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Effects of dietary inclusion of hydrolysed feather meal on faecal fermentation products in adult female dogs

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ABSTRACT

There is growing interest in alternative protein sources to promote a more circular economy within agri-food systems. This study evaluated the impact of replacing 7% of poultry meal (PM) with hydrolysed feather meal (HFM) in canine diet on faecal characteristics. Six adult female English Setter dogs, matched for age, body weight, and body condition score (BCS), were divided into two groups and tested with two diets (PM *vs.* HFM) in a cross-over design. The study included a 7-d adaptation period, followed by a 45-d total faecal collection. A 30-d washout period was used. Digestive fermentation by-products were analysed by using gas and liquid chromatography. Data were analysed using a generalised linear mixed model (GLMM), with diet, time and their interaction as fixed effects, and the experimental phase as a random effect. Faecal scores for both groups remained within the range of 2–3, which is considered 'ideal'. Dogs fed the HFM diet showed significantly higher ($p < 0.001$) faecal concentrations of acetate and isobutyrate, and significantly lower ($p < 0.01$) levels of propionate and butyrate compared to the PM diet. Furthermore, dogs fed HFM diet exhibited significantly increased ($p < 0.001$) concentrations of putrescine and cadaverine and significantly decreased ($p < 0.001$) levels of spermine. The profile of short-chain fatty acids (SCFAs) and biogenic amines differed from that those reported in dogs with inflammatory enteropathies, confirming the maintenance of gastrointestinal health throughout the study. These results suggest that HFM is a promising alternative protein source for dog food formulations, contributing to the sustainable utilisation of animal by-products.

HIGHLIGHTS

1. Diet including 7% hydrolysed feather meal (HFM) was well accepted and the dogs consumed the total amount of the offered food.
2. Faecal scores remained within the ideal range of 2.0–2.5 in both groups, indicating good faecal quality.
3. The organic acid and biogenic amine content confirms the maintenance of gastrointestinal health in the dogs enrolled in this study.

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Introduction

The growing focus on sustainability in the pet food industry has led to increased interest in alternative protein sources derived from animal by-products. In the context of the circular economy, which seeks to minimise waste and maximise the reuse of resources (Korhonen et al. 2018), such by-products represent an opportunity to reduce environmental impact while maintaining nutritional value in companion animal diets.

Animal by-products, as defined in Regulation (EC) 1069/2009, include various parts of animals not intended for human consumption. These materials have long been used in pet food to provide proteins, fats and essential nutrients (Corbin 1992), with poultry by-products in particular offering an economical and abundant source of animal protein (Girotto and Cossu 2017). Among these, poultry feathers, which make up around 7% of a chicken's body weight (Machado et al. 2021), represent a major waste stream, with global production exceeding 40 million tons annually (Peydayesh et al. 2023).

Feathers are primarily composed of keratin, an insoluble protein with very low digestibility in its

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native form. However, processing techniques such as thermal, enzymatic, microbial or chemical hydrolysis can disrupt the keratin structure, thereby improving amino acid availability (Zhang et al. 2014; Vasconcellos et al. 2024). The overall *in vivo* tract digestibility of hydrolysed feather proteins remains relatively low, depending on the degree of hydrolysis and the processing method applied, averaging approximately 67% in dogs (Pacheco et al. 2016). Despite its limited biological value for monogastric species, primarily due to low concentrations of lysine and methionine (Baker et al. 1981; Klemesrud et al. 2000), hydrolysed feather meal (HFM) does contribute essential amino acids such as leucine and isoleucine (Ssu et al. 2004; Pfeuti et al. 2019).

The nutritional limitations of HFM also raise concerns regarding its potential impact on gastrointestinal health. Proteins that escape digestion in the small intestine undergo colonic fermentation, resulting in the production of metabolites such as short-chain fatty acids (SCFAs), biogenic amines, ammonia and phenolic compounds. While some of these metabolites may disrupt gut homeostasis and contribute to dysbiosis (Macfarlane et al. 1988; Bastos et al. 2020; Montegiove et al. 2023), others, such as butyrate, exert beneficial effects by supporting mucosal integrity (Ichikawa and Sakata 1998).

Furthermore, biogenic amines may occur in pet foods at variable concentrations, often associated with microbial contamination and proliferation during processing, handling and storage. To date, however, no regulatory guidelines have been established for biogenic amine concentrations in animal feeds (Montegiove et al. 2023).

A few recent studies have examined HFM in canine diets, with mixed findings. El-Wahab et al. (2022) reported no effect on crude protein digestibility but noted a decline in faecal quality with increasing HFM inclusion. Machado et al. (2021) found no impact on stool consistency, while Pacheco et al. (2016) demonstrated that enzyme-assisted hydrolysis improved digestibility. Although HFM is already employed in certain commercial canine diets, particularly in hypoallergenic formulations, owing to its low protein antigenicity and functional suitability for sensitive animals (Lesponne et al. 2018), evidence regarding its effects on faecal characteristics and fermentation-related markers in healthy adult dogs remains scarce, further research is needed to assess its suitability as a sustainable dietary protein source.

The aim of this preliminary study was to evaluate the effects of a diet containing HFM, compared to a control (CTR) poultry meal (PM)-based diet, on faecal quality parameters and fermentation by-products in healthy adult dogs. We hypothesised that HFM inclusion would not adversely affect faecal consistency or microbial metabolite production, supporting its potential role in sustainable canine nutrition.

Materials and methods

Animal ethics statement

The research was approved by the Ethical Animal Care and Use Committee of the Department of Veterinary Sciences, of the University of Messina, code 01/2023, and the experiment were in compliance with the European guidelines for the care and use of animals in research (European Directive 2010 2018/63/EU) and those on the placing on the market and use of feed (European Union 2009).

Animals

A total of six female English setter dogs with an age of 60 ± 34 months were involved in a cross-over design. The dogs were divided into two groups, CTR and treated (TRT), blocked for body weight (CTR: 16.5 ± 1.18 Kg; TRT: 16.5 ± 0.5 Kg), body condition score (BCS, CTR: 5 ± 0; TRT: 5 ± 0, on a 9-point scale), muscle condition score (MCS, CTR: 1 ± 0; TRT: 1 ± 0, on a 4–point scale) and faecal consistency score (FCS, CTR: 2.5 ± 0; TRT: 2.5 ± 0, on a 5-point scale).

Dog health was assessed by regular clinical assessment. Prophylactic treatments against intestinal worms were administered before each phase of the experimental protocol.

Experimental diets

Before the beginning of the experimental trial, all the dogs fed a commercial extruded dry diet (referred to as the 'Basic diet').

