activity and is a potential nutritional intervention for promoting gut health by modulating the formation of butyrate.

## 1. Introduction

The majority of commercial dry dog feeds are currently produced by the thermoplastic extrusion process (Moscicki and Wójtowicz, 2011; Alvarenga and Aldrich, 2020). During extrusion, thermal and mechanical energy are applied which, together with compression in a laminar flow, promote shear stress and extensive changes in starch and proteins, allowing cooking in a few seconds and with low moisture (Gibson and Alavi, 2013; Pacheco et al., 2018; Baller et al., 2021). This process is energetically efficient, reduces microbial counts, inactivates thermolabile antinutritional factors, and allows the formation and texturizing of feed in a short time (Riaz, 2007; Baller et al., 2018).

One of the main ingredients in dog kibble feeds is cereals, whose endosperm is mainly starch organized in semicrystalline granules (Svihus, 2005). Starch facilitates the extrusion process; in the presence of water, heat, shear and pressure, starch swells, melts and loses its crystalline structure (Zeng et al., 1997). This process increases the viscosity and mass resistance to flow inside the extruder barrel, allowing for mechanical energy transfer from the screw and promoting proper cooking, adequate kibble expansion, cellular structure formation, and kibble crispness (Crane et al., 2000; Monti et al., 2016).

Gelatinized starch is soluble in water and more susceptible to enzymatic degradation than the uncooked starch (Dona et al., 2010). In properly extruded diets, dogs can digest more than 0.98 of the starch from grains and tubers (Carciofi et al., 2008). However, very little is known about the extent of starch gelatinization (SG) needs for adequate nutrient digestibility and faecal formation in dogs. The assumption is that extensive SG during extrusion increases nutrient digestibility and promotes better faecal formation (Alvarenga et al., 2021a). However, without a clear understanding of the implications of SG, it is possible that more energy than necessary has been applied, unnecessarily increasing production costs.

In dog studies, however, it was found that diets with different cereals (maize, broken rice and sorghum) require different degrees of grinding (raw material particle size) and different levels of SG to be properly digested (Bazolli et al., 2015). In that study, the authors found that 75% of SG in diets based on maize or broken rice was enough for proper apparent nutrient digestibility and faeces formation, but for sorghum diets, higher SG and smaller raw material particle sizes were necessary. The degree of grinding and starch cooking level also result in differing organic matter fermentation in the colon, potentially modulating the production of short-chain fatty acids (SCFA) (Bazolli et al., 2015; Ribeiro et al., 2019). This concept was explored in a study in which greater raw material particle size was associated with lower specific mechanical energy implementation, decreased starch cooking and increased amount of resistant starch in the diets, with higher butyrate fecal concentration, supporting better intestinal health in old dogs (Peixoto et al., 2018).

In addition, protein quality can be affected by some chemical reactions induced during extrusion processing, such as protein cross-linking formation (Stanley, 1989; Arêas, 1992), Maillard reactions and advanced glycated product formation (Bjorck and Asp, 1983; Van Barneveld, 1993). Several studies have indicated that overprocessing can negatively affect lysine reactivity and nitrogen digestibility in commercial and experimental dog feeds (Stroucken et al., 1996; Williams et al., 2006; Lankhorst et al., 2007). On the other hand, protein digestibility can be improved by reorganization of the molecular structure and the prehydrolysis by linkage rupture (Singh et al., 2007). Thus, the overall effect of extrusion on protein digestibility is controversial.

Considering that industrial processing requires energy and increases production costs, understanding the influence of SG on apparent nutrient digestibility is important for informing feed companies about processing requirements and conditions. Taking these findings into consideration, the present study evaluated the effect of experimental diets with increasing SG degree on the coefficients of total tract apparent digestibility (CTTAD) of nutrients and energy and on the fecal characteristics and fermentation products in dogs. Additionally, the effect of SG on CTTAD was verified in a meta-analysis using a database of a scientific publication and two databases of commercial extruded diets.

## 2. Material and methods

Two studies were conducted to evaluate the effect of SG. The first study evaluated CTTAD of nutrients, faecal characteristics and fermentation products in dogs fed the same formulation processed under different conditions to obtain four SG degrees. The second study evaluated the relationship between SG and CTTAD in kibble diets using a meta-analysis of data from the literature and two databases from commercial kibble diets for dogs, with a total of 60 diets. This second study was conducted to verify in different cereals (data from the literature) and commercial diets for dogs the impact of SG, as it was observed in the first study.

### 2.1. Study 1

The experimental protocol was in accordance with the ethical principles adopted by the National Council for Animal Experiments Control (CONCEA, Ministério da Ciência, Tecnologia e Inovação, Brasília, Brazil) and was approved by the Ethics Committee on Animal Use (CEUA), Faculdade de Ciências Agrárias e Veterinárias da UNESP, Jaboticabal, Brazil (Protocol 09954/13).

Twenty-four Beagle dogs were used - twelve males and twelve females - with a mean age of  $5.5 \pm 0.8$  years old, and an average body weight of  $11.9 \pm 0.3$  kg. Dogs were considered healthy after physical examination, hemogram, and serum biochemistry analysis (urea,

creatinine, alanine aminotransferase, alkaline phosphatase, and albumin). The experiment followed a randomized block design consisting of two blocks of 12 animals and four diets. Each block included three dogs per feed, for a total of six dogs per diet. Considering the sex of the dogs, the diets were also distributed, resulting in three males and three females being included in each treatment. The blocking factor was the period. Each block lasted 20 days, and was organized as follows: the first 10 days were used for diet adaptation; total faecal collection for digestibility was conducted from the 11th to 16th days; and faecal samples were collected immediately after voiding (within 10 min) from the 18th to 20th days to measure faecal fermentation products. Faecal quality was evaluated during the entire faecal collection period, from the 11th to the 20th day.

