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Characterisation of spray dried hydrolysed chicken liver powder: effects on palatability and digestibility when included as single source of animal protein in dog diets

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ABSTRACT

This study aimed to evaluate a commercial hydrolysed chicken liver powder (HCLP) as a single source of animal protein in diets for adult dogs. A characterisation of the HCLP was followed by assessment of diets palatability and *in vivo* and *in vitro* digestibility. Two extruded isonitrogenous diets were produced: control (poultry byproduct + bovine meat and bone meal) and HCLP. Twenty-two dogs of different breeds were used to test palatability, and twelve Beagle dogs were used to evaluate digestibility. HCLP had high concentrations of lysine, linoleic and arachidonic acids, and most of peptides with molecular weight <10 kDa. HCLP diet had the highest inclusion of the experimental ingredient based on its chemical composition. Dogs did not show preference among diets ($p > .05$). Ash and fat intake were higher in dogs fed the control diet, ($p < .0001$) and ($p = .0135$), respectively. Crude fibre intake was higher in dogs fed the HCLP diet ($p = .0001$). Dogs fed the HCLP diet had similar faecal score ($p > .05$) compared to control diet, although faecal dry matter was reduced ($p = .0321$) and the daily faecal production was increased ($p = .0361$). The diets in vitro digestibility did not differ ($p > .05$). Based on our results, HCLP included up to 26% in diets for adult dogs presented satisfactory results in palatability, digestibility of nutrients and energy, faecal and urinary characteristics. Although dogs fed the HCLP diet produced slightly moist stools, it had no negative impact on faecal score.

HIGHLIGHTS

- A commercial hydrolysed chicken liver powder (HCLP) was evaluated and presented low molecular weight and high amounts of essential nutrients. HCLP, included as a single source of animal protein, had good digestibility and acceptance for adult dogs.
- Despite findings from previous studies, the inclusion of HCLP at the level of 25.8% did not promote diarrhoea and the final faecal score remained within the ideal range.

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Introduction

Adverse food reactions are classified as an abnormal IgE-mediated immune response due to the ingestion of a specific food by sensitive individuals (Cianferoni and Spergel 2009). Recently, hydrolysed protein diets have been highly recommended for the diagnosis and management of adverse food reactions in dogs and cats as an option to homemade diets. Homemade diets were the 'gold standard' in elimination trials since they consist of one protein and one carbohydrate source and both were never consumed previously (Bethlehem et al. 2012). However, it demands full adherence, compliance, and investment of time of owners in order to follow and prepare the prescribed diet with only the selected ingredients. Additionally, if these diets are not nutritionally balanced or complete, they can lead to deficiencies in a long-term feeding period.

Hydrolysis allows the utilisation of ingredients associated with adverse food reactions in dogs, such as beef, chicken, pork, fish, and corn in hypoallergenic diets (Roudebush 2013). In human beings, the main food allergens are water-soluble glycoproteins, with a molecular weight ranging from 10 to 70 kDa, and relatively stable to heat, acid, and protease treatment (Sampson 1999). Hydrolysis breaks large polypeptides chains into smaller peptides and amino acids,

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Table 1. Hydrolysed chicken liver powder ingredient chemical composition.