For the experimental trial, two extruded complete dry foods were used (provided by an Italian petfood manufacturer). A tested diet (HFM) with 7% HFM (GOLDMEHL FM, Gepro, Diepholz, Germany; https://www.lidorr.com/wp-content/uploads/2021/01/Goldmehl-FM.pdf) as partial substitution of PM was compared with a reference diet (PM) containing PM as a main protein source. GOLDMEHL FM derives from fully healthy poultry by-products, and it is considered a processed animal protein, Cat. III material, in accordance with Regulation (EC) No 1069/2009. Both experimental diets contained docosahexaenoic acid (DHA)

sourced from algae (mostly *Schizochytrium*), yeasts: and botanical to support the digestive health. From a qualitative perspective, the two diets included the same ingredients, nutritional additives and antioxidants (Table 1).

The individual maintenance energy requirements were calculated according to the recommendations of FEDIAF - The European Pet Food Industry Federation guidelines (2021) for adult dogs with low physical activity (<1 h/d) and the diets were offered once a day, at the same time each day (8:00 pm), to meet the estimated maintenance requirements. When necessary, the daily food allowance was adjusted to maintain a stable body weight. Water was available *ad libitum* throughout the study.

Analysis of dietary components

Before analysing the chemical composition, the kibbles were ground in a cutting mill with a 1 mm screen sieve (Cyclotec 1093 Sample mill, FOSS Hillerod, Denmark). The chemical composition of HFM and diets (PM and HFM) was analysed in triplicate using procedures outlined by the Association of Official Analytical Chemists (AOAC 2010). The following methods were employed: method no. 934.01 for dry matter (DM); method no. 942.05 for ash content; method no. 990.03 for nitrogen (N) content; method no. 954.02 for acid-hydrolysed fat; method no. 978.10 for crude fibre. Total starch content was determined using a Megazyme Total Starch Assay Kit (Megazyme, NEOGEN, Lansing, MI) following AOAC (2019) method no. 996.11. Crude protein was calculated based on the analysed nitrogen (N) content, using a conversion factor of 6.25 (El-Wahab et al. 2022). Moreover, a Total Dietary Fibre Assay Kit (Megazyme, NEOGEN, Lansing, MI) for the analysis soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) was used according to AOAC (2019) method no. 991.43. Total dietary fibre was calculated from the sum of SDF and IDF. The results were expressed as g/100g (as-fed). The organic matter (OM) was calculated by reducing the amount of DM by the amount of in OM (crude ash) (Cherian 2019).

The metabolisable energy (ME) was calculated using the predictive equation with modified Atwater factors for dogs and cats (3.5 kcal/g protein, 8.5 kcal/g fat and 3.5 kcal/g NFE) as described by AAFCO (2019):

$$ME = (3.5 \times \text{g protein}) + (8.5 \times \text{g fat}) + (3.5 \times \text{g NFE}).$$

Table 1. Chemical composition (g/100g, as-fed) and metabolisable energy (ME) of the hydrolysed feather meal and experimental diets.

Parameters, g/100g, as-fed	Hydrolysed feather meal	Diets[†] PM[‡]	HFM[§]
DM	94.41	90.84	91.92
Moisture	5.59	9.16	8.08
CP	82.64	19.07	19.20
Fat	7.07	15.24	15.00
CF	0.91	2.10	2.00
Ash	1.83	5.45	5.01
Starch	5.63	40.59	40.80
NFE*	1.96	48.98	50.71
OM[#]	92.63	85.39	86.91
TDF	2.38	7.51	6.97
IDF	2.03	6.14	5.98
SDF	0.35	1.37	1.00
ME, Kcal/100g (as-fed)	356	368	368

DM: dry matter; CP: crude protein; CF: crude fibre; TDF: total dietary fibre; IDF: insoluble dietary fibre; SDF: soluble dietary fibre; GE: gross energy; ME: metabolisable energy

*NFE = calculated nitrogen-free extract.

[#]OM = calculated organic matter.

[†]Extruded commercial dry diets; the amount of the ingredients was not available.

Both the experimental diets contained the same ingredients, nutritional additives and antioxidants. Ingredients: cereals pregelatinised (rice 30%, starch digestibility 95%; malted cereals 0.3%), processed animal proteins of poultry origin 16%, Oils and fats–(Algal Omega 3 DHA 0.18%, MCT-medium chain triglycerides 0.2%), vegetables (Chicory-FOS, Pea, Garlic fibre) carob extract (roasted), fish and fish by-products, extruded flax, minerals, yeasts: *Saccharomyces cerevisiae*, cell walls-M.O.S (mannan-oligosaccharides), algae, *Yucca shidigera*; extracts of Andrographis paniculata, *Boerhavia diffusa*, *Physilantus amarus*, *Solanum nigrum*, Silymarin. Additives as reported in the label (mg/kg): Vitamins: 3a672a-Vit. A I.U. 20.000, 3a67-Vit. D3 I.U. 1.600, 3a700-Vit.E mg 240, 3a821-Vit. B1 (thiamine monohydrate) mg 12.50, 3a825ii-Vit. B2 (riboflavin) mg 25, 3a831-Vit. B6 (pyridoxine hydrochloride) mg 7.50, Vit.B12 (cyanocobalamin) mg 0. 009, 3a711-Vit. K3MNB mg 4, 3a312-Vit. C Prot. mg 125, 3a314-Niacin mg 68.50, 3a880-Vit. H (biotin) mg 1, 3a841-Ac. pantothenic mg 35.50, 3a316-Folic Acid mg 1.80, 3a890-Choline Chloride mg 1500, 3a160a-Beta-carotene mg 10, 3a910-LCarnitine mg 100. Minerals: 3b101-Iron (ferrous carbonate) mg 50.50 + 3b103-Iron (Iron sulphate monohydrate) mg 50.50 + 3b105-Iron (fumarate ferrous) mg 35.00 + 3b107-Iron (Iron(II) chelate of glycine hydrate) mg 46.00, 3b502-Manganese (Manganous oxide) mg 76.00 + 3b502-Manganous (manganous oxide) mg 76.00 + 3b5.10-Manganese (Manganese chelate of the hydroxy analogue of methionine) mg 30.25, 3b605-Zinc (Zinc sulphate monohydrate) mg 127.00 + 3b6.10-Zinc (Zinc chelate of the hydroxy analogue of methionine) mg 37.50, 3b405-Copper (Copper sulphate pentahydrate) mg 10.00 + 3b4.10-Copper (Copper chelate of the hydroxy analogue of methionine hydroxy analogue) mg 2.20, 3b203-Iodine (anhydrous calcium iodate) mg 2.50, 3b802-Selenium (Sodium selenite) mg 0.13 + 3b810-Organic selenium, Saccharomyces cerevisiae CNCM I-3060 mg 0.002. Amino acids: 3c301-DL Methionine mg 440 - 3c322-Lysine monohydrochloride mg 130.00, 3c307-Hydroxy analogue of methionine mg 330. Preservatives: 1a300-citric acid mg200, E332 Potassium citrate mg 500; Acidity regulators: 4d8 ammonium chloride mg 800. Antioxidants: 1b3068(i)- extracts of natural origin rich in tocopherol mg320, 3a300-L-ascorbic acid mg255. Organoleptic additive: Chestnut extract mg 2800.