During the adaptation period, dogs remained in kennels of 1.5 m x 4.5 m with a solarium and were released daily to voluntary exercise in a grass area. In the last two days of adaptation, the dogs were restricted to their kennels to avoid eating grass or faeces from other dogs. During the faecal collection periods, the dogs were housed in individual cages of stainless steel, with dimensions of 90 × 90×90 cm in length, and equipped with an apparatus to separate the faeces and urine for collection. The amount of feed fed to the animals was individually calculated according to the energy value of the feed (estimated based on their chemical composition) and the energy needs of kennel dogs (130 kcal/kg body weight<sup>0.75</sup>/day, National Research Council (US), 2006). The dogs were weighed weekly, and the offered amount of feed was adjusted to maintain constant body weight. The total daily amount of feed was divided into two equal meals, provided at 9 h and 16 h, and the dogs were allowed to eat for 30 min. The advised and refused amounts of feed were weighed, and the intake was recorded. Water was offered ad libitum.

### 2.1.1. Experimental diets

The study included four experimental diets, three extruded feeds and one pelleted feed. A single compound feed for dog maintenance was formulated following the nutritional recommendations of FEDIAF (2017), as described in Table 1. From this unique formulation, the four experimental diets were produced by varying the degree of raw material particle size reduction and the extrusion parameters (three diets), and one diet was pelleted to produce diets with a decreasing percentage of SG. The experimental diets were as follows: SG 99% – raw materials ground with a 0.5 mm screen sieve size and extruded with high thermal and high mechanical energy application; SG 76% – raw material ground with a 1.8 mm screen sieve size and extruded with moderate thermal and low mechanical energy application; SG 63% – raw material ground with a 2.0 mm screen sieve size and extruded with low thermal and low mechanical energy application; and SG 26% - raw material ground with a 1.8 mm screen sieve size and pelleted.

All the ingredients were obtained and mixed, forming a single lot of product. The mixture was then divided into three parts and ground in a hammer mill (Tigre, Sao Paulo, Brazil) fitted with a screen sieve size of 0.5 mm, 1.8 mm or 2.0 mm, generating mixtures with three different raw material particle size distributions (Table 2). The raw material mean geometric diameter (MGD) was calculated by the sieve method according to Zanotto and Belaver (1996).

Each one of the raw material mixtures was extruded in a single screw extruder with a processing capacity of 250 kg per hour (MEX 250, Manzoni, Campinas, Brazil). During extrusion, the water addition, feed rate and extruder screw speed variable parameters were

**Table 1**

Ingredient composition of the same formulation for dogs used to produce experimental diets with different starch gelatinization degrees in study 1.

Ingredient	g/kg, as fed
Corn grain	543.5
Poultry by-product meal	250.0
Poultry fat	90.0
Corn gluten meal 60% protein	50.0
Sugarcane fiber	20.0
Liquid palatant <sup>a</sup>	30.0
Vitamin and mineral premix <sup>b</sup>	3.0
Salt	5.0
Potassium chloride	4.8
Choline chloride	2.0
Mold inhibitor <sup>c</sup>	1.0
Antioxidant <sup>d</sup>	0.4
L-lysine	0.3

<sup>a</sup> SPF do Brasil, Descalvado, Brazil.

<sup>b</sup> Addition per kg of product: Iron, 100 mg; Copper, 9.25 mg; Manganese, 6.25 mg; Zinc, 150 mg; Iodine, 1.87 mg; Selenium, 0.13 mg; Vitamin A, 18.750 IU; Vitamin D, 1.500 IU; Vitamin E, 225 mg; Vitamin K, 0.15 mg; Thiamin, 5 mg; Riboflavin, 16 mg; Pantothenic acid, 35.75 mg; Niacin, 62.5 mg; Pyridoxine, 7.5 mg; Cobalamin, 45 µg; Folic acid, 0.75 mg.

<sup>c</sup> Mould Zap: Ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brasil Agroindustrial Ltda, Maringá, Brazil.

<sup>d</sup> Banox: butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda, Maringá, Brazil.

**Table 2**

Particle size distribution of a same formulation for dogs ground in a hammer mill fitted with different screen sieve sizes.

Item	Scree sieve size		
	0.5 mm	1.8 mm	2.0 mm
Scree sieve size (µm)	Percentage of particles retained		
2000	ni <sup>a</sup>	ni	0.10
1400	ni	0.0	0.10
840	ni	0.10	1.50
500	4.30	12.7	24.7
425	0.20	ni	ni
300	4.50	16.3	33.5
210	63.9	29.7	20.4
125	25.9	20.7	18.8
53	1.10	0.90	0.90
Plate	0	0	0
MGD <sup>b</sup> (µm)	224	283	312
Geometric standard deviation (µm)	1.50	1.60	1.70

<sup>a</sup> ni = sieve not included in the test<sup>b</sup> Mean geometric diameter. Values are an average of the analysis of two samples of each scree sieve size.

unchanged for the three extruded diets. The preconditioner temperature was maintained by direct steam injection. To modulate thermal energy application the mean temperature of the mass leaving the preconditioner was 84 °C for the SG 99% diet, 68 °C for the SG 76% diet, and 58 °C for the SG 63% diet. After 45 min of stable processing, the following parameters were measured at every 10 min: preconditioner temperature, moisture content of the preconditioner discharge mass, extruder motor amperage, pressure and temperature of mass inside the extruder barrel, and kibble bulk density. The in-barrel moisture content was the same for the three diets, approximately 25.3 ± 0.5%. The implementation of specific mechanical energy (SME) was determined according to Riaz (2007). SME was modulated by adjusting the extruder die open area to 232 mm<sup>2</sup>/ton/h for the SG 99% diet and 340 mm<sup>2</sup>/ton/h for the SG 76% and SG 66% diets. Due to the interaction between raw material particle size, mass temperature out of the preconditioner and the die open area, SME application was 22.0 ± 0.5 kW-h/ton for the SG 99% diet, 13 ± 0.6 kW-h/ton for the SG 76% diet, and 12 ± 0.4 kW-h/ton for the SG 63% diet. The mean temperature of the mass before the die was 113 °C, 107 °C, and 108 °C, and the mean pressure of the mass was 42 bars, 34 bars, and 38 bars, respectively, for the SG 99%, SG 76%, and SG 63% treatments.

Additionally, for the manufacture of the fourth treatment, one portion of the raw material mixture ground with the 1.8 mm screen sieve size was pelletized in a horizontal rind die equipment with a processing capacity of 300 kg per hour (California Pellet Mill, Blaine, USA). The pelletizer preconditioner temperature was kept at approximately 79 °C by direct steam injection, and the pellet temperature outside the pelletizer was 78 °C. After processing, the kibbles and pellets were dried for 20 min in a continuous forced air dryer system, heated to 105 °C, and subsequently coated with fats and palatants. The chemical composition of experimental the diets is presented in Table 3).

