

Article

Assessing Enzymatically Pre-Treated, Vacuum Paddle-Dehydrated Tomato Pomace as a Sustainable Ingredient in Dog Diets

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Abstract

Industrial processing of tomato generates large amounts of tomato pomace (TP), whose disposal and stabilization are challenging due to its high moisture content. This study evaluated, for the first time, the effects of increasing inclusion levels of enzymatic pre-treatment, vacuum paddle-dehydrated TP (ETP), in extruded diets for adult dogs. Three diets containing 0%, 2% or 4% ETP, replacing wheat bran and sunflower meal, were produced. Three two-bowl tests assessed palatability, and a feeding trial was performed using a four 3 × 3 Latin square design with 12 healthy adult Beagle dogs across three 28-day periods. Inclusion of ETP had negligible effects on the chemical composition of diets and kept unaffected palatability, body weight, food intake, fecal consistency and output. Fecal pH was lower, and valerate proportion was higher, in dogs fed the 2% ETP diet. All diets exhibited high digestibility (>90%) without effects of dietary treatments. Overall, ETP, a locally sourced co-product of the food industry, may represent a more sustainable alternative to imported raw materials, although further studies are needed to explore its effects on fecal microbiota and health-related parameters.

Keywords: circular economy framework; dog nutrition; tomato pomace

1. Introduction

The food industry faces a significant challenge due to the accumulation of processing waste [1], which can be redirected into animal nutrition within a circular economy framework, thereby contributing to more sustainable livestock and companion animal sectors. In this context, the pet food industry represents a promising avenue for the valorization of such co-products as ingredients or supplements with high nutritional and functional values [2,3].

Tomato (*Solanum lycopersicum* L.) is one of the most important cultivated species in the Solanaceae family and a fundamental element of the global horticultural economy [4]. According to the most recent 2023 statistics, tomato production accounted for 192 million tons, making it the most produced vegetable worldwide [5]. In 2024, Portugal was the third-largest producer of tomatoes in the European Union (EU), producing about 10% of the EU's total tomato production, corresponding to approximately 1.7 million tons [6]. During the industrial processing of tomatoes for products such as juice, paste, sauces and canned goods, numerous by-products are generated, notably TP [7]. Tomato pomace consists mainly of tomato skins, pulp, and seeds, in proportions that vary depending on the



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production method applied [8]. Large quantities of TP are produced annually, particularly during seasonal processing, and considering that TP represents 3–5% of the total weight of processed tomatoes [9], Portugal is estimated to produce 51,000–85,000 tons of TP. Its high moisture content makes disposal and stabilization challenging, with sun and oven drying being the most frequently described treatments for TP [10,11]. An alternative technological approach has emerged to address the challenges associated with high moisture content and structural complexity. Enzymatic pre-treatment of TP may improve drying efficiency by promoting the depolymerization of the fibrous matrix of the plant cell walls, potentially reducing viscosity and water-holding capacity and enhancing heat and mass transfer during drying.

Dried TP is rich (dry matter, DM, basis) in fiber (21–55%), crude protein (CP; 15–27%), ether extract (EE; 10–20%), and ash (3–6%; ref. [10–13]). The variability reported can be due to the different analytical methods employed as well as the raw material composition and processing conditions applied. Indeed, despite the most predominant component of TP being fiber, its concentration varies depending on the proportion of each component [10]. While tomato skins are high in fiber (41%), followed by protein (14%) and fat (3%), seeds are characterized by a high protein content (32%) as well as considerable amounts of fat (27%) and fiber (18%; ref. [14]). Other constituents of TP include sugars, vitamins, flavonoids, carotenoids, and nucleosides [8]. Among the bioactive compounds present in TP, lycopene is the most abundant natural carotenoid [15] and is responsible for the red pigmentation of tomatoes and tomato-derived products. Due to its high antioxidant capacity, lycopene helps protect lipids, proteins, and DNA from oxidative damage [16]. In canine nutrition, lycopene is used not only as a food coloring but also plays a significant role in bone and joint health [17].

Within a circular economy framework, recovering and reusing TP as a functional ingredient in pet food represents a sustainable strategy to reduce food processing waste while producing value-added products. This study aimed, for the first time, to investigate the effects of increasing inclusion levels of enzymatic pre-treatment, vacuum paddle-dehydrated TP (ETP), in extruded diets for adult dogs.

2. Materials and Methods

All procedures were approved by the Animal Ethics Committee of the School of Medicine and Biomedical Sciences, University of Porto (ICBAS-UP), under permit number P489-2023. All animal manipulations adhered to the guidelines for good animal welfare practices established by the EU (Directive 2010/63/EU) and were carried out by trained researchers in laboratory animal science (FELASA, Category C certification). Before the start of the trial, each dog was clinically examined by a veterinarian to confirm its suitability to participate in the studies.

2.1. Animals, Housing and Care Routine

Twelve adult Beagle dogs (six neutered males and six spayed females) of 6.3 (SD = 0.43) years old, weighing 12.9 (SD = 2.01) kg and with a body condition score (BCS) of 6.1 (SD = 0.47; assessed according to a 9-point scale [18]), were used in the experimental trials. Animals were housed in the kennel of the ICBAS-UP. To encourage socialization and promote environmental enrichment, dogs were housed in pairs in communicating boxes comprising an interior and exterior area of 1.8 m² and 3.5 m², respectively, with sliding doors for individual feeding. All dogs were leash walked for at least 30 min once a day. Animals were allowed to exercise and socialize under supervision in an outdoor park between meals. During the fecal collection period, dogs were individually housed at night and the supervised access to the outdoor park was maintained between daily meals.