[‡]PM diet: diet with poultry meal (16%).

[§]HFM diet: diet with poultry meal (9%) and hydrolysed feather meal (7%).

Nitrogen-free extract (NFE) is obtained by subtracting percent protein, fat, crude fibre, moisture and ash from 100%.

The chemical composition and the ME content of HFM and diets (PM and HFM) are reported in Table 1.

Table 2. Amino acid composition of the experimental diets (g/100g, as-fed).

	Diets		
	PM[‡]	HFM[§]	*p* Value
Essential amino acid, g/100g, as-fed			
Arginine	1.62	1.67	0.321
Histidine	0.37	0.36	0.519
Isoleucine	0.90	0.91	0.519
Leucine	1.49	1.50	0.225
Lysine	0.80	0.81	0.184
Methionine	0.40	0.44	0.053
Phenylalanine	1.01	1.03	0.036
Tryptophan	0.27	0.27	0.742
Threonine	0.72	0.73	0.057
Valine	1.23	1.26	0.281
Non-essential amino acid			
Serine	2.13b	2.20a	0.002
Proline	1.65	1.72	0.075
Alanine	0.65	0.68	0.092
Glycine	1.63	1.66	0.244
Aspartic acid + Asparagine	1.23	1.25	0.139
Hydroxyproline	0.51	0.53	0.038
Glutamic acid + Glutamine	1.82	1.91	0.222
Hydroxylysine	0.45	0.46	0.101
Tyrosine	0.57	0.58	0.057
Cysteine	0.41	0.42	0.134

[‡]PM diet: diet with poultry meal (16%).
[§]HFM diet: diet with poultry meal (9%) and hydrolysed feather meal (7%).

The composition of amino acids in the two diets was analysed following Oteri et al. (2021) by using a Trace 1310 chromatograph (Thermo Fisher, Waltham, MA) with a flame ionisation detector (FID) and a ZB-AAA Amino Acid column (10 m × 0.25 mm internal diameter); the oven temperature was programmed from 110 °C to 320 °C at 32 °C/min, with a final isotherm of 320 °C (1 min). During the acid hydrolysis, the asparagine and glutamine were converted to aspartic and glutamic acids; therefore, they were calculated as the sum of the aspartic acid plus asparagine and of the glutamic acid plus glutamine. The analysis was performed in triplicate for both diets and the results were expressed as g/100g (as-fed) (Table 2).

The biogenic amine (tryptamine, phenethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) concentrations in both diets were analysed according to the method reported by Chiofalo et al. (2019) by using a HPLC (Shimadzu Italia, Milan, Italy), coupled to a fluorescence an RF-20A detector (FLD, Shimadzu Italia, Milan, Italy) at 320 nm excitation and 523 nm emission (Preti et al. 2015). Chromatographic separations were obtained in the SUPELCOSIL LC-18 column (Supelco, Bellefonte, PA) in an oven (CTO-20A, Shimadzu Italia, Milan, Italy) set at 35 °C. A gradient elution of HPLC water and acetonitrile at a constant flow of 0.8 mL/min using a DGU-20A5R degasser (Shimadzu Italia, Milan, Italy) and an LC-20AD pump (Shimadzu Italia, Milan, Italy) was used. Total analysis time was 35 min. Identification and quantification of biogenic amines were performed using external calibration with biogenic amine standards (Merck, Darmstadt, Germany). Standard solutions were first prepared at a concentration of 1 mg/mL for each biogenic amine in perchloric acid. These standard solutions were then diluted to concentrations of 0.0005, 0.001, 0.005, 0.010, 0.025 and 0.050 mg/mL to generate the calibration curves. In the standard solution the limit of quantification (LOQ) was 0.001 mg/g for all amines. All samples were analysed in triplicate and the results expressed as mg/g (Table 3).

Table 3. Biogenic amine content in the experimental diets (mg/g, as-fed).

	Diets		
Parameters, mg/g, as-fed	PM[‡]	HFM[§]	*p* Value
Tryptamine	0.012	0.023	0.016
Phenethylamine	0.014	0.029	0.019
Putrescine	0.140	0.171	0.025
Cadaverine	0.082b	0.124a	0.009
Histamine	0.007	0.005	0.312
Tyramine	0.008	0.017	0.057
Spermidine	0.004	0.004	0.699
Spermine	0.192	0.210	0.087
Sum of biogenic amines	0.458	0.583	0.020

[‡]PM diet: diet with poultry meal (16%).
[§]HFM diet: diet with poultry meal (9%) and hydrolysed feather meal (7%).
a,b for $p \leq 0.01$.

Study design

The study followed a crossover experimental design involving two groups of six dogs each: a CTR group and a treated group (TRT) (Figure 1). Two dietary treatments were tested across two consecutive phases (R1 and R2): a diet containing PM and a diet containing HFM. During the first phase (R1), for 45 d, dogs in the CTR group received the PM diet, while those in the TRT group received the HFM diet. The first phase (R1) was followed by a 30-d washout period, during which all dogs fed the basic diet previously used prior to the start of the experimental trial. After the washout, the dietary treatments were crossed over: dogs originally in the CTR group received the HFM diet, and those in the TRT group received the PM diet for an additional 45-d period (R2). Each phase (R1 and R2) was preceded by a 7-d of adaptation period to the experimental diets, with the objective of acclimatising the test animals to the diet and adjusting food intake to maintain body weight.

The amount of food offered during the first and the second phase was constant. Food intake was recorded throughout both stages.