### 2.1.2. Determination of CTTAD of nutrients and metabolizable energy (ME)

The CTTAD of nutrients and energy of the diets were determined by the method of total collection of faeces without urine collection, following the recommendations and calculation procedures of FEDIAF (2017). To calculate the feed ME content, energy excretion in urine was estimated based on the digestible protein intake of the dogs (FEDIAF, 2017). Feed intake was recorded daily, by weighing the feed quantities offered and refused during every meal. For five consecutive days, total faecal excretion were collected at 08:00 h and at 17:00 h, after which the samples were weighed and stored in a freezer (−20 °C) for later analysis.

At the end of the collection period, the faeces were thawed and homogenized, compounding one sample per dog and per period. The faeces were then dried in a forced-air oven (Model 320-SE, FANEM, São Paulo, Brazil) at 55 °C for 72 h. The dried faeces and diets

**Table 3**

Starch gelatinization (SG) degree and chemical composition of a single formulation for dogs processed under different conditions.

Item	Diets <sup>a</sup>			
	SG 26%	SG 63%	SG 76%	SG 99%
Starch Gelatinization, %	26.3	62.6	76.0	99.9
Chemical composition, g/kg as fed				
Dry matter	923.0	952.0	953.0	952.0
Ash	80.0	80.0	86.0	81.0
Crude protein	271.0	270.0	271.0	270.0
Fat	145.0	143.0	142.0	141.0
Starch	415.0	394.0	395.0	395.0

<sup>a</sup> SG 26% - raw material ground with a 1.8 mm screen sieve size and pelletized; SG 63% – raw material ground with a 2.0 mm screen sieve size and extruded with low thermal and mechanical energy application; SG 76% – raw material ground with a 1.8 mm screen sieve size and extruded with moderate thermal energy and low mechanical energy application; SG 99% – raw material ground with a 0.5 mm screen sieve size and extruded with high thermal and mechanical energy application. Values are an average of the analysis of three samples of each diet.

were ground in a knife type mill (Model MOD 340, ART LAB, São Paulo, Brazil), fitted with a 1 mm screen sieve size and analysed according to the methodology described by the Association of Official Analytical Chemists (AOAC International, 2010) for the determination of dry matter (DM) by oven-drying the sample (method 934.01), by muffle furnace incineration for ash content (method 942.05), by the Kjeldahl method for crude protein (CP) (method 954.01), and by Soxhlet apparatus extraction for acid-hydrolysed ether extract (method 954.02). The organic matter (OM) content of the samples was calculated as DM minus ash. The starch content was determined according to the method of Hendrix (1993), and the SG degree was measured by the amyloglucosidase method (Hoseney, 1994) with modifications proposed by Sá et al. (2013). The gross energy (GE) content was determined by an adiabatic calorimetry pump (1281, Parr Instruments, Moline, USA). All laboratory analyses were conducted in duplicate and repeated when the variation between duplicates was greater than 5%.

### 2.1.3. Faecal characteristics and fermentation products

Faecal quality was indirectly assessed by the following score (Carciofi et al., 2008): 0 = liquid faeces; 1 = soft faeces, formless; 2 = soft faeces, malformed, which adopts the shape of the container; 3 = soft, formed faeces and wet, which retains its shape; 4 = well-formed and consistent faeces, which does not stick to the floor; and 5 = dry and hard faeces, with reduced volume.

Fresh samples of faeces, collected and processed at a maximum of 10 min after defecation, were used to measure pH and to determine SCFA, branched-chain fatty acids (BCFA), lactate and ammonia. The pH was measured in two grams of faeces diluted (1:3 w/w) in six ml of Milli-Q water, and the pH was measured with a pH meter (Digicrom Analytical Inc., model DM20; São Paulo, Brazil). The procedure was performed on each of the three collection days, and the mean values were used in the study.

For the analysis of SCFA and BCFA, 10 g of fresh faeces was homogenized and mixed with 30 ml of 4.2 N formic acid (1:3 w/w). The procedure was repeated for each of the three days of collection. The mixture was centrifuged three times at 4500 xg for 15 min at 15 °C, after which the obtained supernatant was collected. The supernatant was then centrifuged at 20,000 xg for 1 h at 5 °C. After extraction, the samples were stored in a freezer (−20 °C). The concentrations of SCFA and BCFA were determined by gas chromatography according to Erwin et al. (1961) in a chromatograph (Shimatzu, CG-2014, Shimatzu Scientific Instruments, Tokyo, Japan).

For lactic acid analysis, approximately three grams of fresh faeces was rapidly homogenized and mixed with nine millilitres of distilled water (1:3 w/v). The mixture was kept at 4 °C for one day, and then centrifuged three times at 4500 xg and 15 °C, for 15 min. The supernatant was tapped, and the sediment was discarded. Lactic acid was analysed according to the method of Pryce (1969) by the spectrophotometric method with readings at 565 nm (QUICK-Lab, Drake, São José do Rio Preto, Brazil). The samples were quantified by comparing the readings with a standard lactic acid solution of 0.08%. Faecal ammonia was measured based on a methodology adapted from Vieira (1980), using the extracts obtained for the determination of SCFA. The extracts were thawed at room temperature and diluted in distilled water (2:13 v/v), after which the ammonia was distilled in a nitrogen purification system (TE Tecnal - 036/1, Piracicaba, Brazil).

## 2.2. Study 2

To study the relationship between SG degree and nutrient digestibility, data from the literature and two databases of commercial extruded diet digestibility were used. From the literature, the study of Bazolli et al. (2015) was reviewed and the data were combined for a new evaluation, not presented by the authors, which is presented in this manuscript. Briefly, the study included three formulations with similar compositions, varying the source of starch among broken rice, corn and sorghum. Each formulation was ground to produce raw material mixtures with three different MGD. The mixture was then extruded under similar conditions, resulting in nine

**Table 4**

Chemical composition, coefficient of total tract apparent digestibility (CTTAD) of nutrients and starch gelatinization (SG) degree of prepared diets for dogs evaluated at UNESP (Universidade Estadual Paulista, Jaboticabal, Brazil, n = 18).