Therefore, all feces were individually collected, identified and stored. Dogs always had access to drinking water and were individually fed the daily ration in two equal meals, at 8:30 am and 5:00 pm. Diets were fed as extruded kibbles. Daily food allowance was determined for each individual, according to their body weight (BW) and BCS [18], to ensure metabolizable energy (ME) requirements [19].

2.2. Tomato Pomace and Experimental Diets

The ETP was supplied by Simplyeast Lda (Póvoa de Varzim, Portugal), a company dedicated to the valorization of co-products. According to the manufacturer, for the enzymatic treatment, a broad-spectrum endoprotease and two multienzyme preparations were used, which included cellulases, hemicellulases, pectinases, and β -glucanases. Enzymatic hydrolysis occurred for 3 h at an approximate temperature of 55 °C, at the natural pH of tomato pomace (pH: 4.3), using an enzyme inclusion rate of 0.2% (DM basis). The solid-to-liquid ratio was approximately 1:3 ($w v^{-1}$). This was followed by vacuum paddle-dehydration at an approximate temperature of 88 °C, under a vacuum pressure of approximately 100 mbar, for ~19 h, with constant agitation to ensure stable temperature conditions.

Three complete extruded diets for medium-sized adult dogs were formulated according to the current formulation matrices to be iso-energetic, iso-lipidic, and iso-nitrogenous. Diets presented the same ingredients except for the replacement of half or the total amount of wheat bran and sunflower meal with 2% and 4% of ETP, respectively. Diets were produced by Sorgal Pet Food (S. João Ovar, Portugal) and, therefore, due to the proprietary formulation, the detailed composition of the diet ingredients cannot be disclosed. A diet with no inclusion of ETP was used as control. Due to the fiber content of ETP, low inclusion levels (2% and 4%) were selected.

2.3. Palatability Trials

Three two-bowl tests [20] were performed to determine palatability by the pairwise comparison between all diets. Each test was carried out during the morning meal and for two consecutive days, before starting the feeding trial. Each animal ($n = 12$) was given the choice between two diets in two different bowls, separated by 45 cm. The bowls were positioned on opposite sides between days to control side bias. Each bowl contained a quantity of food calculated to meet the ME requirement of each dog for a single meal [19], with the amount offered determined using the nutritional composition of the final diet formulations provided by the manufacturer, such that each dog was offered a quantity of food equivalent to twice its ME requirement. Tests ended after 30 min or when one of the diets present in one of the bowls was completely ingested. The bowl that was first approached and the first food tasted were registered and the consumption amount of each diet was recorded to calculate the intake ratio of the two diets.

2.4. Feeding Trial

The twelve adult Beagle dogs that had previously participated in the palatability trials were assigned to four Latin squares 3×3 , with three diets (without and with ETP included at 2% and 4%) and three experimental periods of 28 days each. Each experimental period was structured into two stages: 6 days of diet transition and 22 days of full diet consumption. During days 1 to 6, dogs underwent a gradual diet transition in which 25% of the previous diet was progressively replaced by the new diet. Total feces collection was performed during 5 days of the last week of each experimental period. Once a week, before the morning feeding, the dogs were weighed, and their BCS was assessed in order to adjust their daily food allowance [21]. The total fecal collection procedure was employed to determine the apparent total tract digestibility (ATTD) of control and experimental diets. During each 5-day fecal collection period, the number of defecations from each dog was

recorded. Individual fecal samples were immediately collected after defecation, scored on a 5-point scale (1 = watery diarrhea; 2 = soft, unformed stools; 3 = soft, formed and moist stools; 4 = hard, well-formed and dry stools; 5 = hard, dry and powdery stools; ref. [22]) and weighed. Each sample was then homogenized and representatively subsampled and stored at -20°C until analysis of Kjeldahl nitrogen (N) and fermentation metabolites [23]. The remaining subsamples were dried individually at 65°C to determine DM content. Subsequently, the dried samples were ground through a 1 mm sieve and stored in airtight containers, properly labeled and composited by experimental period and dog, in a cool, dry environment until further analysis.

2.5. Analytical Procedures

2.5.1. Proximate Chemical Composition and Gross Energy

The proximate chemical composition of the ETP, control and experimental diets, and feces (all dried at 65°C and ground through a 1 mm sieve) were analyzed in duplicate according to official methods [24] to determine DM (ID 934.01), ash (ID 942.05), EE (ID 920.39) and Kjeldahl N (ID 990.03) contents. Fecal N content was determined in fresh, thawed samples. Crude protein was calculated as Kjeldahl N $\times$ 6.25 (ID 990.03). Neutral detergent fiber (NDF) was determined with thermostable α -amylase and without sodium sulfite and expressed exclusive of residual ash [25]. The acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were also determined in diets and ETP [26]. Control and experimental diets were also analyzed for crude fiber, with a prior extraction of fat using petroleum ether [27]. Soluble and insoluble fiber contents (ID 993.19 and ID 991.42, respectively) were determined for all diets and ETP previously ground to 0.5 mm and defatted. For diets and feces, starch content was determined in samples ground to 0.5 mm as described by Salomonsson et al. [28]. The gross energy (GE) of all samples was determined using an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany).