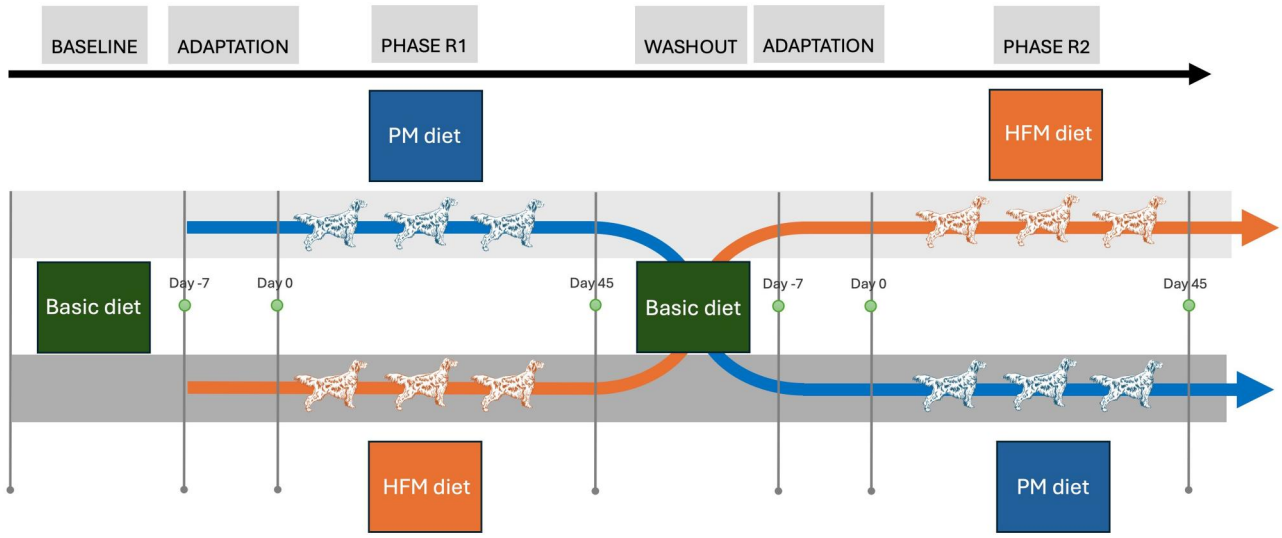

Figure 1. Schematic design of the crossover study. Participant dogs switched diets after a washout period. Sample collection (5 time-points). Each stage of the trial consisted in a 45–day period.

Breeding facility and faeces collection

The dogs were privately owned by the same person and housed in the same place. During the test period, the dogs were housed in cages with a natural light-dark cycle (natural rhythms from dawn to dusk). Exercise was provided twice daily (morning and evening, for approximately half an hour each time) in an outdoor area; all dogs had regular opportunities to socialise with each other and with members of the owner's family.

Preliminary observation conducted prior the trial began, enabled us to identify the dogs' defaecation patterns. No defaecation events were recorded during the night and no instances of coprophagia were observed throughout the trial. For additional assurance, the owner installed security cameras in both the outdoor spaces and the cages, which allowed us to constantly monitor the dogs' behaviour, both before the start of the trial and during the trial. Consequently, during the test period, the presence of a trained technician, consistently the same individual, was scheduled from 8:00 am to 10:00 pm to collect faeces and to evaluate FCS according to the table proposed by Waltham Faecal Score (Moxham 2001) (1: dry stool; 5: liquid stool), also assigning a score of a quarter of a point (0.25).

During each phase (R1 and R2), on days 0, 3, 7, 15 and 45, a trained technician assessed the FCS and collected faecal samples to assess faecal fermentation products. Three faecal samples per group (CTR and TRT) were collected within 15 min of defaecation and promptly transported in a polystyrene cooler to the laboratories located nearby the breeding facility to determine the concentration of fermentation products. Upon arrival, faeces were pooled according to dietary treatment (TRT), homogenised, and, in a subsample of 10% of each fresh faeces, the DM was determined as described for the diets (method no. 934.01; AOAC 2010). Thereafter, faecal samples were divided into two aliquots.

One aliquot of faeces was diluted to 1:5 (wt./vol) in metaphosphoric acid (25%) for the extraction of SCFAs and branched-chain fatty acids (BCFAs) (Chiofalo et al. 2019). The diluted samples centrifuged at 20,000 *g* for 20 min at 4 °C. The supernatant was collected and kept at −20 °C pending analysis.

The second aliquot, collected in test tubes and kept at −20 °C until analysis, was used for determination of biogenic amine concentrations.

Analyses of faecal fermentation products

SCFA and BCFA were studied by using a gas chromatograph Dani Master GC1000 (Dani Instrument, Milan, Italy) equipped with a FID (Dani Instrument, Milan, Italy) (Chiofalo et al. 2019). A fused-silica capillary column SUPELCO SPB (Supelco, Bellefonte, PA) was used. Helium was used as the carrier gas at a constant linear speed of 30 cm/s. The initial oven temperature was 40 °C maintained for 3 min, raised to 160 °C at 20 °C/min, then increased to 245 °C at 40 °C/min and finally held at 245 °C for 2 min. The temperatures of the FID and the injection port were 280 °C. Data handling was carried out with Clarity Chromatography Software version 4.0.2 (Dani Instrument, Milan, Italy). Identification and quantification of acetate, propionate, butyrate and isobutyrate

were carried out by comparison with the retention times of reference compounds (Merck Spa, Sigma-Aldrich, Milan, Italy) in the respective external analytical curves. In the standard solutions, the LOQ (signal to noise ratio >10) was 1 mmol/g for all analytes. The total run time was 12 min. All samples were analysed in triplicate and the results of acetate, propionate, butyrate, isobutyrate and total short chain fatty acids (SCFAs) and the sum of organic acids (SOAs) were expressed as micromol/g.

Biogenic amines (tryptamine, phenethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) were extracted according to Flickinger et al. (2003). Briefly, 2 g of faeces were mixed with 15 mL of perchloric acid (0.4 mol/L) and centrifuged at 2000 *g* for 10 min at 4 °C. After recovering the supernatant, 7 mL of perchloric acid (0.4 mol/L) were added to the remaining faecal pellet, mixed thoroughly, and centrifuged at 2000 g for 10 min at 4 °C. The supernatants were combined and centrifuged at 12,500 *g* for 5 min at 4 °C and analysed by using a HPLC (Shimadzu Italia, Milan, Italy) coupled to a fluorescence an RF-20A detector (FLD, Shimadzu Italia, Milan, Italy), following the same methods described for the diets. All samples were analysed in triplicate and the results expressed as mg/g.

Statistical analysis

Significant differences in the organic acids, biogenic amines and FCS in faeces between the TRT and CTR groups were assessed using a generalised linear mixed model (GLMM). Each focal analytical parameter served as response variable, with diet (PM or HFM), time (day 0, 3, 7, 15, 45) and their interaction as fixed effects, and the experimental phase as random variable. Ordinal response variables (FCS) were modelled using the 'clmm' function within the 'ordinal' version 2023.12-4.1 R package (Christensen 2023). For continuous, zero-bounded variables, the 'glmer' function of the 'lme4' version 1.1–37 R package (Bates et al. 2014) was used with a Gamma family with inverse link function. Model selection was based on the Akaike information criterion (AIC), and the model assumptions were verified through graphical assessment of residuals. The 'emmeans' version 1.11.1 R package (Bates et al. 2014) was used to compute the estimated marginal means and evaluate the contrast among TRTs. Statistical significance for the GLMM tests was assumed at 0.05. Differences between amino acid and biogenic amines of the experimental diets were assessed using a two sample *t*-test assuming a more stringent significance at 0.01 due to the small number of replicates available.