Item	Mean	Min – max	SEM <sup>a</sup>
Chemical composition, g/kg, as-fed			
Dry matter	937	909 – 981	3.5
Crude protein	287	203 – 429	14.2
Fat	140	96.0 – 217	7.4
Ash	89.0	67.0 – 126	4.8
Crude fiber	32.0	18.0 – 55.0	2.7
Nitrogen-free extract <sup>b</sup>	388	190 – 521	24.6
Starch	305	162 – 379	13.7
Starch gelatinization degree (%)	89.5	81.7 – 99.8	1.69
CTTAD			
Dry matter	0.775	0.706 – 0.864	0.006
Organic matter	0.831	0.759 – 0.911	0.008
Crude protein	0.826	0.741 – 0.902	0.008
Fat	0.894	0.784 – 0.932	0.007
Nitrogen-free extract	0.854	0.770 – 0.958	0.009

<sup>a</sup> SEM – standard error of mean (n = 18 feeds)

<sup>b</sup> Nitrogen-free extract was calculated as: DM – (CP + Fat + Ash + crude fiber). It was used for digestibility calculation due to the lack of information on starch digestibility of the feeds from the database.

experimental diets. The diets had SG degrees ranging from 62% to 90%, determined by the same methodology described previously (Hoseney, 1994; Sá et al., 2013). Each diet was fed to six dogs, after which the CTTAD of the nutrients was calculated.

To complement the evaluation of the relationship between SG and nutrient digestibility, two databases of commercial extruded diets for dogs were used. One group was composed of 18 commercial extruded diets for dogs from Brazil (Table 4; digestibility tests carried out at UNESP, Jaboticabal, Brazil), and the other was composed of 33 commercial extruded diets for dogs from Spain (Table 5; digestibility study conducted at the University of Zaragoza, Zaragoza, Spain). For all the diets, the chemical composition and CTTAD of the nutrients were obtained. All digestibility trials were carried out in a minimum of six dogs, including a minimum of five days of adaptation and five days of total faecal collection. In addition, for all the diets the SG degree was determined by the amyloglucosidase method, as previously described, and this analysis was carried out in the same laboratory of UNESP, Jaboticabal, Brazil. No information about the processing conditions or ingredient composition of the feeds was available for either database of commercial diets.

### 2.3. Statistical analysis

The assumptions of homogeneity of variance and normality of errors were verified for all variables. Study 1 was analysed in accordance with a randomized block design, with four diets and six dogs per diet. The experimental unit was considered the dog. The data were subjected to analysis of variance and the model sums of squares were separated into treatment (diet) and block effects. When treatment effects were verified via the F test, means were compared via polynomial contrasts according to the SG degree of the diets. The databases from study 2 were analysed by polynomial contrasts considering the SG degree of the diets as the independent variable and the nutrient digestibility as the dependent variable. The experimental unit was considered the prepared feed. For both studies P-values at  $P < 0.05$  were considered significant. The statistical procedures were performed using SAS Institute Inc (2004).

## 3. Results

### 3.1. Study 1

All the diets, including the pelleted diet, were adequately eaten, without differences in nutrient intake among the treatments ( $P > 0.05$ ; Table 6). A linear increase in the CTTAD of crude protein was observed ( $R^2 = 0.40$ ;  $P = 0.002$ ), and a quadratic increase in the starch CTTAD ( $R^2 = 0.58$ ;  $P < 0.001$ ) was observed as the SG degree of the diets increased. The CTTAD of the other nutrients and the metabolizable energy did not differ between treatments.

Faecal production (as-is basis) decreased quadratically (Table 7) as the SG degree increased ( $R^2 = 0.23$ ;  $P = 0.045$ ). However, differences accounted were less than 1 g of faeces per kg per day and were not significant when expressed on a DM basis ( $P = 0.933$ ). Although the faecal score remained adequate (range 3 to 4), faecal score increased ( $R^2 = 0.29$ ;  $P = 0.012$ ). The differences in faecal moisture were not significant ( $P = 0.268$ ). In fact, the mean values of faecal production and score were altered only in dogs fed the pelleted diet.

The acetate (acetate =  $208.1 - 0.691SG$ ;  $R^2 = 0.30$ ), propionate (propionate =  $145.9 - 0.281SG$ ;  $R^2 = 0.68$ ), butyrate (butyrate =  $59.63 - 0.053SG$ ;  $R^2 = 0.46$ ), total amount of SCFA (total SCFA =  $418.9 - 0.30SG$ ;  $R^2 = 0.49$ ), and valerate (valerate =  $2.77 - 0.017SG$ ;  $R^2 = 0.27$ ) content in the faeces decreased linearly as the SG degree of the diets increased ( $P < 0.01$ ). In particular, the lactic acid content in the faeces of dogs fed the SG 26% diet was 6.8 times greater than that in the faeces of dogs fed the SG 99% diet (lactate =  $71.6 - 1.58SG + 0.01 SG^2$ ;  $R^2 = 0.67$ ;  $P < 0.001$ ). No differences in fecal ammonia concentration were observed.

**Table 5**

Chemical composition, coefficient of total tract apparent digestibility (CTTAD) of nutrients, and starch gelatinization (SG) degree of prepared diets for dogs evaluated at the University of Zaragoza (Zaragoza, Spain,  $n = 33$ ).

Item	Mean	Min – max	SEM <sup>a</sup>
Chemical composition, g/kg, as-fed			
Dry matter	921	897 – 948	2.0
Crude protein	296	255 – 406	4.9
Fat	184	106 – 281	5.8
Ash	78.0	98.0 – 65.0	1.5
Crude fiber	23.0	10.0 – 59.9	1.9
Nitrogen-free extract <sup>b</sup>	340	201 – 532	18.6
Starch	322	208 – 422	8.2
Starch gelatinization degree (%)	85.6	72.4 – 95.9	1.19
CTTAD			
Dry matter 0.823 0.723 – 0.879			0.005
Organic matter	0.867	0.776 – 0.915	0.005
Crude protein	0.841	0.758 – 0.896	0.005
Fat	0.929	0.865 – 0.964	0.003
Nitrogen-free extract	0.898	0.809 – 0.944	0.005

<sup>a</sup> SEM – standard error of mean ( $n = 33$  feeds)

<sup>b</sup> Nitrogen-free extract was calculated as:  $DM - (CP + Fat + Ash + crude\ fiber)$ . It was used for digestibility calculation due to the lack of information on starch digestibility of the feeds from the database.