2.5.2. Amino Acid Profile

The amino acid content of the ETP, control and experimental diets was determined in duplicate using reversed-phase ultra-high-performance liquid chromatography (UHPLC). Prior to analysis, the samples underwent hydrolysis with 6 M hydrochloric acid at 116°C for 48 h, followed by pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ Fluor reagent, Waters, Milford, MA, USA) according to the AccQ Tag method. Norvaline was used as an internal standard to ensure accurate quantification. Amino acid separation and detection were performed on a Waters UHPLC system configured for reversed-phase amino acid analysis. The resulting peaks were processed using EMPOWER software, version 3.8.1.1 (Waters; ref. [29]).

2.5.3. In Vitro Digestibility

The in vitro DM, organic matter (OM), and CP digestibility of the ETP and diets was determined using the two-step multienzyme incubation method described by Boisen & Fernández [30], with adaptations proposed by Hervera et al. [31] to better simulate the digestive physiology characteristics of dogs. Analysis was performed in quadruplicate with a blank and a control sample included in each series. The entire procedure was performed under low-light conditions to prevent the photo-oxidation of lycopene and other photosensitive components. Dry matter digestibility was calculated from the weight of the residue obtained after the in vitro digestion and the undigested fraction was analyzed for ash and Kjeldahl N to determine OM and CP digestibility, respectively. The recovered supernatants were stored in the dark at -20°C until the antioxidant activity analysis was performed.

2.5.4. Antioxidant Activity

The antioxidant activity of the ETP and diets was evaluated in supernatants obtained from each replicate ($n = 4$) of the *in vitro* digestibility assay. Supernatants were properly diluted to ensure that the absorbance values were within the linear range of each method. All procedures were carried out under low-light conditions, and all analyses were performed in triplicate. The following methods were used: decolorization of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS $\bullet^+$ assay; ref. [32]), and Folin–Ciocalteu reducing capacity (FC assay; ref. [33]). For the ABTS $\bullet^+$ assay, a solution of ABTS $\bullet^+$ was prepared by reacting equal volumes of 7 mM of ABTS (Sigma-Aldrich, A-1888, Saint Louis, MO, USA) with 2.45 mM of potassium persulfate. The solution was then left to stand overnight in the dark at room temperature. Before use, the solution was diluted with water to achieve an absorbance of 0.80 to 0.90 at 734 nm. In a 96-well microplate, 100 μ L of the supernatant from each solution and the blank were added, along with 100 μ L of the ABTS $\bullet^+$ solution. After incubating for 20 min at 22 °C in the dark, the absorbance was analyzed at 734 nm. The antioxidant activities of the ABTS $\bullet^+$ assay were calculated using Trolox calibration curves dissolved in 50% aqueous ethanol. The results were expressed in milligrams of Trolox equivalents per gram of DM. Finally, the FC assay was performed by adding 100 μ L of each sample and the blank to 20 μ L of Folin–Ciocalteu reagent (Sigma-Aldrich, F-9252) in a 96-well microplate. After a few min, 100 μ L of 0.4 M sodium hydroxide solution was added. The incubation period in the dark was 20 min, after which the absorbance was read at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of DM. A gallic acid calibration curve was used.

2.5.5. Fecal pH and Metabolites

The pH of thawed and homogenized fecal samples was measured using a potentiometer (pH and Ion-Meter GLP 22, Crison, Barcelona, Spain). The concentration of fecal ammonia-N was determined following the Chaney and Marbach method [34]. In brief, fecal samples were diluted at a 1:10 ($w v^{-1}$) ratio in 2 M potassium chloride and then subjected to a 60 min centrifugation process at $5251 \times g$ at 4 °C. The resulting supernatant was subsequently filtered using a 0.45 μ m polyethersulfone syringe filter (Filter-Lab, Barcelona, Spain). Absorbance was read at 550 nm using a Synergy HT multimode microplate reader (BioTek Instruments, Winooski, VT, USA) after the indophenol colorimetric reaction was catalyzed for 10 min at 37 °C. Ammonia-N content was determined using an external ammonia standard solution (0–32 mg dL $^{-1}$). For short-chain fatty acids (SCFA) analyses, 0.12 g of dog feces, rigorously weighted, were transferred into a centrifuge tube and thoroughly mixed with 1.00 mL of 0.3 mol L $^{-1}$ ortho-phosphoric acid (ref. 30417, Honeywell, Lincolnshire, IL, USA) solution containing 1.0 mmol L $^{-1}$ 3-methyl valeric acid (ref. 222453, Sigma-Aldrich Inc., St. Louis, MO, USA) as internal standard. The mixture was centrifuged at $4500 \times g$ for 6 min. The supernatant was analyzed by gas chromatography using a Shimadzu GC-2010 Plus (Shimadzu Corporation, Kyoto, Japan) equipped with a capillary column (HP-FFAP, 30 m $\times$ 0.25 mm $\times$ 0.25 μ m; ref. 19091F-443, Agilent Technologies, Santa Clara, CA, USA), and a flame ionization detector. The injector and detector temperatures were held at 250 °C and 260 °C, respectively. The oven temperature started at 100 °C for 5 min, increased at 5.5 °C per min to 220 °C, which remained during an additional 5 min. Helium was used as carrier gas at a column flow rate of 0.64 mL per min. The injection volume was 1 μ L and the split ratio was 50. Short-chain fatty acids were identified by comparison of the retention times with standards (acetic acid, ref. 49199, Fluka, Seelze, Germany; propionic acid, ref. 81910, Sigma-Aldrich Inc.; iso-butyric acid, ref. 58360, Fluka; butyric acid, ref. B103500, Sigma-Aldrich Inc.; iso-valeric acid, ref. 129542, Sigma-Aldrich