Nutrient profile [g/kg, as fed basis]	
Water	61.0
Protein	619
Fat	224
Ash	59.0
Amino acids [g/kg]	
Alanine	38.4
Arginine	37.5
Aspartic acid	56.0
Cystine	4.6
Glutamic acid	78.6
Glycine	31.7
Histidine	14.0
Isoleucine	29.2
Leucine	54.6
Lysine	58.1
Methionine	14.8
Phenylalanine	28.7
Proline	26.8
Serine	27.4
Threonine	28.6
Tryptophan	8.9
Tyrosine	23.1
Valine	37.2
Fatty acids [g/kg]	
C08:0 Octanoic (caprylic)	<0.20
C10:0 Decanoic (capric)	<0.20
C11:0 Undecanoic (hendecanoic)	<0.20
C12:0 Dodecanoic (lauric)	<0.20
C14:0 Tetradecenoic (myristic)	0.60
C14:1 Tetradecenoic (myristoleic)	<0.20
C15:0 Pentadecanoic	<0.20
C15:1 Pentadecenoic	<0.20
C16:0 Hexadecanoic (Palmitic)	41.60
C16:1 Hexadecenoic (Palmitoleic)	5.90
C16:2 Hexadecadienoic	<0.20
C16:3 Hexadecatrienoic	<0.20
C16:4 Hexadecatetraenoic	<0.20
C17:0 Heptadecanoic (margaric)	0.20
C17:1 Heptadecenoic (margaroleic)	<0.20
C18:0 Octadecanoic (stearic)	26.60
C18:1 Octadecenoic (oleic + isomers)	55.50
C18:2 Octadecadienoic (linoleic + isomers)	38.40
C18:2 Octadecadienoic omega 6 (linoleic)	37.70
C18:3 Octadecatrienoic (linolenic + isomers)	2.20
C18:3 Octadecatrienoic Omega 3 (alpha linolenic)	1.70
C18:3 Octadecatrienoic Omega 6 (gamma linolenic)	0.50
C18:4 Octadecatetraenoic Omega 3 (stearidonic)	<0.20
C20:0 Eicosanoic (arachidic)	0.30
C20:1 Eicosanoic (Gondoic + isomers)	0.80
C20:2 Eicosadienoic Omega 6	0.50
C20:3 Eicosatrienoic	1.60
C20:3 Eicosatrienoic Omega 3	<0.20
C20:3 Eicosatrienoic Omega 6	1.60
C20:4 Eicosatetraenoic (arachidonic + isomers)	11.60
C20:4 Eicosatetraenoic Omega 3	<0.20
C20:4 Eicosatetraenoic Omega 6 (arachidonic)	11.50
C20:5 Eicosapentanoic Omega 3	0.30
C21:5 Heneicosapentaenoic Omega 3	<0.20
C22:0 Docosanoic (behenic)	0.40
C22:1 Docosenoic (erucic + isomers)	<0.20
C22:2 Docosadienoic Omega 6	<0.20
C22:3 Docosatrienoic Omega 3	<0.20
C22:4 Docosatetraenoic Omega 6	1.40
C22:5 Docosapentaenoic	1.60
C22:5 Docosapentaenoic Omega 3	0.60
C22:5 Docosapentaenoic Omega 6	1.00
C22:6 Docosahexaenoic Omega 3	1.20
C24:0 Tetracosanoic (lignoceric)	0.20
C24:1 Tetracosenoic (nervonic)	0.20
Minerals [mg/kg]	
Calcium	240
Phosphorus	8830
Magnesium	620
Sodium	2710
Potassium	8360
Chloride	6100
Zinc	68.00
Copper	6.80
Iron	329
Manganese	7.30
Selenium	1.84

Amino acids and fatty acids values reported as a % of the total product.

reducing molecular weight and therefore decreasing the antigenicity of the original protein, preserving its nutritional properties. Properly hydrolysed proteins contain peptides with reduced molecular weight that do not allow the IgE cross-linking, therefore, preventing degranulation and release of mediators (Corlde 1994 ; Verlinden et al. 2006).

In this way, testing novel hydrolysed protein sources have become an important way to validate their availability for inclusion in commercial diets specific for dogs with adverse food reaction and gastrointestinal disorders, as well as in premium commercial diets for healthy dogs. Thereby, the present study aimed to describe the nutritional composition of a commercial hydrolysed chicken liver powder ingredient, followed by the evaluation of palatability, and digestibility in vivo and in vitro when hydrolysed protein replaces the most traditional sources of animal protein in diets for dogs: poultry byproduct + bovine meat and bone meals.

Materials and methods

All animal care and handling procedures were approved by The Institutional Animal Care and Use Committee at the Universidade Federal do Rio Grande do Sul (UFRGS), protocol number 36,138.

Nutritional characterization of the hydrolyzed chicken liver powder

Chemical analysis

Hydrolysed chicken liver powder (HCLP) was obtained from a commercial industry (PROSURANCE® CHX Liver HD, Kemin® Industries). The product is reviewers a spray-dried protein hydrolysate produced by enzymatic hydrolysis under controlled temperature and pressure conditions. The final product is a fine caramel brown powder with low molecular weight protein and low ash content (Table 1). Using Size Exclusion

Table 2. Ingredients and chemical composition of experimental diets.

Items	Treatments	
	Control	HCLP*
Ingredient [g/kg, as fed basis]		
Brewers rice	527	527
Full-fat rice bran	80.00	80.00
Poultry byproducts meal	186	–
Bovine meat and bone meal	50.00	–
HCLP†	–	258
Cellulose	33.40	42.20
Poultry fat#	87.50	43.00
Soybean oil#	–	18.00
Canola oil#	13.70	4.60
L-lysine	6.30	–
DL-methionine	3.30	2.70
Potassium chloride	2.30	0.70
L-tryptophan	0.30	–
Limestone	–	20.10
Premix mineral/vitamin$	5.00	5.00
Salt	5.00	5.00
Analysed composition [g/kg, DM-basis]		
Dry matter	941	945
Organic matter	943	954
Ash	56.70	46.20
Crude protein	248	248
Acid-hydrolysed fat	151	140
Crude fibre	37.50	46.70
Gross energy [kJ/g]	20.70	21.20
Peroxide index [meq/kg] ‡	2.23	3.83