Results

All the dogs remained healthy throughout the study, with no occurrence of vomiting and diarrhoea. Moreover, dogs were provided with equal amounts of the experimental diets (PM and HFM), which were formulated to be isoenergetic, isonitrogenous and isolipidic (Table 1); mean values of weight variations between the beginning and end of each stage (R1 and R2) are shown in Table 4. The entire daily ration was consistently consumed, with no leftovers observed throughout the trial; as a result, nutrient intake remained stable over the entire experimental period (Table 4).

Dry matter content in faeces and FCS

Table 5 represents the DM and the faecal score of dogs fed experimental diets during the trial, along with the probability values for the effects of diet, time and their interaction (diet × time). Diet had a significant effect ($p < 0.001$) on faecal DM. The effect of time on faecal DM was significant ($p = 0.04$) only within the treated group. Concerning faecal score, no significant differences were observed in relation to the diet and time. Additionally, there was no significant interaction ($p = 0.088$) between diet and time for either faecal DM content or faecal scores.

Table 4. Mean values of weight, food and nutrient intake during the two stages of the total collection period (±SD).

		Collection period	
	Group*	Stage 1** R1	Stage 2# R2
Body weight, kg			
	CTR	16.76 ± 1.40	16.74 ± 1.46
	TRT	16.91 ± 1.42	16.83 ± 1.54
Daily intake, g/d			
Food	CTR	220	220
	TRT	220	220
CP	CTR	41.96	41.96
	TRT	42.24	42.24
Fat	CTR	33.53	33.53
	TRT	33.00	33.00
Starch	CTR	89.30	89.30
	TRT	89.76	89.76
OM	CTR	187.86	187.86
	TRT	191.20	191.20
DM	CTR	199.85	199.85
	TRT	202.23	202.23

SD: standard deviation; CP: crude protein; OM: organic matter; DM: dry matter
*Group: CTR group, dogs fed with PM diet (diet with poultry meal); TRT group, dogs fed with HFM diet (diet with hydrolysed feather meal).
**Stage 1: $n = 3$ dogs per group (CTR and TRT).
#Stage 2: $n = 3$ dogs per group (CTR and TRT).

Faecal organic acid content

Table 6 reports the concentrations of acetate, propionate, butyrate, isobutyrate, total SCFAs, and the SOAs in faecal samples, together with the probability values for the effects of diet, time, and their interaction (diet × time). Throughout the trial, diet exerted a significant effect ($p < 0.001$) on faecal organic acid concentrations. Specifically, the faeces of animals in the CTR group showed higher levels of propionate and butyrate compared with those of the TRT group. In contrast, acetate, isobutyrate, total SCFA and SOA concentrations were significantly higher ($p < 0.001$) in the faeces of the TRT group than in those of the CTR group.

The effect of time on organic acid concentrations was statistically significant ($p < 0.01$) for all organic acid, except for acetate, which remained stable ($p = 0.574$) throughout the trial in the faeces of both CTR and TRT groups.

A significant interaction ($p < 0.05$) between diet and time was observed for propionate, butyrate, isobutyrate and total SCFA content (Figure 2), whereas no significant interaction was found for acetate ($p = 0.763$) and SOA ($p = 0.142$).

Table 5. Faecal score and dry matter (g/100g) of dogs fed diets with or without hydrolysed feather meal during the trial*.

		Time points#					p Value$		
Item	Groups**	T0	T3	T7	T15	T45	D	T	DxT
DM (g/100g)									
	CTR	29.3	29.4	29.9	29.4	29.6	<0.001	0.004	0.088
	TRT	29.8b	31.2a	30.9a	30.9a	31.2a			
FCS									
	CTR	2.50	2.42	2.75	2.50	2.70	0.895	0.297	0.416
	TRT	2.50	2.13	2.50	2.38	2.67			

DM: dry matter
*Values are given as least square means (LSM).
**Groups: CTR group, $n = 6$ dogs fed with PM diet (diet with poultry meal); TRT group, $n = 6$ dogs fed with HFM diet (diet with hydrolysed feather meal).
#Time points: Collection days of faeces (T0-T45).
$D and T refer to diet and time, respectively. Probability values for the effects of diet, time and diet × time.
[a,b]Within row, means with different superscript letter within each parameter were significantly different ($p < 0.05$) due to the time.

Faecal biogenic amines

Table 7 presents the concentrations of the most abundant biogenic amines identified in faecal samples, together with the probability values for the effects of diet, sampling time and their interaction (diet × time). Data for histamine, tyramine and spermidine were not included, as their concentrations were below the LOQ (0.001 mg/g). Similarly, tryptamine and phenethylamine were excluded from the table because their concentrations were below 0.05 mg/g of dry faeces. The discussion below focuses on the most abundant biogenic amines, putrescine, cadaverine and spermine, as well as on the sum of biogenic amines (SBA). Throughout the trial, diet had a significant effect ($p < 0.001$) on all individual biogenic amines and on

Table 6. Faecal concentration of organic acid content (μmol/g dry faeces) of dogs fed diets with or without hydrolysed feather meal during the trial*.

		Time points#					p Value$		
Item, μmol/g dry faeces	Group**	T0	T3	T7	T15	T45	D	T	D x T
Acetate									
	CTR	469	475	474	469	465	<0.001	0.574	0.763
	TRT	562	562	562	561	556			
Propionate									
	CTR	130.5a	136.2a	118.6b	108.4b	117.3a	<0.001	<0.001	0.049
	TRT	107.1b	115.2a	109.4ab	96.6b	90.3c			
Butyrate									
	CTR	75.8c	94.3b	106.0b	105.9b	112.1a	<0.001	<0.001	<0.001
	TRT	47.0d	67.2c	79.8b	103.4a	84.4b			
Isobutyrate									
	CTR	28.3d	33.5c	35.1bc	39.4ab	40.2a	<0.001	<0.001	0.014
	TRT	39.0b	43.3a	42.6a	44.9a	44.6a			
Total SCFA									
	CTR	675b	706a	699a	683ab	694ab	<0.001	0.001	0.013
	TRT	716b	744ab	752a	761a	730ab			
SOA									
	CTR	703b	739a	734a	723ab	734a	<0.001	<0.001	0.142
	TRT	755b	787a	794a	806a	775ab			

Total SCFA: sum of the short chain fatty acids (C2:0 + C3:0 + C4:0); SOA: sum of organic acid (C2:0 + C3:0 + C4:0 + isoCa4:0).
*Values are given as least square means (LSM).
**Groups: CTR group, $n = 6$ dogs fed with PM diet (diet with poultry meal); TRT group, $n = 6$ dogs fed wit HFM diet (diet with hydrolysed feather meal).
#Time points: Collection days of faeces (T0-T45).
$D and T refer to diet and time, respectively. Probability values for the effects of diet, time and diet × time.
[a–d] Within row, means with different superscript letter within each metabolite were significantly different ($p < 0.05$) due to the time.