Inc.; valeric acid, ref. 240370, Sigma-Aldrich Inc.) and quantified based on the internal standard addition method. All analyses were performed in duplicate.

2.5.6. Calculations and Statistical Analysis

Fecal production (%) was calculated as:

$$\text{Fecal production}(\%) = \frac{\text{Dried feces output (g d}^{-1}\text{)}}{\text{Dry matter intake (g d}^{-1}\text{)}} \times 100$$

Apparent total tract digestibility (%) of the different diets was calculated using the following equation:

$$\text{ATTD}(\%) = \frac{\text{Nutrient intake (g d}^{-1}\text{)} - \text{Fecal output (g d}^{-1}\text{)}}{\text{Nutrient intake (g d}^{-1}\text{)}} \times 100$$

The ME (MJ kg⁻¹ DM) of the diets was estimated according to Hall et al. [35]:

$$\text{ME (MJ kg}^{-1}\text{ DM)} = \frac{(\text{GE intake (MJ d}^{-1}\text{)} - \text{GE fecal (MJ d}^{-1}\text{)}) - (\text{CP intake (g d}^{-1}\text{)} - \text{CP fecal (g d}^{-1}\text{)}) \times 5.23}{\text{DM intake (g d}^{-1}\text{)}}$$

All data were analyzed using the SAS[®] OnDemand for Academics, version 9.4 (SAS Institute Inc., Cary, NC, USA, 2026). The chi-square test was used to evaluate data from the first approach and first taste ($n = 12$), while the consumption ratio ($n = 12$) was evaluated using Student's *t*-test. Data from the feeding trial were analyzed according to a mixed model with the fixed effects of the square, period, and diet, dog within square as a random effect, and the random residual error. Linear and quadratic effects of diet were evaluated using orthogonal contrasts when the effect of diet was significant. The resulting *p*-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Data on the number of defecations were checked for normality of distribution, and as they did not follow a normal distribution, a non-parametric test (Friedman test) was used. Fecal consistency scores, an ordinal variable, were also analyzed using the Friedman test. For all analyses, significance was set at $p < 0.05$.

3. Results

3.1. Chemical Composition

The ETP presented (DM basis) 202.4 g kg⁻¹ CP, 618.2 g kg⁻¹ NDF and 618.2 g kg⁻¹ insoluble fiber (Table 1). Replacing wheat bran and sunflower meal with ETP did not greatly alter the proximate composition of the diets (Table 1). For ETP and all diets, leucine and arginine were the most abundant essential amino acids, while glutamic acid and glutamine predominated among the non-essential amino acids (Table 2).

Table 1. Proximate composition (g kg^{−1} dry matter, DM) and gross energy (MJ kg^{−1} DM) of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace (ETP) and experimental diets with different levels of ETP inclusion.

Item	ETP	Diet ¹		
		0%	2%	4%
DM, g kg ^{−1}	947.8	978.5	974.0	979.3
Ash	31.9	86.6	75.2	89.0
Crude protein	202.4	283.9	301.1	293.6
Ether extract	108.1	113.9	125.9	131.9
Crude fiber	ND ²	33.1	31.0	44.4
Neutral detergent fiber	618.2	164.7	157.7	171.9
Acid detergent fiber	536.1	55.2	55.9	60.3
Acid detergent lignin	317.0	15.9	19.5	22.2
Insoluble fiber	643.1	255.4	243.9	251.7
Soluble fiber	34.4	7.2	11.6	8.1
Starch	ND ²	343.7	337.5	311.9
Gross energy	23.96	20.02	20.51	20.22

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace.

² ND, not determined.

Table 2. Amino acid profile (g kg^{−1} DM) of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace (ETP) and experimental diets with different levels of ETP inclusion.

Item	ETP	Diet ¹		
		0%	2%	4%
Essential amino acids				
Arginine	14.5	18.4	19.2	18.7
Histidine	3.34	6.37	6.75	6.42
Lysine	8.33	15.2	16.7	15.3
Threonine	8.18	12.6	13.3	12.7
Isoleucine	9.92	14.6	15.0	14.7
Leucine	12.5	25.8	26.7	26.4
Valine	9.69	15.7	16.3	15.9
Methionine	10.3	10.8	11.3	11.2
Phenylalanine	11.6	16.2	16.6	16.3
Total	88	136	142	138
Non-essential amino acids				
Cystine	6.52	6.88	7.04	6.92
Tyrosine	16.4	16.6	17.0	16.9
Aspartic acid + Asparagine	18.5	22.9	25.0	23.9
Glutamic acid + Glutamine	31.6	43.8	46.1	44.5
Alanine	7.92	19.1	20.2	19.7
Glycine	9.01	21.1	22.4	22.3
Proline	9.79	20.9	21.6	21.5
Serine	8.97	15.0	15.5	15.1
Taurine	0.79	1.72	2.00	1.73
Total	109	168	177	173

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace.