*HCLP: hydrolysed chicken liver powder diet; †HCLP: hydrolysed chicken liver powder ingredient; #Added on top; $Premix mineral/vitamin (supplied per kilogram of diet): vitamin A (10,800U), vitamin D3 (980 U), vitamin E (60 mg), vitamin K3 (4.8 mg), vitamin B1 (8.1 mg), vitamin B2 (6.0 mg), vitamin B6 (6.0 mg), 12 vitamin (30 mcg), pantothenic acid (12 mg), niacin (60 mg), folic acid (0.8 mg), biotin (0.084 mg), manganese (7.5 mg), zinc (100 mg), iron (35 mg), copper (7.0 mg), cobalt (10 mg), iodine (1.5 mg), selenium (0.36 mg), choline (2.400 mg), taurine (100 mg), and, antioxidant BHT (150 mg); ‡milliequivalents of active oxygen/kg of sample.

Chromatography (Kemin Nutrisurance Proprietary Method), the HCLP was analysed for protein molecular weight. Also, the HCLP was analysed for dry matter (DM—AOAC 934.01), crude protein (CP—AOAC 954.01; model TE 036/2, Tecnal, Piracicaba, Brazil), acid-hydrolysed fat (AHF—AOAC 954.02; model 170/3, Fanem, São Paulo, Brazil), ash (AOAC 1995), amino acids (AOAC 994.12 [Alt. III]; AOAC 994.12 [Alt. I]; AOAC 988.15 [mod]), fatty acids (AOAC 985.01 [mod]), and minerals (AOAC 985.01 [mod]). All analyses were performed in duplicate, assuming a coefficient of variation of <5% for all analyses. The amino acid score (AAS) was calculated based on the equation described by Kerr et al. (2013), using minimal requirements for the growth of puppies and kittens provided by NRC–National Research Council (2006) as reference values:

$$AAS = \frac{mg\ of\ limiting\ AA\ in\ 1g\ of\ test\ protein}{mg\ of\ limiting\ AA\ in\ 1g\ of\ reference\ protein} \times 100$$

For the *AAS* calculation, the amino acid content of poultry byproduct and bovine meat and bone meals were obtained from Rostagno et al. (2017).

Diets

Two experimental diets were formulated and extruded (model 2000, TNL Tecnal, Ourinhos, Brazil) to be isonitrogenous, differing only in the animal protein added: control (poultry byproduct + bovine meat and bone meals) and HCLP (hydrolysed chicken liver powder) (Table 2). For the palatability assay, diets were coated with a mix of soybean and canola oil at 2% instead of poultry fat. For the digestibility assay, diets were coated with poultry fat only. No commercial palatability enhancer was added, as these contain amino acids and peptides that alter the molecular weight and mono-protein concept of the HCLP diet.

Palatability assay

Animals

Twenty-two healthy adult dogs of different breeds (2 Rottweilers, 4 Labradors, 4 Siberian Huskies, 2 Basenjis, 4 Beagles, 2 Shih Tzus, and 4 Spitzes) were used to evaluate the palatability of the experimental diets. The dogs were allocated into individual kennels, and two diets were offered at the same time. During the rest of the period, the dogs were maintained outdoors. Water was provided *ad libitum*.

Assessment of palatability

Palatability was determined using the '2-pan' choice method (Griffin 2003). One comparison was made to evaluate dietary preference: Control vs. HCLP in a completely randomised design, with two comparisons and four meals for a total of 88 observations. During the testing phase, at 8:00 h, each diet used in the comparison was offered side by side, simultaneously, in identical feeders for 30 min. After this period, the dogs were released outdoors. At 18:00 h, the dogs were placed back into their metabolic cages, where they stayed until the following morning and were fed the experimental diets. The leftovers were collected, weighed, and discarded. The feeders were alternated for every meal to eliminate any bias effects. Food intake and first choice were observed during the trial. Food intake was calculated based on the total consumption of each diet. The first choice, observed when food was first offered, is the number of times that a given diet was chosen first.

Statistical analyses

The results of the palatability assessment were tested for homogeneity of variances and normality of errors and then subjected to Student's *t*-test ($p < .05$) to determine whether the food intake differed for dietary comparison. The first choice was analysed using a

Chi-square test ($p < .05$) based on the frequency of each meal choice using Statistix 10 (Analytical Software, Tallahassee, FL, USA). Based on the number of dogs used in this test, values of first choice and feed intake of >.80 were considered to be significant at a p value of <.05, according to the method described by Griffin (2003).