**Table 6**

Body weight, feed intake, coefficient of total tract apparent digestibility (CTTAD) of nutrients, and metabolizable energy (ME) content of a same formulation for dogs processed under different conditions to obtain four starch gelatinization (SG) degrees.

Item	SG degree of diets <sup>a</sup>				SEM <sup>b</sup>	P-value	Contrasts <sup>c</sup>	
	SG 26%	SG 63%	SG 76%	SG 99%			Linear	Quadratic
Body weight (BW, kg)	12.4	12.3	10.5	12.4	0.27	0.012	- <sup>d</sup>	-
Feed intake (g/kg BW/day)								
Dry matter	15.6	15.6	15.9	15.1	0.22	0.593	-	-
Crude protein	4.2	4.2	4.3	4.2	0.06	0.572	-	-
Fat	2.3	2.2	2.1	2.1	0.04	0.600	-	-
Crude fiber	0.4	0.4	0.4	0.3	0.01	0.276	-	-
Starch	6.5	6.2	6.3	5.9	0.10	0.215	-	-
CTTAD								
Dry matter	0.796	0.804	0.795	0.796	0.003	0.536	-	-
Organic matter	0.848	0.854	0.843	0.849	0.002	0.375	-	-
Crude protein	0.837	0.858	0.861	0.867	0.003	0.010	0.002	0.213
Fat	0.923	0.921	0.922	0.925	0.002	0.849	-	-
Starch	0.997	0.998	0.998	0.998	0.000	0.004	0.002	< 0.001
Gross energy	0.857	0.862	0.857	0.857	0.002	0.448	-	-
ME (kcal/g of DM)	4.1	4.2	4.1	4.1	0.09	0.056	-	-

<sup>a</sup> SG 26% - raw material ground with a 1.8 mm screen sieve size and pelleted, 26.3% of SG; SG 63% – raw material ground with a 2.0 mm screen sieve size and extruded with low mechanical energy application, 62.6% of SG; SG 76% – raw material ground with a 1.8 mm screen sieve size and extruded with low mechanical energy application, 76.0% of SG; SG 99% – raw material ground with a 0.5 mm screen sieve size and extruded with high mechanical energy application, 99.9% of SG.

<sup>b</sup> SEM – standard error mean (n=6 dogs per diet)

<sup>c</sup> Linear or quadratic effects of SG degree

<sup>d</sup> means are not statistically different

**Table 7**

Fecal characteristics and concentration of fermentation products in feces of dogs fed a same formulation processed under different conditions to obtain four starch gelatinization (SG) degrees.

Item	SG degree of diets <sup>a</sup>				SEM <sup>b</sup>	P-value	Contrasts <sup>c</sup>	
	SG 26%	SG 63%	SG 76%	SG 99%			Linear	Quadratic
Feces								
g/kg body weight/day (As-is basis)	8.0	7.0	7.1	7.1	0.13	0.029	0.026	0.045
g/kg body weight/day (DM basis)	3.7	3.2	3.1	3.3	0.08	0.933	- <sup>d</sup>	-
Moisture (g/kg, as is)	590	583	521	567	3.7	0.268	-	-
Score	3.8	3.9	3.9	3.9	0.02	0.028	0.012	0.089
Ph	6.44	7.00	7.11	7.32	0.07	< 0.001	< 0.001	0.003
Fermentation products (mMol/kg of feces DM)								
Acetate	309.2	304.2	258.2	252.7	10.32	0.013	0.002	0.988
Propionate	167.7	143.4	122.9	111.5	4.56	< 0.001	< 0.001	0.308
Butyrate	72.5	68.7	60.6	44.3	2.91	< 0.001	< 0.001	0.165
Total SCFA <sup>e</sup>	549.9	527.2	441.7	410.1	15.01	< 0.001	< 0.001	0.840
Isobutyrate	7.6	7.5	7.6	7.9	0.37	0.959	-	-
Isovalerate	11.7	11.6	11.3	11.9	0.52	0.897	-	-
Valerate	2.6	1.9	1.4	0.9	0.20	0.122	0.001	0.783
Total BCFA <sup>f</sup>	21.8	20.9	20.3	20.8	0.93	0.766	-	-
Total volatile fatty acids	571.8	548.5	462.0	430.4	15.43	< 0.001	< 0.001	0.850
Ammonia	148.3	145.1	141.8	142.1	2.37	0.771	-	-
Lactate	36.9	6.0	8.5	5.4	3.29	< 0.001	< 0.001	0.002

<sup>a</sup> SG 26% - raw material ground with a 1.8 mm screen sieve size and pelleted, 26.3% of SG; SG 63% – raw material ground with a 2.0 mm screen sieve size and extruded with low mechanical energy application, 62.6% of SG; SG 76% – raw material ground with a 1.8 mm screen sieve size and extruded with low mechanical energy application, 76.0% of SG; SG 99% – raw material ground with a 0.5 mm screen sieve size and extruded with high mechanical energy application, 99.9% of SG.

<sup>b</sup> SEM – standard error mean (n=6 dogs per diet)

<sup>c</sup> Linear or quadratic effects of SG degree

<sup>d</sup> means are not statistically different

<sup>e</sup> SCFA – short-chain fatty acids

<sup>f</sup> BCFA - branched-chain fatty acids

### 3.2. Study 2

With the database of the study of [Bazolli et al. \(2015\)](#), significant polynomial contrasts were established between SG degree and the CTTAD of OM (CTTAD of OM =  $5.71 + 1.91SG - 0.01SG^2$ ;  $R^2 = 0.63$ ;  $P = 0.048$ ), CP (CTTAD CP =  $-15.28 + 2.25SG - 0.01SG^2$ ;  $R^2 = 0.91$ ;  $P < 0.001$ ), fat (CTTAD Fat =  $44.31 + 1.18SG - 0.006SG^2$ ;  $R^2 = 0.84$ ;  $P = 0.003$ ), starch (CTTAD Starch =  $66.14 + 0.80SG - 0.004SG^2$ ;  $R^2 = 0.95$ ;  $P < 0.001$ ), and GE (CTTAD GE =  $20.27 + 1.51SG - 0.008SG^2$ ;  $R^2 = 0.65$ ;  $P = 0.04$ ), as shown in [Fig. 1](#). These data were not explored in that publication. When the optimal amount of SG was deducted from the polynomial equations generated, an SG degree of 95.5% resulted in a maximum OM CTTAD, 86.5% in maximum CP CTTAD, 85.5% for maximum fat CTTAD, 82.8% for maximum starch CTTAD and 86.4% for maximum GE CTTAD.