3.2. In Vitro Digestibility

Table 3 presents the results of the in vitro digestibility of DM, OM, and CP for the ETP and experimental diets. The control diet (0%) presented numerically higher in vitro digestibility of DM, OM and CP.

Table 3. In vitro digestibility (%) of dry matter (DM), organic matter (OM) and crude protein (CP) of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace (ETP) and experimental diets with different levels of ETP inclusion.

Item	ETP	Diet ¹		
		0%	2%	4%
DM	44.1 (1.46)	85.0 (0.22)	83.6 (1.29)	83.0 (0.96)
OM	41.8 (1.92)	84.1 (0.21)	83.1 (1.73)	82.4 (1.28)
CP	62.9 (0.97)	83.6 (0.24)	82.1 (1.41)	79.5 (1.16)

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace. Values are presented as mean with standard deviation in parentheses ($n = 4$).

3.3. Antioxidant Activity

The antioxidant activity and total phenolic content of the ETP and the experimental diets are shown in Table 4. The ETP exhibited higher numerical values in both assays than experimental diets. Among diets, the one with 2% ETP presented the lowest and the highest numerical values respectively for ABTS and total phenolics content.

Table 4. Antioxidant activity of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace (ETP) and experimental diets with different levels of ETP inclusion.

Item	ETP	Diet ¹		
		0%	2%	4%
ABTS (mg TE ² g ^{−1})	2.51 (0.055)	2.39 (0.118)	1.95 (0.073)	2.19 (0.125)
Total phenolics (mg GAE ³ g ^{−1})	14.1 (0.44)	16.4 (0.53)	14.8 (0.74)	15.2 (0.57)

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace. ² TE, trolox equivalents. ³ GAE, gallic acid equivalents. Values are presented as mean with standard deviation in parentheses ($n = 4$).

3.4. Palatability Trials

Table 5 presents the results of the two-bowl tests. The first diet approached, tasted and the intake ratio was similar between diets ($p > 0.05$).

Table 5. Diet first approached and first tasted frequency and intake ratio of diets containing enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace (ETP) compared to a control diet.

Item	Diet ¹		<i>p</i> -Value	Diet ¹		<i>p</i> -Value	Diet ¹		<i>p</i> -Value
	0%	2%		0%	4%		2%	4%	
<i>n</i>	12	12		12	12		12	12	
First approach	54.2	45.8	0.683	45.8	54.2	0.683	45.8	54.2	0.683
First taste	54.2	45.8	0.683	45.8	54.2	0.683	45.8	54.2	0.683
Intake ratio	50.5	49.5	0.904	49.8	50.2	0.798	52.9	47.1	0.085

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace.

3.5. Feeding Trial

3.5.1. Body Weight, Body Condition Score and Nutrient Intake

All dogs remained healthy throughout the study with no episodes of vomiting, diarrhea, or food refusals. The dogs' BW remained stable throughout the experimental period (Table 6), with the median BCS of dogs during the study being 4.9 out of 9 (interquartile range of 1.0) for all diets. No significant differences were observed in food, nutrient and energy intake among diets.

Table 6. Body weight, diet, nutrient and energy intake of dogs fed experimental diets with different levels of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace (ETP) inclusion.

Item	Diet ¹			SEM ²	p-Value
	0%	2%	4%		
<i>n</i>	12	12	12		
Body weight	12.2	12.4	12.2	0.42	0.425
Diet intake					
g diet d ⁻¹ , as-is	170	158	157	13.3	0.625
g diet d ⁻¹ , DM	162	150	151	12.7	0.611
Nutrient intake, g d ⁻¹					
Organic matter	148	139	137	11.6	0.646
Crude protein	46.1	45.2	44.2	3.68	0.897
Ether extract	18.5	18.9	19.9	1.51	0.682
Neutral detergent fiber	26.7	23.6	25.8	2.06	0.381
Starch	55.8	50.6	47.0	4.31	0.206
Gross energy, MJ d ⁻¹	3.25	3.08	3.05	0.256	0.736

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace. ² SEM, standard error of the mean.

3.5.2. Fecal Output, Characteristics and Metabolites

The increasing level of ETP inclusion kept the fecal consistency unaffected, with fecal scores of 3.2–3.3 out of 5 for all diets (Table 7). No differences were observed in fecal output, characteristics and metabolites, except for fecal pH and valerate concentration that were, respectively, lower and higher when dogs were fed the diet with 2% inclusion of ETP (Tables 7 and 8).

Table 7. Fecal consistency score and number of defecations of dogs fed experimental diets with different levels of enzymatic pre-treatment, vacuum paddle-dehydrated tomato pomace inclusion (mean ± standard deviation).

Item	Diet ¹			p-Value
	0%	2%	4%	
Fecal consistency score	3.3 (0.42)	3.2 (0.36)	3.3 (0.32)	0.903
Defecations, number d ⁻¹	1.8 (0.36)	1.9 (0.25)	2.0 (0.40)	0.991

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace. Values are presented as mean with standard deviation in parentheses.