In vivo digestibility, fecal, and urinary characterization

Animals

Twelve healthy, intact adult Beagles (six males and six females) from the Animal Science Department, UFRGS, Porto Alegre, Brazil, were used in this study. All individuals were 5 years of age, weighing 11.8 ± 1.45 kg, with a body condition score (BCS) ranging from 5 to 6 out of 9 points (Laflamme 1997), and were free of endo- and ectoparasites. All dogs were regularly immunised and submitted to clinical and laboratory tests to measure complete blood count (CBC) and to perform biochemical and coproparasitological analyses before the start of the study. The dogs were housed and kept in conditions similar to those used in the palatability study. Dogs were fed experimental diets twice a day (at 08:30 h and 17:00 h) to meet their daily maintenance energy requirements (110 kcal of metabolisable energy × body weight $(kg)^{0.75}$/day), as recommended by the NRC– National Research Council (2006). Water was provided *ad libitum* throughout the experiment.

Experimental procedures

Digestibility was measured using the total faecal collection method. The assay was conducted as a randomised block design with two treatments and six dogs per treatment for a total of six replicates per treatment according to the American Association of Feed Control Officials protocol (AAFCO – Association of American Feed Control Officials 2020). Sex (female and male) was used as a criterion for blocking, and body weight was used to randomise the treatments. The experimental period lasted 10 days, with 5 days for adaptation to the cage and experimental diet, followed by 5 days of total faeces and urine collection and measurement of urinary pH.

Sample procedure

To establish the beginning and the end of each period of faeces and urine collection, gelatine capsules containing 1 g of iron oxide (III) Fe_2O_3 were orally administered to the dogs. Faeces were collected for five consecutive days, every 3 h except at night (0 AM/midnight), and scored as follows: 1 = very hard and dry stool, 2 = hard, dry, firm stool, 3 = soft, moist stool, well formed, 4 = soft and shapeless stool, and 5 = liquid stool and diarrhoea. Faecal score analysis was conducted by a single trained person using the WALTHAM Faeces Scoring System (Moxham 2001). After daily collection, faeces were weighed and stored in a freezer at −20 °C until the end of the trial for analysis. Total urine collection was performed daily in the morning and then stored in plastic bottles containing 0.1 g of thimol/100 mL of urine (EXÔDO CIENTÍFICA®, Hortolândia, Brazil), an aliquot of urine was used to measure the pH using a bench pH metre (AKSO® pH Plus, São Leopoldo, Brazil) and urinary density by a portable refractometer (BEL ENGINEERING® model RPI).

Chemical analysis

Stools from each dog were thawed, homogenised, and dried in a forced-air oven at 55 °C for 72 h, according to the recommendations of the AOAC (1995). Faeces and diets were ground through a 1 mm screen in a Wiley hammer mill (DeLeo Equipamentos Laboratoriais, Porto Alegre, Brazil), and analysed for DM (AOAC 934.01), AHF (AOAC 954.02; model 170/3, Fanem, São Paulo, Brazil), CP (AOAC 954.01; model TE 036/2, Tecnal, Piracicaba, Brazil), crude fibre (CF—AOAC 962.10; model MA 450/8, Marconi, Piracicaba, Brazil), and ash (AOAC 985.01 [mod]). The total urine produced was thawed, homogenised, and 150 mL aliquots were lyophilised (Micromodulyi-Fis; Thermo Fisher Scientific Inc., Lanham, MD, USA) for analysis of DM. Another 50 mL aliquot was collected for the analysis of CP. Dietary and faecal GE were determined using isoperibolic bomb calorimetry (calorimeter model C2000 basic, Ika-werke, Staufen, Germany). All analyses were performed in duplicate, assuming a coefficient of variation <0.01 for energy and <0.05 for the other analyses.

Statistical analyses

Data were tested for homogeneity of variances and normality of errors, and then analysed using ANOVA in SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Means were compared using Tukey's test ($p < .05$).

In vitro digestibility assay

Chemical analysis

The *in vitro* digestibility of dry matter (IVDDM) and organic matter (IVDOM) was determined based on the method proposed by Hervera et al. (2007) using 1 g of

Table 3. Nutrient intake, coefficient of total tract apparent digestibility, metabolisable energy, faecal and urinary characteristics of dogs fed experimental diets.