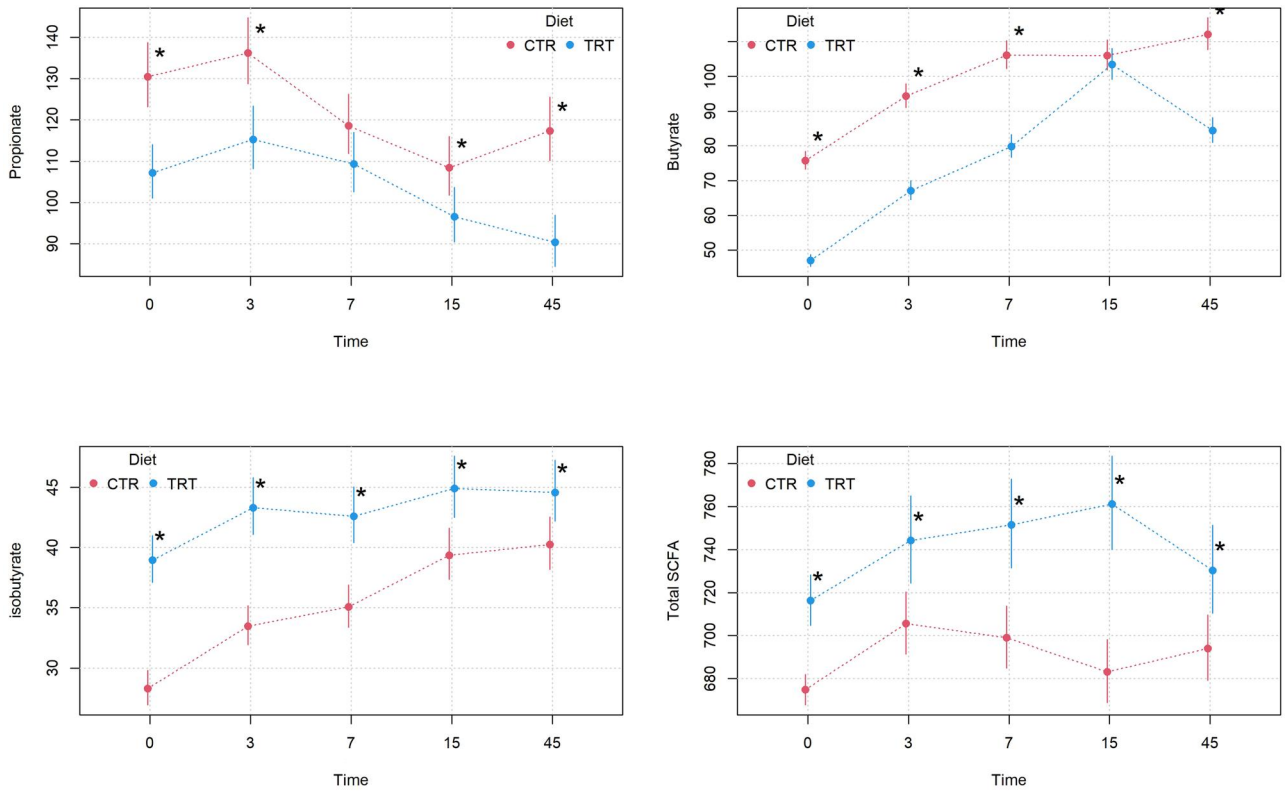

Figure 2. Temporal profiles of faecal organic acid concentrations in control (CTR) and treated (TRT) groups (expressed in μmol/g dry faeces), where a significant diet × time interaction was detected ($p < 0.05$). Solid points and vertical bars represent mean values ±95% confidence intervals. Dashed lines connect time points within each diet group to illustrate temporal trends. Asterisks indicate specific time points where CTR and TRT differ significantly ($p < 0.05$), illustrating where the interaction occurs.

Table 7. Faecal concentration of biogenic amine content (mg/g dry faeces) of dogs fed diets with or without hydrolysed feather meal during the trial*.

		Time points#					p Value$		
Item, mg/g dry faeces	Groups**	T0	T3	T7	T15	T45	D	T	D x T
Putrescine									
	CTR	0.276a	0.274a	0.237b	0.254ab	0.261ab	<0.001	<0.001	<0.001
	TRT	0.378a	0.395a	0.379a	0.417a	0.306b			
Cadaverine									
	CTR	0.133d	0.160c	0.186b	0.198b	0.230a	<0.001	0.034	<0.001
	TRT	0.316a	0.308a	0.309a	0.297a	0.253b			
Spermine									
	CTR	0.394b	0.519a	0.460ab	0.372b	0.127c	<0.001	<0.001	<0.001
	TRT	0.203d	0.277c	0.504a	0.386b	0.092e			
Sum of biogenic amines									
	CTR	0.823b	0.999a	0.935ab	0.862b	0.627c	<0.001	<0.001	<0.001
	TRT	0.916c	1.036b	1.237a	1.212a	0.662d			

*Values are given as least square means (LSM).
**Groups: CTR group, $n = 6$ dogs fed with PM diet (diet with poultry meal); TRT group, $n = 6$ dogs fed wit HFM diet (diet with hydrolysed feather meal).
#Time points: Collection days of faeces (T0-T45).
$D and T refer to diet and time, respectively. Probability values for the effects of diet, time and diet × time.
a–e Within row, means with different superscript letter within each metabolite were significantly different ($p < 0.05$) due to the time.

the SBA. Putrescine and cadaverine, along with SBA, showing a higher content in the faeces of the TRT group compared to the CTR group. In contrast, spermine levels were significantly higher ($p < 0.001$) in the faeces of the CTR group compared to the TRT. At the end of the trial (T45), SBA did not show significant differences ($p > 0.05$) between the two groups.

The effect of time on biogenic amine and SBA concentrations was statistically significant ($p < 0.05$) across the trial period in the faeces of both CTR and TRT groups.

With regard to the most abundant biogenic amines, a significant interaction ($p < 0.001$) between diet and time was observed for putrescine, cadaverine, spermine and the total SBA content (Figure 3).