Table 8. Fecal parameters, metabolites and proportions of short-chain fatty acids (SCFA) of dogs fed experimental diets with different levels of enzymatic pre-treatment, vacuum paddle-dehydrated tomato pomace inclusion.

Item	Diet ¹			SEM ²	p-Value
	0%	2%	4%		
Fecal output and characteristics					
g feces d ⁻¹ , as-is	122	119	114	12.5	0.745
g feces d ⁻¹ , DM	41.1	36.0	37.9	3.82	0.298
Gross energy, MJ d ⁻¹	0.615	0.580	0.591	0.0591	0.753
Production, %	73.7	76.9	75.5	3.22	0.725
DM, %	34.4	32.9	34.4	0.73	0.105
Fecal metabolites					
pH ³	6.5	6.3	6.5	0.05	0.037
Ammonia-N, mg kg ⁻¹ DM	223.7	257.1	223.2	18.74	0.206

Table 8. *Cont.*

Item	Diet ¹			SEM ²	p-Value
	0%	2%	4%		
Total SCFA (mmol kg ⁻¹ DM)	53.98	54.34	48.17	2.795	0.169
SCFA molar proportions (% of total SCFA)					
Acetate	57.08	53.56	54.03	1.319	0.106
Propionate	26.04	26.40	27.44	1.046	0.465
Butyrate	11.42	13.91	12.45	0.810	0.054
Iso-butyrate	1.64	1.66	1.75	0.077	0.496
Iso-valerate	2.11	2.17	2.19	0.082	0.765
Valerate ⁴	1.39	2.08	1.77	0.267	0.020
Iso-caproate	0.32	0.26	0.36	0.057	0.146

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace.

² SEM, standard error of the mean. ³ Linear ($p = 0.929$) and quadratic ($p = 0.022$) contrasts. ⁴ Linear ($p = 0.105$) and quadratic ($p = 0.036$) contrasts.

3.5.3. Apparent Total Tract Digestibility and Metabolizable Energy

No significant differences were observed in the ATTD or in the ME content of the different diets (Table 9).

Table 9. Apparent total tract digestibility (ATTD) and metabolizable energy of dogs fed experimental diets with different levels of enzymatic pre-treatment, vacuum paddle-dehydrated tomato pomace inclusion.

Item	Diet ¹			SEM ²	p-Value
	0%	2%	4%		
ATTD, %					
DM	75.1	76.4	74.8	1.08	0.505
Organic matter	81.0	81.5	80.4	0.87	0.674
Crude protein	69.4	70.1	69.9	1.50	0.923
Ether extract	94.1	94.5	94.8	0.25	0.133
Neutral detergent fiber	53.9	55.7	51.8	2.04	0.413
Starch	99.8	99.7	99.7	0.05	0.066
Gross energy	81.4	81.6	80.6	0.85	0.678
ME, MJ kg ⁻¹	15.3	15.6	15.2	0.15	0.130

¹ Diets with 0, 2 and 4% inclusion of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace.

² SEM, standard error of the mean.

4. Discussion

4.1. Chemical Composition of Tomato Pomace and Diets

Enzymatic pre-treatment of TP may promote the depolymerization of the fibrous matrix of the plant cell wall, potentially increasing the bioavailability of its components. However, tomato peel, rich in lycopene, consists of a rigid matrix formed by several layers of tissue, externally covered by a layer of cutin [36]. Cutin, a complex lipid biopolymer, can only be degraded by cutinase enzymes [37,38]. As canines do not produce these enzymes, the complexity of the plant cell wall matrix may limit access to the cell's contents, including lycopene.

To the best of the author's knowledge, no study evaluated the chemical composition of ETP, precluding the comparison of the results obtained herein. Data on dried TP have shown that chemical composition varies according to tomato variety, growing conditions, processing methods and proportions of seeds and peels [39]. In the present study, the parameters analyzed for the chemical composition of ETP were generally within the range reported by other studies on dried TP. Indeed, ash content falls within the range reported

earlier for sun-dried and oven-dried TP (30.8 to 60.8 g kg⁻¹ DM; ref. [10]). The CP content of TP depends on seed proportion and fruit ripeness [40], and has been reported to range from 150.8 to 246.7 g kg⁻¹ DM [41], which is in agreement with the values obtained in the present study. Tomato seeds and peels contribute differently to the amino acid profile, with seeds generally providing higher concentrations of amino acids, especially glutamic acid, aspartic acid, arginine, leucine, glycine, alanine, phenylalanine, lysine, serine, and proline, and peels in glutamic acid, aspartic acid, arginine, leucine, isoleucine, alanine, and glycine [42]. In the present study, the amino acid profile of the ETP was consistent with previously reported values for dried TP [43]. The fat content of dried TP, largely due to tomato seeds, averages 100 to 150 g kg⁻¹ DM, but can range from 50.4 to 200.5 g kg⁻¹ DM [10], with the latter being when a high proportion of seeds is present [13]. The EE content of ETP fell within that range. Generally, ETP presented slightly higher NDF, ADF, and ADL contents than those reported by other authors for sun-dried, oven-dried or freeze-dried TP [10,44]. Although the high fiber content of ETP might limit digestibility, it can increase fecal volume and intestinal motility [45]. In dog nutrition, dietary fiber is typically divided into insoluble and soluble fiber. Minimally fermentable insoluble fiber includes hemicellulose, cellulose, and lignin, while highly fermentable soluble fiber encompasses various non-cellulosic polysaccharides and oligosaccharides [46]. Compared to the seeds, tomato peels are richer in dietary fiber, with the insoluble fiber fraction prevailing [41]. However, the proportion of fiber in peels and seeds can vary depending on the geographic region, growing conditions, and production process [47]. In the ETP analyzed, both fiber fractions remained within the reference ranges described by other authors for dried TP [10,41]. Soluble fiber increases the viscosity of gastric and intestinal contents, delaying gastric emptying and increasing transit time in the small intestine [46,48]. It also limits intestinal enzymatic activity, reduces postprandial glucose levels, and increases the production of SCFA [46,48]. In contrast, insoluble fiber increases fecal volume and decreases intestinal transit time [48]. Generally, increasing fiber in diets reduces caloric density, improving satiety [46,48]. Despite the scarcity of studies, the GE values of the treated TP sample fall within the expected range for dried TP [42,49].