	Treatments			
Item	Control	HCLP*	*p*-Value	SEM†
Nutrient intake [g/d]				
Dry matter (DM)	171	167	.4749	9.29
Organic matter	161	159	.6978	8.78
Ash	9.71[a]	7.72[b]	<.0001	0.52
Crude protein	42.5	41.50	.4835	2.29
Acid-hydrolysed fat	25.8[a]	23.30[b]	.0135	1.39
Nitrogen-free extract	93.2	94.60	.6472	5.12
Crude fibre	6.42[b]	7.81[a]	.0001	0.38
Gross energy [kJ/d]	3546	3536	.9329	193
Apparent total tract digestibility [%]				
Dry matter	87.2	86.10	.3037	1.67
Organic matter	90.0	89.10	.2637	1.33
Crude protein	88.0	89.30	.2335	1.74
Acid-hydrolysed fat	92.4	90.80	.0928	1.47
Nitrogen-free extract	90.3	88.60	.0964	1.57
Gross energy	89.8	89.00	.3636	1.36
Digestible energy [kJ/g]	18.6	18.80	.2454	0.30
Metabolisable energy [kJ/g]	17.4	17.60	.2273	0.27
Faecal characteristics				
Faecal score#	1.98	2.17	.1071	0.18
Faecal DM [%]	51.7[a]	43.30[b]	.0321	5.76
Faecal output [g/d]	42.9[b]	53.70[a]	.0361	7.63
Faecal output [g/d, DM]	29.6	27.70	.5719	5.61
Urinary characteristics				
Volume [mL/d]	219	159	.4431	130
Dry matter [%]	6.87	7.85	.6113	3.22
Nitrogen [g/d]	3.48	2.93	.4296	1.15
Urine pH	7.85	8.33	.1981	0.60
Urine density	1032	1034	.7196	10.90

*HCLP: hydrolysed chicken liver powder diet; †SEM: standard error of the mean; #Scored as follows: 1 = very hard and dry stool, 2 = hard, dry, firm stool, 3 = soft, moist stool, well formed, 4 = soft and shapeless stool, 5 = liquid stool, diarrhoea.
[a,b]Means in the same row with different lowercase letters are significantly different ($p < .05$).

each experimental diet. The analysis was conducted in duplicate. This method simulates the stomach and small intestine compartments with the action of pepsin followed by pancreatin.

Results

The HCLP had high concentrations of protein, fat, and amino acids lysine, aspartic acid, and leucine, and low ash content (Table 1). Formulation adjustments were necessary in order to formulate the diet to contain an exclusive source of animal protein as HCLP or the most common sources based in beef and poultry byproducts meal (Table 2).

The experimental diets had similar chemical compositions, with small differences on ash and fibre content (Table 2), specially on HCLP diet which increased differences in nutrient intake for the diets (Table 3). The intake of ash ($p<.0001$) and fat ($p = .0135$) was higher in dogs fed the control diet. Dogs fed the HCLP diet consumed more crude fibre ($p = .0001$), probably due to the higher addition of cellulose in this diet.

The AAS of the HCLP showed high amounts of all amino acids based on the minimal requirement for puppies and kittens (Table 4), except for combination of Met + Cys and Phe + Tyr for kittens that had AAS values under 100. The HCLP contained considerable amount of essential fatty acids, mainly the polyunsaturated fatty acids (Table 1). The AAS of the bovine meat and bone meal showed values below 100 of Leu, Met, Met + Cys, Phe + Tyr, Thr, and Try for dogs, and Met, Met + Cys, Phe + Tyr, and Try for cats. The poultry byproducts meal had AAS below 100 of Try for dogs and Met, Met + Cys, and Phe + Tyr for cats.

The molecular weight profile differed between samples (Table 5), especially in content (%) <10 kDa, in which HCLP had 57%, poultry byproducts meal had 41%, bovine meat and bone meal had 35%, control diet had 39% and HCLP diet had 59%.

The dogs consumed the experimental diets, without refusals and leftovers. There were not significant differences in feed intake and first choice between both experimental diets (Table 6). The inclusion of HCLP at a level of 258 g/kg did not affect the palatability of the diet, and no vomit, diarrhoea, or other gastrointestinal clinical signs were observed during the study.

Consumption of the HCLP diet promoted an increase in the faecal water content ($p = .0321$) and increased daily faecal production ($p = .0361$) (Table 3). In spite of these changes, the mean faecal score did not differ between diets (average score 2.2), resulting in well formed, but slightly moist stools. No changes were observed in the urine volume, dry matter, nitrogen, pH, and density ($p > .05$).