In the first database of prepared extruded diets ([Fig. 2](#)), SG explained some of the variation in CTTAD for OM (CTTAD OM =  $48.3 + 0.38SG$ ;  $R^2 = 0.26$ ;  $P = 0.032$ ), CP (CTTAD CP =  $50.2 + 0.36SG$ ;  $R^2 = 0.26$ ;  $P = 0.031$ ), fat (CTTAD Fat =  $-343.9 + 9.19SG - 0.048SG^2$ ;  $R^2 = 0.46$ ;  $P = 0.009$ ), and nitrogen-free extract (CTTAD NFE =  $46.3 + 0.42SG$ ;  $R^2 = 0.23$ ;  $P = 0.042$ ). However, the coefficients of determination were less than 50%. For the CTTAD of OM, CP, and NFE a linear relationship with SG was found ( $P < 0.05$ ), suggesting that nutrient digestibility increased with increasing SG. Fat digestibility was fitted to a quadratic equation, with an SG of 95% resulting in maximum CTTAD.

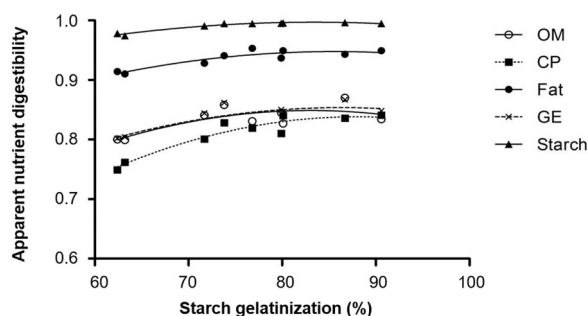
In the second database of prepared feeds similar findings were obtained, except for fat. The SG accounted for some of the variation in CTTAD of OM (CTTAD OM =  $-208.0 + 6.99SG - 0.04SG^2$ ;  $R^2 = 0.34$ ;  $P = 0.001$ ), CP (CTTAD CP =  $-229.9 + 7.27SG - 0.041SG^2$ ;  $R^2 = 0.55$ ;  $P < 0.001$ ), and NFE (CTTAD NFE =  $-165.3 + 6.08SG - 0.036SG^2$ ;  $R^2 = 0.26$ ;  $P = 0.009$ ), as shown in [Fig. 3](#). When the optimal amount of SG was deducted from the polynomial equations, an SG of 84.9% resulted in a maximum CTTAD of OM, 86.7% for the maximum CTTAD of CP and 84.4% for the maximum CTTAD of NFE. Fat CTTAD was not related to SG in this database.

## 4. Discussion

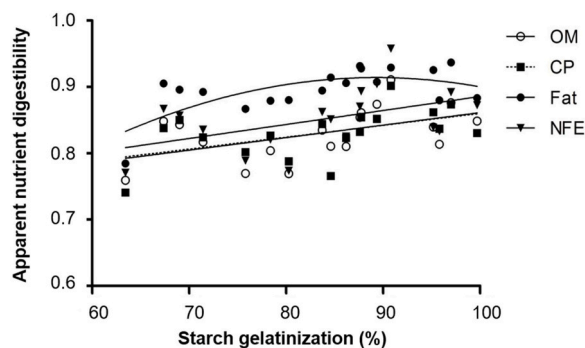
In Study 1, it was observed that the degree of grinding associated with the application of mechanical and thermal energy during processing resulted in marked differences in the SG degree of a single diet formulation for dogs. Treatment with the smallest MGD and highest energy application resulted in almost complete SG, which was greatly reduced by the combination of a greater particle size and lower thermal and mechanical energy application. The SG concentration was particularly low in the pelletized feed, as expected, due to the limited application of energy and shear and the limited residency time in this system ([Abdollahi et al., 2013](#)). One consideration is that this experimental pelletized diet might differ from the prepared pelletized diets for dogs, in which the starch sources used might be pregelatinized before being included in the formulation, therefore, in this commercial type of product, the SG can be greater.

The influence of the raw material particle size on the extent of SG was previously observed by [Bazolli et al. \(2015\)](#). In their study, the particle size of cereals (rice, sorghum and maize) was strongly correlated ( $R^2 = 0.85$ ;  $P < 0.001$ ) with the SG in extruded dog feeds. Based on these findings, the authors of the present study decided to associate the raw material particle size and extrusion conditions to modulate the starch cooking at the extruder. The influence of thermal energy application on starch cooking was evaluated in dogs ([Pacheco et al., 2018](#)) and cat feeds ([Baller et al., 2021](#)), and a linear relationship was observed in both situations. Based on these findings, the authors selected the target mass temperature at the preconditioner by modulating the steam addition to achieve the range of starch cooking desired in the present experiment. Finally, the SME implementation was modulated by changing the extruder open area, altering the resistance to mass flow, a key factor for energy transfer and the consequent transformation of the raw material ([Moscicki and Wójtowicz, 2011](#)). The extruder die open area increased by approximately 46%, reducing the mechanical energy transfer by approximately 40%, compared the SG 99% diet with the SG 76% and SG 63% treatments.