The chemical composition of the experimental diets ensured the nutritional requirements for adult dogs [19] and including 2% or 4% ETP, replacing wheat bran and sunflower meal, had a minimal impact on their chemical composition. Conversely to ETP, for experimental diets, crude fiber content was also analyzed. Differences in crude fiber, NDF, and soluble and insoluble dietary fiber values arise from the distinct analytical methods and fiber fractions quantified. Crude fiber, determined by sequential acid and alkaline digestion, measures only a limited portion of structural carbohydrates, mainly cellulose and part of lignin, and underestimates total dietary fiber [50]. Moreover, despite crude fiber being considered undigestible by dogs, hemicellulose and cellulose are partially digested by gastrointestinal fermentation in the large intestine of companion animals; the extent of fermentation largely depends on the residence time of digesta [51,52]. Despite its limitations, crude fiber continues to be used in pet food due to the legal requirement to indicate it on the labels of compound feeds and to estimate the ME content of diets. In contrast, NDF quantifies a broader cell wall fraction, including cellulose, hemicellulose, and lignin, and therefore typically yields higher values. The determination of soluble and insoluble dietary fiber relies on enzymatic–gravimetric methods that separate fiber components based on solubility, capturing both structural and non-structural polysaccharides such as pectins and gums [53]. Consequently, differences among these measurements reflect the specific fiber fractions present in tomato pomace and the analytical principles underlying each method.

4.2. *In Vitro* Digestibility and Antioxidant Activity

The high fiber content of ETP might have limited the *in vitro* digestibility of DM, OM, and CP. The use of an enzymatic pre-treatment can promote the depolymerization of the fibrous matrix of the plant cell wall, favoring the release of compounds, namely proteins integrated into the fiber, and, consequently, increasing their bioavailability [54]. However, to the best of the authors' knowledge, there are no studies evaluating the *in vitro* digestibility of TP through canine digestion simulation. The *in vitro* digestibility values of the experimental diets decreased as the level of inclusion of ETP increased, showing that ETP acts as a dilution factor for digestibility, due to its matrix rich in structural carbohydrates and lignin [55,56].

In the present study, the antioxidant activity was measured in the supernatants from the *in vitro* digestibility to better mimic the antioxidant potential of compounds absorbed by the animal. Tomato contains a wide range of phytochemicals, with research primarily focusing on lycopene, the major carotenoid and a potent antioxidant [36]. In addition to lycopene, tomato provides α - and β -carotene and lutein [57], which exhibit provitamin A and antioxidant activities [58] as well as various phenolic compounds, mainly flavonoids and hydroxycinnamic acid derivatives [59]. According to the supplier, ETP has a significantly higher lycopene concentration than TP dried at higher temperatures (339 ppm vs. 200 ppm, respectively). This difference aligns with the effects of temperature on the decomposition of temperature-sensitive compounds, such as lycopene, as well as with the potential role of enzymatic hydrolysis on increased lycopene bioavailability [60,61]. However, a higher lycopene content in the ingredient does not necessarily equate to greater antioxidant activity, as the effectiveness of enzymatic pre-treatment in degrading the plant cell wall and consequently releasing its encapsulated compounds may be limited. In the present study, the antioxidant activity observed in the ETP may be related to the presence of lycopene, since tomato peels are the part of the fruit with the highest lycopene content and, therefore, with the greatest antioxidant capacity [36]. Inclusion of ETP at low levels (2% and 4%) did not reflect in an increased antioxidant activity of diets. Furthermore, since lycopene is hydrophobic, the low values of the ABTS assay could be explained by the fact that the ABTS radical was in an aqueous medium [15].

4.3. *Palatability*

Palatability is the perceptual response to food consumption that influences intake and allows the assessment of acceptance or preference relative to other foods [20]. It depends on taste, odor, appearance, temperature, size, texture, and consistency, as well as previous experiences [62,63]. Given its influence on food intake, palatability may affect animal's nutritional status and well-being, as well as the owner's attitude and opinion about their pet's diet [64]. Preference tests are commonly used to assess food preferences under a controlled environment [65]. Among the different methods, the two-bowl test is the most commonly applied approach to comparing preferences for different diets [66]. To our knowledge, no study has evaluated the palatability of ETP in dog food using standardized preference tests. The replacement of wheat bran and sunflower meal with ETP did not affect the first approach, taste and intake ratio, likely because these ingredients share similar sensory characteristics and do not substantially modify the concentration of compounds associated with flavor perception. Palatability in dogs is largely influenced by free amino acids and small peptides related to savory or umami taste, particularly glutamic and aspartic acids [67], whose contents were similar between diets. Additionally, studies on diets containing fiber-rich fruit by-products suggest these ingredients do not affect dogs' food preferences [68]. However, depending on the variety and level of inclusion, fiber can

decrease the diet palatability of canine diets [69]. In the present study, fiber content did not greatly vary among diets.