The comparison between *in vivo* and *in vitro* digestibility of dry matter and organic matter was slightly different for each diet tested (Table 7). The *in vitro* method was effective for estimating the digestibility of the ingredient. The *in vitro* method underestimated the digestibility of DM and OM of control diet (84.5 and 84.0%, respectively) and HCLP diet (84.4 and 84.3%, respectively).

Discussion

The growing demand for therapeutic diets for dogs and cats diagnosed with adverse food reactions and food sensitivities highlights the importance of investigating new protein sources. The presence of thermal, chemical, and enzymatic resistant glycoproteins associated with the high molecular weight of these compounds, are the main factors which limit the inclusion of regular sources of protein in diets for sensitive

Table 4. Amino acid score (AAS) of the protein ingredients.

	AAS								
	Puppies 4–14w*			Puppies > 14w†			Kittens#		
Amino acid	HCLP$	PBM‡	BMBM§	HCLP$	PBM‡	BMBM§	HCLP$	PBM‡	BMBM§
Arginine	173	203	211	160	187	195	142	166	173
Histidine	131	111	100	158	133	121	157	132	119
Isoleucine	163	139	101	165	140	102	197	168	122
Leucine	154	124	99	190	153	122	156	125	100
Lysine	241	148	136	235	144	132	248	153	140
Methionine	154	123	87	159	127	90	123	98	70
Methionine + cystine	101	109	75	104	113	78	81	87	60
Phenylalanine	160	143	102	162	145	104	209	186	133
Phenylalanine + Tyrosine	145	120	86	146	121	87	98	81	59
Threonine	128	113	92	129	115	93	160	142	114
Tryptophan	144	94	54	144	94	54	199	130	75
Valine	200	169	148	187	158	138	212	179	157

*Calculated based on the minimal requirement for growing puppies 4–14 weeks old as reference values (NRC– National Research Council 2006); †Calculated based on the minimal requirement for growing puppies 14 weeks and older as reference values (NRC– National Research Council 2006); #Calculated based on the minimal requirement for growing kittens as reference values (NRC– National Research Council 2006); $HCLP: chicken liver powder ingredient; ‡PBM: poultry byproducts meal; §BMBM: bovine meat and bone meal.

Table 5. Molecular weight profile of the protein ingredients.

	Percentage of total sample [%]				
	Ingredients			Diets	
Molecular weight [kDa]	HCLP*	PBM†	BMBM#	Control	HCLP$
<1	31.0	14.0	17.0	15.0 (14.6)‡	31.0 (31.0)‡
1–10	26.0	27.0	18.0	24.0 (25.1)‡	28.0 (26.0)‡
10–20	16.0	27.0	23.0	24.0 (26.2)‡	17.0 (16.0)‡
>20	27.0	32.0	42.0	37.0 (34.3)‡	24.0 (27.0)‡

*HCLP: chicken liver powder ingredient; †PBM: poultry byproducts meal; #BMBM: bovine meat and bone meal; $HCLP: hydrolysed chicken liver powder diet, ‡Values within parentheses were estimated based on food formulation.

Table 6. Preference of experimental diets in adult dogs from different breeds.

	Treatments		
Item	Control	HCLP*	*p*-Value
Feed intake [g]	195	202	.2405
First choice [%]	45.5	54.6	.5465

*HCLP: hydrolysed chicken liver powder diet.

Table 7. *In vitro* coefficient of digestibility of experimental diets.

	Treatments	
Item	Control	HCLP*
Dry matter [%]	84.5	84.4
Organic matter [%]	84.0	84.3

*HCLP: hydrolysed chicken liver powder diet.

patients. Thus, the present study aimed to describe the chemical composition of a commercial HCLP and its acceptability and availability of nutrients and energy when it is replacing the most common sources of protein in diet for dogs: poultry byproduct meal and bovine meat and bone meal. And, finally, to compare *in vivo* and *in vitro* digestibility of the diet based on HCLP.

Adverse food reaction may occur due to an exacerbated immune response to food antigens (Mueller and Unterer 2018). The gastrointestinal tract has some mechanisms to avoid the entrance of foreign bodies into the bloodstream. The gut associated lymphoid tissue (GALT) is one of them, providing an active barrier to harmful substances such as food antigens. However, due to the higher permeability of the intestinal mucosa some food antigens may pass through inducing an immune response (Verlinden et al. 2006). The absorbed antigen cross-links between two high-affinity IgE receptor (FCεRI) present on the surface of mast cells and basophils, eliciting the release of mediators, such as histamine, prostaglandins, enzymes, and cytokines (Cave 2006).