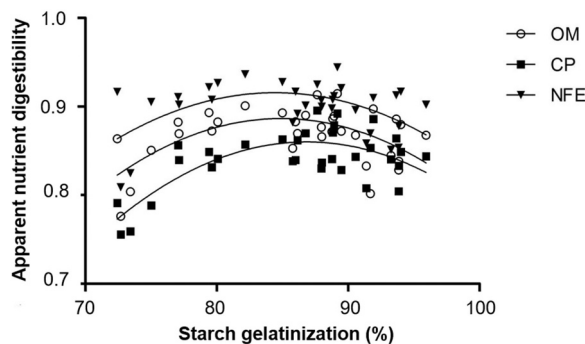
Although a wide range of SG was obtained, the impact on nutrient digestibility was smaller than expected. The starch CTTAD remained higher than 99%, the CP CTTAD above 83%, and the faecal quality was within the optimum range for all the treatments, including the pelleted diet. The most impacted nutrient was protein, whose CTTAD increased three percentage points with an increase in SG from 26 to 99%. This effect of SG on CP CTTAD was previously observed in two studies that compared a corn-based recipe processed with different thermal and mechanical energy applications and raw material particle sizes, obtaining limited (62%) and fully



**Fig. 1.** Relationship between starch gelatinization (%) and the coefficient of total tract apparent digestibility of organic matter (OM), crude protein (CP), fat, gross energy (GE) and starch, obtained with the data from study of [Bazolli et al. \(2015\)](#).



**Fig. 2.** Relationship between starch gelatinization (%) and the coefficient of total tract apparent digestibility of organic matter (OM), crude protein (CP), fat, nitrogen-free extract (NFE) of prepared diets for dogs evaluated at UNESP (Universidade Estadual Paulista, Jaboticabal Brazil; n = 18).



**Fig. 3.** Relationship between starch gelatinization (%) and the coefficient of total tract apparent digestibility of nutrients of prepared diets for dogs evaluated at the University of Zaragoza (Zaragoza, Spain; n = 33).

gelatinized starch (99%). The CP CTTAD was 2.2% points (Peixoto et al., 2018) and 1.6% points greater (Ribeiro et al., 2019) in dogs fed the fully gelatinized diets.

The effect of raw material particle size and SG degree on nutrient digestibility has been shown in several species (Owsley et al., 1981; Fadel et al., 1988; Healy et al., 1994; Amerah et al., 2007; Hilcko et al., 2009; Di Santo et al., 2018). Both the nutritional value and the efficiency of processing are dependent on these raw material transformations (Wondra et al., 1995; Pacheco et al., 2018; Baller et al., 2021). For dogs, however, the available information on this topic is scarce, and companies do not always have sufficient data to establish informed decisions about processing objectives and parameters (Alvarenga et al., 2021b).

In a study in dogs where only thermal energy application was altered, Pacheco et al. (2018) did not observe differences in CTTAD of nutrients using a single formulation with corn as the carbohydrate source, with SG varying from 68.9% to 89.3%. A similar finding was observed in cats (Baller et al., 2021), in which the percentage of SG was varied from 75% to 81% after thermal energy modulation. Therefore, in the present study, a lower percentage of SG (26.3% and 62.6%) than in the above mentioned studies was induced in two of the treatments to better evaluate its importance, but even under these conditions the impact on apparent digestibility was limited. Looking deeper into the data of Bazolli et al. (2015), the authors observed lower CTTAD for the corn-based diet associated with an SG lower than 70% only when the MGD of the raw material was greater than 450  $\mu\text{m}$ ; this condition was not evaluated in the present study, in which the coarser raw material particle size was  $312 \pm 1.7 \mu\text{m}$ .

Additionally, the study of Bazolli et al. (2015) highlighted the differences among cereals in terms of processing requirements, as for broken rice all the diets presented similar CTTAD, for corn-based recipes only the grossly ground (screen sieve size of 3 mm; MGD of  $619 \pm 2.3 \mu\text{m}$ ) treatment presented lower a CTTAD, whereas for the recipes formulated with sorghum, linear relationships were observed between raw thematerial particle size and CTTAD of nutrients. This emphasizes the differences in cereals regarding processing requirements and that the application of the data obtained in Study 1 requires consideration of the carbohydrate source of the diet.

The implications of SG were also observed for prepared diets. According to database one of Study 2, this relationship was predominantly linear, for which higher cooking would result in a greater CTTAD of nutrients. According to this database, fat CTTAD also increased with SG ( $R^2 = 0.46$ ), although the response was quadratic, as was also observed in Bazolli et al. (2015). This greater fat digestibility could be explained by an increased exposure of the cell content in better processed material, favoring the formation of micelles and absorption of fats as previously observed in dogs (Peixoto et al., 2018). The fact that fat CTTAD did not change in Study 1 or database two is unclear but may be due to differences in the proportions of internal fat (in the extruded ingredients) and external fat, added by coating after extrusion. The fact that elevated SG did not reduce fat CTTAD indicates that the fat-carbohydrate complexation

that is induced during extrusion (Gibson and Alavi, 2013) did not reduce apparent fat digestibility in dogs.

The quadratic relationships observed in database two indicate maximum OM, CP, and NFE CTTAD in diets with 84% to 87% of SG. In general, the observed coefficients of determination were moderate to low (from 0.40 to 0.25), except for the crude protein CTTAD, for which a remarkable proportion of the variation in crude protein digestibility was explained by SG ( $R^2 = 0.55$ ). The low values of  $R^2$  values may be explained by the differences in the prepared feeds evaluated which largely differed in chemical composition, fibre content (Castrillo et al., 2001; Loureiro et al., 2017), raw material sources (Maria et al., 2017; Alvarenga and Aldrich, 2020) and processing conditions. Considering all these sources of variation, it is quite remarkable that a positive effect of cooking degree was also observed on the CTTAD of nutrients. The use of these two databases, therefore, was important for complementing the results obtained in a laboratory-controlled situation with a single formulation in Study 1, making it possible to explore the implications of SG for two commercial extruded diet databases for dogs.

The effects of SG on the CTTAD of nutrients can be explained by the nutritional unavailability of partially processed cereals and native starch, which remain in their crystalline form. Under these conditions, limiting contact between enzymes and nutrients in the animal digestive tract may result in incomplete digestion and absorption in the small intestine (Wondra et al., 1995; Svihus et al., 2005; Amerah et al., 2007; Dona et al., 2010).