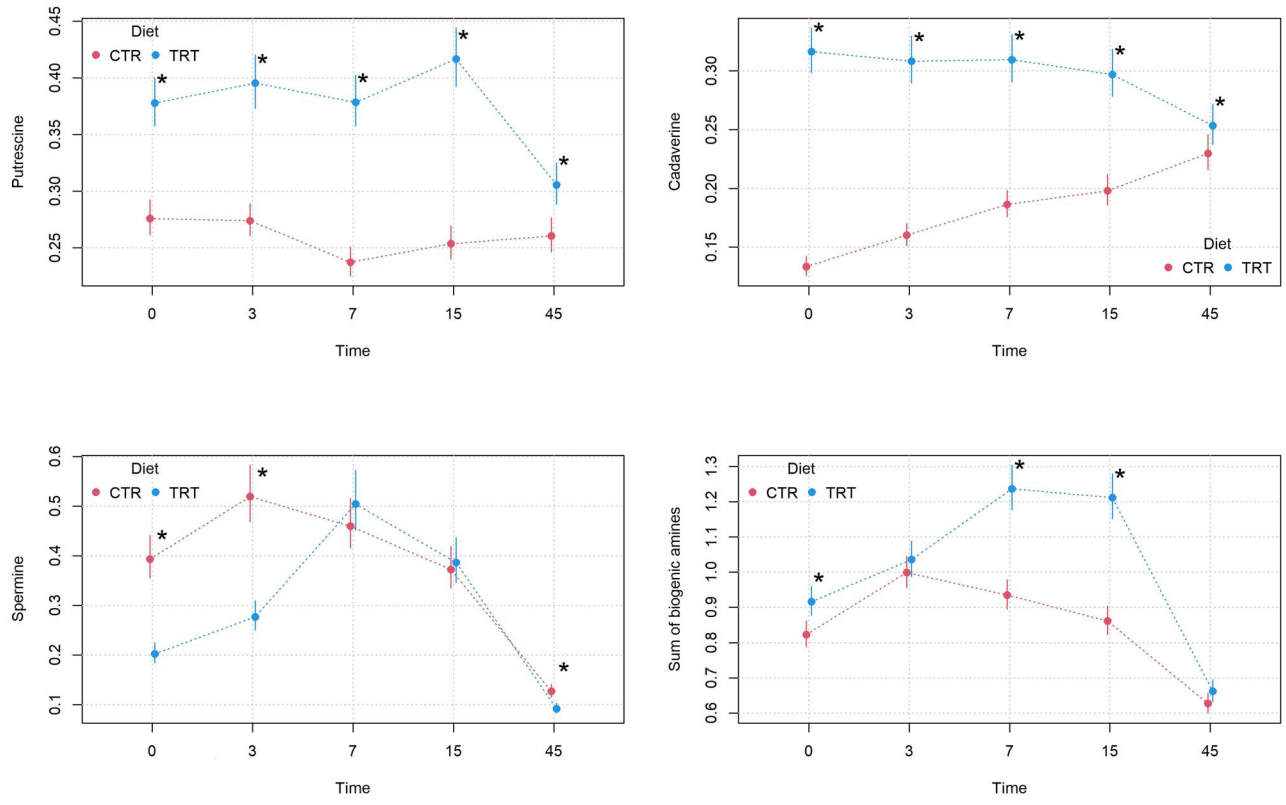

Figure 3. Temporal profiles of the most represented faecal biogenic amine concentrations in control (CTR) and treated (TRT) groups (expressed in mg/g dry faeces), where a significant diet × time interaction was detected ($p < 0.05$). Solid points and vertical bars represent mean values ±95% confidence intervals. Dashed lines connect time points within each diet group to illustrate temporal trends. Asterisks indicate specific time points where CTR and TRT differ significantly ($p < 0.05$), illustrating where the interaction occurs.

Discussion

Although the number of animals used in the study was relatively limited (six per TRT group), which may reduce the overall statistical power, the results nonetheless provide valuable insights and allow for preliminary hypotheses regarding the adaptability of healthy dogs to the inclusion of HFM in their diet.

The feather-based diet was well accepted by all dogs, which readily consumed the entire daily portion (220 g/dog) throughout the trial. No refusals or reductions in feed intake were recorded in either the CTR or TRT groups. Moreover, the inclusion of feather meal in the diet did not induce altered gastrointestinal function (e.g. vomiting, diarrhoea) or other adverse effects (Freeman et al. 2011), as confirmed by the physical examinations and consistent feed intake throughout the trial.

Faecal scores remained within the ideal range of 2–3, as defined by Moxham (2001), for both groups. The inclusion of HFM in the diet did not significantly affect faecal consistency; however, it did lead to variations in faecal DM content, indicating a potential effect of diet composition on faecal moisture regulation.

Results are in accordance with those reported by Pacheco et al. (2016), who observed appropriate faecal scores in adult Beagles fed diets containing 7.5% HFM. El-Wahab et al. (2022) reported increased faecal scores in Beagle dogs fed basal diets supplemented with 5%, 10% and 20% HFM. In contrast to these findings, this study demonstrated that inclusion of HFM at 7% resulted in lower, therefore better, FCSs (2.27 *vs.* 2.67).

Several factors may influence faecal quality, including food intake, dietary composition, and ingredient quality. Additionally, the composition of the gastrointestinal microbiota (Wakshlag et al. 2011; Do et al. 2021) can play a significant role. Faecal quality is related to the metabolites produced by gut bacteria during fermentation which directly influence faecal characteristics, including the consistency of faeces. High fermentative activity results in increased bacterial biomass and significant production of fermentation-derived metabolites. The increase in concentration of fermentation end-products could shift the osmotic balance in the colon, ultimately favouring water and sodium transport towards the lumen (El-Wahab et al. 2022). The great osmotic power of these compounds

could increase intraluminal osmotic pressure resulting in secretion and/or retention of water in the colon (Macfarlane and Cummings 1991).

In this study, considering that both diets contained the same ingredients except for the source of animal-derived proteins (HFM *vs*. PM), our focus was on protein fermentation and the resulting production of its end by-products. Similar to fibre fermentation, protein fermentation produces SCFAs (Diether and Willing 2019); however, these are accompanied by branch-chained fatty acids, ammonia, amines, hydrogen sulphide, phenols and indoles (Macfarlane et al. 1992). BCFAs are reliable markers of proteolytic fermentation as they are produced exclusively through the fermentation of branched-chain amino acids such as valine, leucine and isoleucine (Diether and Willing 2019). Many of these products are also being identified as bioactive molecules. Isobutyrate is the BCFA with the greatest importance, due to its similarities with butyrate (Charney et al. 1999). Higueras et al. (2024) report anti-inflammatory properties of the isobutyrate through inhibition of nuclear factor kappaB, a group of proteins that regulate gene expression and cell functions.