The most common ingredients associated with adverse food reactions in dogs are beef, dairy products, chicken, and wheat. Less frequently are chicken egg, soy, lamb, pork, fish, and corn (Roudebush 2013). Through chemical analysis investigation, the HCLP provided adequate nutritional composition to be used as protein source in diets for dogs with no prior adverse reactions.

Molecular weight is one of the main tools for selecting protein sources, since small peptides can retain allergenicity and induce adverse food reactions (Cave 2006). In humans, peptides with molecular weight between 10 and 70 kDa are absorbed entirely by the enteric mucosa inducing an allergic reaction by IgE binding (Sampson 1999). In dogs, the molecular weight associated with allergenicity remains unknown, but the selection of new and/or low molecular weight ingredients is recommended for adverse food reaction in dogs. The ingredients used in the experimental diets had different proportions of molecular weights. The control diet had 39% proteins with molecular weight <10 kDa, and the HCLP diet had 59%, based on the calculation according to the molecular weight

present in the ingredients. Thus, seems that the extrusion process did not alter the fraction of molecular weight <10 kDa in both diets, as predicted in the estimated calculation (39.7% proteins with molecular weight <10 kDa on control diet and 57% on HCLP diet). Combined with the hydrolysed chicken liver powder, HCLP diet included brewers rice and full-fat rice bran, which contributed to increase the fraction of proteins with molecular weight >10 kDa. However, adverse food reactions related to rice are rare in dogs and cats (Roudebush 2013).

Cave and Guilford (2004) evaluated the molecular weight profile of a hydrolysate derived from chicken heart and liver, that presented 96.9% of its molecular weight <10 kDa. Compared with the intact protein, the chicken hydrolysate showed a residual antigenic mass of 1.5% analysed by the inhibition ELISA using IgG. According to De Jaham (2000), peptides with a molecular weight >45 kDa are still capable of eliciting an immune response in dogs. Differences in molecular weight profile in protein hydrolysates varies with the type of protein material used and the degree of hydrolysis applied to the protein material. Olson et al. (2000) recommends that at least 50% of the protein material should be hydrolysed to prevent allergic reaction in dogs.

The hydrolysis process of the chicken liver powder was conducted enzymatically and, following this was spray dried. The spray drying technique consists of producing a dry powder from a liquid by drying with a hot gas. This process allows the preservation of functional characteristics of the original raw material compared to the conventional drying process applied to meat by-products that may impact negatively on the nutritional content (Murray et al. 1997). The experimental diet based on HCLP was formulated to meet the complete nutritional requirements for adult dogs, and based on its amino acids content, such as lysine (58.1 g/kg), we were able to include the HCLP at a level of 258 g/kg. Analysis of subsequent batches of HCLP indicate that there was a reduction in the lysine concentration in the ingredient, with an average level of 48.0 g/kg of lysine. In addition, chemical analyzes of new batches of HCLP showed high concentration of taurine (5.00 g/kg) and choline (5120 mg/kg). Thus, HCLP becomes a viable option in diets for cats due to their high requirement for these nutrients. Finally, in order to attain the complete protein requirement, we selected rice, added as brewers rice and full-fat rice bran, due to its low association with dogs with adverse food reactions in dogs, as mentioned above.

The HCLP ingredient had all amino acid scores (AAS) above 100, based on the minimum requirement for growth of puppies and kittens, except for the combined requirement for Met + Cys and Phe + Tyr for kittens. In comparison, the poultry byproduct meal and the bovine meat and bone meal had more than one amino acid scored below 100 for dogs and cats, which indicates fewer limiting amino acids present on the HCLP ingredient evaluated in this study. Kerr et al. (2013) evaluated raw meat diets based on beef, bison, elk and horse and verified that in all diets the first limiting amino acid was the combined requirement of Met + Cys, scored below 100 (AAS ranged from 81 to 95) based on the minimum requirement for growth of kittens. Based on these findings, the HCLP ingredient presents high levels of most amino acids, which indicates high protein quality of the ingredient.

The palatability of the HCLP diet was not affected by the high inclusion of hydrolysed chicken liver powder (258 g/kg) compared to the control diet based on poultry byproducts and bovine meat and bone meals. Hydrolysis reduces the size of protein chains in small peptides and free amino acids, with the goal to reduce the molecular weight to avoid the protein recognition as an antigen by the immune system (Cave 2006). However, hydrolysis can expose side chain peptides, especially the hydrophobic side chain, which elicits the bitterness of some hydrolysed proteins. Cho et al. (2004) evaluated two commercial soy protein hydrolysates and noted that bitterness increased as the molecular weight of the peptide ranged between 4 to 2 kDa, and peptides with molecular weight <1 kDa showed the lowest bitterness. At the same time, hydrolysed protein sensorial characteristics are associated with mixture of peptides and the original protein source that highly affects palatability (Adler-Nissen 1986). Despite reports of bitterness associated to hydrolysed proteins, previous studies showed an adequate consumption of commercial dog diets containing chicken and soy isolate hydrolyzates (Biourge et al. 2004; Loeffler et al. 2004), indicating good palatability. Indeed, hydrolysed proteins have been long used as palatability enhancers in commercial diets for dogs and cats. Additionally, the peroxide index of both experimental diets remained in acceptable range.