In both in controlled studies and in the prepared feed database, the SG degree affected the crude protein CTTAD more than the organic matter CTTAD. At least two hypotheses may explain the higher CTTAD of crude protein in the diets with higher SG. The first is related to a true increase in digestion and absorption in the small intestine; thus, a more intense extrusion may result in more intense protein denaturation and linear reorientation of the amino acid chains along the mass flow inside the extruder. This may be achieved by prehydrolysis and rupture of the peptides chains, favouring protein hydrolysis and amino acid absorption in the intestine (Hullar et al., 1998; Singh et al., 2007; Tran et al., 2008; Milani et al., 2022). The second hypothesis is related only to an apparent effect; with higher organic matter digestibility in the small intestine, less digesta flows into the colon, resulting in less fermentative activity in the large intestine and lower faecal excretion of microbial nitrogen (Kawauchi et al., 2011). In this respect, although there were no significant differences in ammonia or BCFA concentrations between the present study, indicative of decreased protein fermentation with increasing SG degree, there were lower SCFA levels in the faeces of dogs fed diets with higher SG degree, which is interesting to discuss.

Notably, as only CTTAD was measured in the present study it is not possible to distinguish between a true effect or only apparent effect of SG on crude protein digestibility. The same consideration is necessary to interpret starch CTTAD. This is highlighted in studies with swine and extrusion technology, which revealed higher ileal digestibility of starch, crude protein and energy after extrusion, however when CTTAD was measured, no differences were found between treatments revealing the importance of nutrient fermentation in the colon (Fadel et al., 1998; Milani et al., 2022).

In Study 1, the greater organic matter flow to the colon and its fermentation by the gut microbiota in diets with lower SG explained the decrease in faecal pH and the increase in volatile fatty acids and lactate concentrations. The total volatile fatty acids content increased by approximately 33%. These alterations are parallel to those observed in studies in dogs after the addition of fermentable fibre to their diets (Kawauchi et al., 2011; Volpe et al., 2021; Pacheco et al., 2021) and in studies where different SG were compared in dogs (Ribeiro et al., 2019) and cats (Baller et al., 2021).

Among the increased SCFA, the butyrate content was 63% greater in the pelletized treatment (SG 26%) and 55% greater in the SG 63% than in the SG 99% treatment. Butyrate is an important fermentation product that stimulates the proliferation, maturation and differentiation of colonocytes, facilitating the secretory functions and normal colon absorption (Topping and Clifton, 2001; Swanson et al., 2002; Haenen et al., 2013). Assuming that only approximately 5% of the SCFA produced are recovered in the faeces, with most butyric acid being absorbed by the epithelial cells (Macfarlane and Macfarlane, 2012), the influence of SG on SCFA and butyrate production by the microbiota should be even greater.

The promotion of butyrate production is one of the targets of adding prebiotics to dog diets (Theodoro et al., 2019). However, an increase in this short-chain fatty acid is not always observed, and more modest increases in comparison with those in the present study have been reported, as in the study of Theodoro et al. (2019), in which an increase of only 22% in the faecal concentration of butyrate was observed. Butyrate contributes to the modulation of inflammation and immunity through effects on gut-associated lymphoid tissue (Schley and Field, 2002; Roberfroid et al., 2010). Previous studies in dogs evaluating processing conditions that induce limited SG demonstrated an increase in resistant starch content. The authors linked the formation of this prebiotic to increased faecal butyrate levels, suggesting a potential improvement in gut health parameters (Peixoto et al., 2018; Ribeiro et al., 2019; Alvarenga et al., 2021a). The increase in fecal lactate in dogs fed a less SG treatment (the pelletized diet) may also be linked to intestinal health improvement due to its ability to reduce faecal pH and its potential conversion to butyrate by the gut microbiota (Bourriaud et al., 2005; Louis et al., 2007). However, it is also important to note that reduced crude protein and fat digestibility can also promote the formation of undesirable microbial fermentation products in the intestine, such as biogenic amines, indoles, and hydroxylated fatty acids (Pinna et al., 2016). As they were not investigated in the present study, the impact of these components on the microbiome and host health remains unclear and might deserve investigation.

Usually, the effects of higher organic matter fermentation in the colon can be followed by increased faecal moisture and, consequently, the production of moist and more soft faeces (Kawauchi et al., 2011; Fischer et al., 2012). However, in the present study, SG was reduced, and the consequent increase in fermentation products did not change faecal moisture; moreover, although an effect was observed in the faecal score, this was minor, and all the faeces remained close to the ideal (faeces score of 4). Some limitations must be considered, however, in the evaluation of these results. Medium to small breeds of dogs, including Beagles, usually produce firmer faeces that are less impacted by the diet characteristics. Large dog breeds, on the other hand, are more susceptible to alterations in the diet that result in greater organic matter fermentation in the colon and are more prone to present softer and moister unshaped faeces (Weber et al., 2017).

Additionally, Study 1 was limited to a corn-based formulation, and the impact of SG might be different from that of other cereals or tubers used as starch sources or may differ between formulations with different fibre contents. Therefore, additional studies are needed before extrapolating the feed processing conditions adopted in the present study to other dog breeds or recipes. The data obtained in Study 2 were interesting because they complement the observations made with other cereals (rice and sorghum) and because of the wider setting of chemical compositions and processing conditions, allowing us to verify that SG analysis is important for accessing the quality of the processing parameters adopted in the preparation of kibble diets for dogs.

## 5. Conclusion

The coefficient of total tract apparent digestibility of crude protein was the most influenced by the degree of starch gelatinization, as its digestibility increased with increasing starch gelatinization under all the evaluated conditions. The influence of starch gelatinization on the coefficient of total tract apparent digestibility of starch (or nitrogen-free extract) and fat is less clear, as this was only significant for some databases. Although a linear increase response was described for some databases, overall, starch gelatinization from 83% to 87% seems to maximize nutrient digestibility. Only the intake of the feed processed by pelletization (26% starch gelatinization) resulted in higher faecal production, although it did not alter fecal quality in the Beagle dogs. Less gelatinized starch is more readily fermented by the colon microbiota, increasing the faecal concentration of lactate and most short-chain fatty acids, including butyrate. Diets with a limited degree of starch gelatinization induce a substantial increase in the butyrate content in the faeces, an aspect that can be explored as a nutritional intervention to promote gut homeostasis.

## Authors Statement

This declaration is to certify that **all authors have seen and approved** the revised version of the manuscript *Starch gelatinization implications for nutrient digestibility and fermentation products in the faeces of beagle dogs*, submitted to *Animal Feed Science and Technology*.

## Declaration of Competing Interest

The authors report no grants to conduct the study, that was designed and funded independently. Authors also confirm that there are no known conflicts of interest associated with this publication, and that the manuscript has been read and approved by all named authors. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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