In our trial, the faecal concentrations of organic acids, including total SCFAs, BCFAs and the overall SOAs, were significantly higher in dogs fed HFM compared to those fed PM. This likely indicates increased fermentative activity, potentially associated with differences in microbial diversity and the presence of specific bacterial taxa within the canine gut microbiota. Whether this can be associated to a higher amount of protein escaping small intestine digestion for the HFM diet in comparison to the PM diet is not easy to assess. However, feathers were treated with pressure and steam, a process that is known to modify several amino acids, with a production of new disulphide linkages, amine bonds, that reduce the enzymatic hydrolysis in the gastrointestinal tract (Qi et al. 2018). These factors can affect the extent of undigested protein and the type of peptide into the bowel and, in turn, influence the microbiota composition (Wu et al. 2022). A previous study (Balouei et al. 2024), aiming to investigate changes in the canine gut microbiota in response to the effects of dietary intervention with and without HFM, highlighted nine significant taxa and six of them were significantly different at the Kruskal–Wallis test between diets (PM and HFM). Specifically, a significant higher Relative Abundance (RA) was reported for Streptococcus, *Collinsella stercoris*, *Ruminococcus gnavus* and *Bacteroides coprophilus* in the dogs fed the basal diet without HFM. Higher RA for Peptostreptococcaceae and *Bacteroides uniformis* was observed in the dogs fed the diet with HFM. These observations could explain the recorded values for butyrate and isobutyrate in this study. A lower mean value of butyrate in the faeces of the TRT group could be due to the lower RA for the *Ruminococcus gnavus* in the faeces of the TRT group than that of the CTR group, considering that this species is a butyrate-producing bacteria (Louis et al. 2004). A higher mean values of isobutyrate in the faeces of the TRT group to be due to the greater RA for the Peptostreptococcaceae in the faeces of TRT group, considering that this taxon is associated with isovalerate and isobutyrate in the faeces of cats (Bermingham et al. 2018). Furthermore, the results obtained in this study agree with those of El-Wahab et al. (2022) who observed an increased levels of isobutyrate in the faeces of dogs fed a diet containing HFM.

According to the literature (Minamoto et al. 2019; Kilburn et al. 2020; Pan et al. 2025), healthy dogs exhibit proportions of individual SCFAs, acetate, propionate and butyrate, relative to total SCFA concentrations that are consistent with the findings of this study: acetate 67–78%, propionate 12–20% and butyrate 7–16%. These values differ significantly from those reported in dogs with inflammatory enteropathies (Higueras et al. 2024), further supporting the gastrointestinal health of the dogs enrolled in this study.

The dietary-induced changing of the microbiota composition could also explain the influence of diet on polyamine levels (Holmes et al. 2017). The rise in proteolytic bacteria can contribute to increased polyamine levels in the colon by increasing substrate availability for polyamine production (Bekebrede et al. 2020). Biogenic amines (putrescine, spermine and spermidine) are non-volatile low molecular weight nitrogenous organic bases, originating from the decarboxylation of amino acids by colon bacteria. In humans and animals, biogenic amines are present in all living cells and are necessary for metabolic activity and the growth process of tissues and organs in the body (Bardócz et al. 1993); however, a high concentration of amino acid fermentation metabolites, including biogenic amines, may exert negative effects on the colonic epithelium and intestinal function (Blachier et al. 2007).

Animal by-products, especially poultry and meat by-products, commonly used in pet diets due to their high nutritional value and acceptability (Pinto et al. 2023), could be considered a potential source of biogenic amines (Feddern et al. 2019). Among poultry by-products, the increased use of hydrolysed proteins, driven by their high content of bioactive peptides with functional properties such as antimicrobial,

antioxidant, antihypertensive and immunomodulatory activities (Cave 2006; Hou et al. 2017), may lead to higher consumption of biogenic amines in dogs due to the high availability of free amino acids generated from the hydrolysis process (Feddern et al. 2019, Ruiz-Capillas and Herrero 2019).

Pinto et al. (2023) hypothesised that diets containing hydrolysed PM may lead to increased dietary intake and subsequent faecal excretion of biogenic amines. In this study, diet exerted a significant effect on all measured biogenic amines. Cadaverine, putrescine and spermine were the predominant amines detected in faecal samples. Cadaverine and putrescine and tended to be elevated in dogs fed the HFM diet, whereas spermine tended to be elevated in dogs fed the PM diet. Moreover, dogs receiving the HFM diet exhibited higher faecal concentrations of tryptamine and phenethylamine. These findings suggest that the increased levels of biogenic amines observed in the faeces of the TRT group may be attributed to the higher dietary intake associated with the HFM-based diet. Nonetheless, recent studies on the *in vitro* toxicity of biogenic amines to intestinal cells have shown that the cytotoxic levels in food are much higher than those observed in the diet containing 7% HFM (Montegiove et al. 2023). Bastos et al. (2020) reported cadaverine concentrations ranging from 0.297 to 0.433 mg/g dry faeces in healthy dogs, while Grandi et al. (2018) and de Melo Santos et al. (2021) observed putrescine concentrations ranging from 0.307 to 0.446 mg/g dry faeces in the same species.

Sánchez-Pérez et al. (2022) demonstrated that cadaverine and putrescine can reduce histamine metabolism in the epithelium as both are also converted by diamine oxidase. Deloyer et al. (2000) reported that putrescine and spermine are considered beneficial, if present in small quantities, as they are associated with normal cell turnover and involved in apoptosis. Löser et al. (1999) observed that rats fed a polyamine-deficient diet for an extended period showed colonic mucosal hypoplasia, revealed that luminal polyamines in the diets are important factors for colonic mucosal renewal. Overall, the levels of biogenic amines observed in this study are consistent with those previously reported in healthy dogs (Grandi et al. 2018; Bastos et al. 2020; de Melo Santos et al. 2021) and are considerably lower than the concentrations considered harmful to pet health (Montegiove et al. 2023).

Conclusions

This preliminary study aimed to evaluate the effects of including HFM in the diet of healthy adult dogs, compared with a CTR PM-based diet, focusing on faecal quality parameters and fermentation by-products. Despite the relatively small number of animals per group, the findings provide useful insights into the adaptability of dogs to this alternative protein source. The diet containing 7% HFM was well accepted, with full consumption of the offered food. Faecal scores remained within the ideal range (2.0–2.5) in both groups, and the profiles of organic acids and biogenic amines indicated the maintenance of gastrointestinal health.

Overall, these results support the hypothesis that moderate inclusion of HFM does not adversely affect faecal consistency or microbial metabolite production. As a poultry by-product, HFM represents a promising ingredient for the sustainable valorisation of animal by-products in the pet food industry. Further research is warranted to confirm these findings under different dietary conditions and at varying inclusion levels.

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Ethical statement

The research was approved by the Ethical Animal Care and Use Committee of the Department of Veterinary Sciences, of the University of Messina, code 01/2023, and the experiment were in compliance with the European guidelines for the care and use of animals in research (European Union 2023) and those on the placing on the market and use of feed (European Union 2009).

Disclosure statement

Authors declare no competing financial interests or personal relationship that could potentially affect outcomes reported in this manuscript.

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Data availability statement

Data are available from the corresponding author upon reasonable request.

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