Small peptides from partially hydrolysed proteins are more efficiently absorbed from the intestine and have a higher nutritional value than free amino acids (Monchi and Rerat 1993). Thus, the digestibility of protein hydrolysates was expected to be superior to the intact protein (Cave 2006). However, the digestibility of both experimental diets, control and HCLP, did not differ.

According to Rouanet et al. (1990), hydrolysate diets containing di- and tripeptides are efficiently digested, but not better utilised than diets composed of the original protein in healthy growing rats. Hekman (2003) found an apparent ileal protein digestibility of 82.4% for a commercial diet based on hydrolysed chicken. Compared to our results, both diets showed superior results (88.0% for the control diet and 89.3% for the HCLP diet). In order to attain some amino acids requirements and to obtain isonitrogenous diets, synthetic amino acids were added to the control diet, which may have improved its digestibility due to the bioavailability of these components. However, differences in digestibility occurred to a small extent. Though, we use the apparent total tract digestibility that does not account for part of the metabolism of nutrients in the large intestine, mainly due to the microbiota metabolism and epithelial desquamation. Thus, this method may increase the digestibility coefficient of protein due to the microbiota degradation (Zebrowska 1975). Although the diets were formulated to be isonutritive, some differences were observed, such as a lower fat and a higher crude fibre content of the HCLP diet. In addition, the HCLP diet had a lower ash content compared to the control diet (10.5 g/kg less), which is a favourable aspect to the selection of the HCLP ingredient for addition in high digestibility diets.

The increase on the faecal water content and daily faecal production in dogs fed HCLP diet may be due to the hydrolysed chicken liver powder osmolarity. High osmolarity solutions attract water to the intestinal luminal promoting severe diarrhoea and it is increased with hydrolysis. Therefore, extensively hydrolysed proteins could promote diarrhoea in some dogs. In a study conducted by Loeffler et al. (2004) with 46 dogs fed a commercial diet with chicken hydrolysate, only 4 developed soft faeces. In addition, 21 of the 46 dogs had gastrointestinal symptoms prior to the study and all showed improvement on these signs. In our research, one of our first concern was the high inclusion of the HLCP (258 g/kg) that could promote severe diarrhoea in dogs as reported by Hekman (2003), in which dogs fed a commercial diet based on hydrolysed chicken showed diarrhoea. However, despite changes in faecal DM and faecal volume, the faecal output in dry matter did not differ between the dietary treatments, and the final faecal score remained within the recommended by Moxham (2001).

Ribeiro et al. (2019) observed that poultry by-product meals from two integrated rendering plants showed different *in vitro* digestibility of organic matter (IVDOM) based on their oxidative stability. From 100 samples analysed, 18 had 84.8% of IVDOM, classified by the authors as high IVDOM. The control diet showed 84.0% of IVDOM and the HCLP, 84.3% of IVDOM. We did not analyse the ingredients separately, only the complete diets. Biagi et al. (2016) analysed the *in vitro* digestibility of dry matter (IVDDM) of commercial diets for dogs in two different durations of gastric digestive phase and found that IVDDM for 2 h of incubation was 86.4% and for 4 h of incubation was 84.2%. According to these authors, *in vitro* digestibility method is a quick procedure to predict the digestibility of commercial diets, thus reducing the need for *in vivo* assays. However, more assays and replicates are necessary to guarantee the accuracy of this method.

Conclusions

Based on the current high demand for protein hydrolysates of high nutritional value and acceptable for dogs as a viable option in therapeutic diets, the hydrolysed chicken liver powder evaluated in this study has nutritional characteristics compatible with those required for adult dogs, especially due to the high content of some essential amino acids and fatty acids. The inclusion level of 258 g/kg of hydrolysed chicken liver powder promoted good acceptance and digestibility, and did not promote diarrhoea in dogs fed the HCLP diet. Further studies are needed to evaluate its effects in dogs diagnosed with adverse food reactions and gastrointestinal disorders.

Disclosure statement

No potential conflict of interest was reported by the author(s). Marcelino Bortolo and Fábio Ritter Marx are employees of Kemin Industries.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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