4.4. Feeding Trial

The daily food allowance was calculated to meet energy requirements and adjusted based on ideal BW and BCS; consequently, these parameters remained stable throughout the study, with dogs presenting an ideal BCS, defined by palpable ribs without excess fat, waist behind the ribs (viewed from above), and retracted abdomen (viewed from the side; ref. [18]). As the diets had similar energy content and digestibility, the amount of food required to meet energy needs did not differ among diets, and dogs tolerated the inclusion of ETP well. Nutrient and energy intake were not affected by diet.

Experimental diets had no impact on fecal production and characteristics. The average number of defecations and fecal DM and output aligned with values expected for pet foods [70], with stool consistency averaging 3.3 out of 5, representing soft, formed and moist stools. A significant quadratic effect on fecal pH was observed, which decreased with a 2% inclusion of ETP. Although this effect could be attributed to a higher intestinal fermentation promoted by the higher content of soluble fiber of this diet [71,72], no effects were observed on total SCFA production. Although no significant effects were observed on CP intake and digestibility, and fecal ammonia-N concentration, the observed effect on valerate concentration suggests that a higher amount of undigested protein has reached the colon with 2% ETP diet. Although no studies are available on the effects of ETP on fecal metabolites, the results obtained agree with earlier studies on other fiber sources such as dried carrot pomace, dried grape pomace, and dried pea hulls [73].

Differences between *in vitro* and *in vivo* digestibility values can be attributed to the limitations of *in vitro* methods in reproducing the complexity of the canine gastrointestinal environment. *In vitro* assays simulate digestion under controlled enzymatic and physico-chemical conditions, but they cannot fully replicate physiological processes such as gastric emptying, intestinal motility, nutrient absorption, endogenous secretions, and microbial fermentation in the large intestine [31]. In contrast, *in vivo* digestibility reflects the integrated response of the whole animal, including host–microbiota interactions and individual variability. Furthermore, factors such as ingredient matrix effects and processing-induced structural changes may influence nutrient digestion differently under simulated versus physiological conditions. Therefore, although *in vitro* methods are valuable for rapid screening and comparative evaluation of diets, they may overestimate or underestimate digestibility relative to *in vivo* measurements in dogs. All diets exhibited *in vivo* DM and CP digestibility values of approximately 80%, which are considered desirable for nutrient digestibility in dry foods for dogs [74]. The high fiber content of pomace, particularly pectins, is known to reduce the digestibility of protein and fat [75]. However, in the present study, the inclusion of ETP did not affect the digestibility of CP. Metabolizable energy was similar across diets. A previous study, presumably with dry TP included up to 8%, revealed a decrease in energy digestibility [75]. Compared with other fiber sources, such as oven-dried citrus pulp, oven-dried apple pomace and dried grape pomace, ETP appears to be a more favorable ingredient for maintaining overall diet digestibility in dogs [71,75].

5. Conclusions

This study shows that the inclusion of ETP in extruded dog food was safe and well tolerated by the animals and did not affect palatability, intake, fecal traits, metabolites, or digestibility. The increase in fecal valerate concentration observed with the 2% ETP diet suggests a potential modulation of microbial pathways, which should be confirmed through further detailed gut microbiota studies. Although systemic health biomarkers

were not evaluated in the present study, further *in vivo* investigations are needed to assess the bioavailability of bioactive compounds, strategies to enhance their efficacy, the optimal inclusion level, and their effects on animal health. Overall, this study confirms the promising potential of ETP as a sustainable ingredient for extruded dog food, supporting the valorization of this co-product within a circular economy framework, particularly in countries with high tomato production, such as Portugal.

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Conflicts of Interest: Tiago Aires is an employee of Sorgal Pet Food, the company that manufactured the experimental diets used in this study. The authors declare that this affiliation did not influence the study design, data collection, analysis, interpretation, or the decision to publish. The authors further declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ATTD	Apparent total tract digestibility
BCS	Body condition score
BDL	Below detection limits
BW	Body weight
CP	Crude protein
DM	Dry matter
EE	Ether extract
ETP	Enzymatic pre-treated tomato pomace
EU	European Union

FC	Folin–Ciocalteu reducing capacity
FDR	False discovery rate
GAE	Gallic acid equivalents
GE	Gross energy
ICBAS-UP	School of Medicine and Biomedical Sciences, University of Porto
NDIgA	Immunoglobulin A
ME	Metabolizable energy
N	Nitrogen
ND	Not determined
NDF	Neutral detergent fiber
OM	Organic matter
PBS	Phosphate-buffered saline
SCFA	Short-chain fatty acids
SEM	Standard error of the mean
TE	Trolox equivalents
TP	Tomato pomace
UHPLC	Ultra-high-performance liquid chromatography

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