



# **Evaluation Evaluation of the Nutritional Value of Insect-Based Complete Pet Foods**

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Abstract: Since the legalization of insect protein in pet food, a variety of products incorporating this ingredient have emerged on the market. Although edible insects are acknowledged for high protein content, chitin can also elevate the quantity of indigestible carbohydrates. The objective of this study was to evaluate the nutritional adequacy of fourteen complete dog foods containing edible insects in accordance with the FEDIAF nutritional guidelines. Due to the use of insects as the predominant animal component in all diets, analyses of dietary fiber fractions were carried out to estimate the content of indigestible carbohydrates. The analyses included the assessment of chemical composition, calcium, and phosphorus levels and metabolizable energy. The findings were then compared with the data provided by the manufacturers. All diets were found to meet the minimum recommended levels from the FEDIAF nutritional guidelines for protein (18.0 g/100 g DM) and fat (5.5 g/100 g DM). However, discrepancies were noted between the label data and analysis results. The results for the dietary fiber fraction differed from the crude fiber content, which is consistent with the imprecision inherent to the crude fiber determination method. In one food, there was a discrepancy of up to 19.21 g between the NDF fraction and the crude fiber content. Calcium levels were inadequate in two foods, and furthermore, twelve foods exhibited an abnormal calcium/phosphorus ratio. These findings indicate that while edible insects can be a valuable protein source, their inclusion may lead to increased indigestible carbohydrates, potentially causing digestive issues and gastric discomfort in dogs.

**Keywords:** chemical composition; complete pet foods; dietary fiber fractions; edible insects; food labeling; indigestible carbohydrates; novel protein sources

## 1. Introduction

The contemporary pet food market is distinguished by accelerated growth, diversification, and evolving consumer preferences, which mirror broader tendencies associated with heightened concern for the well-being, advancement, and humanization of animals. The rise in the number of pets globally is contributing to substantial growth in the sector, with the global pet food industry estimated to be worth approximately USD 115 billion, with dog and cat food alone accounting for approximately 70% of the value [1]. It is anticipated that growth in value will persist, driven by heightened awareness of animal nutrition and health as well as evolving consumer preferences regarding pet food ingredients and origins. A noteworthy trend within the sector is the growing prevalence of specialized diets, including vegan, grain-free, and those comprising unconventional animal proteins. The preference for plant-based diets is largely driven by pet owners' concerns about farm animal welfare and environmental sustainability as well as a desire to align their dietary choices with the nutrition of their pets [2]. The popularity of grain-free foods has increased in response to concerns about contaminants, food allergies, and intolerances in animals [3]. The livestock sector is a significant contributor to global environmental degradation, accounting for approximately 14.50% of all anthropogenic greenhouse gas emissions [4]. The carbon footprint of meat production represents a pivotal element in the evaluation of its environmental

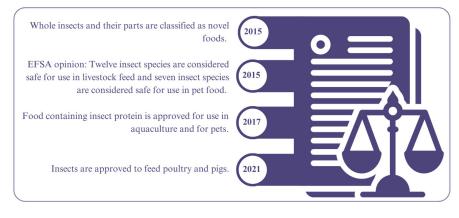


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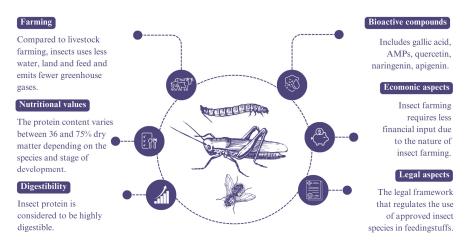
**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). impact, as it directly influences climate change through the emission of gases into the atmosphere [5]. The production of meat is associated with a considerable demand for water, which in turn contributes to the loss of biodiversity [5]. The water footprint of meat production, defined as the volume of water consumed in the process, is a crucial element to be considered when evaluating the environmental consequences of meat consumption [6]. The increasing popularity of insect consumption has significant implications for the pet food industry. This is primarily driven by an increase in demand for sustainable protein sources, the nutritional benefits of insects, and changing consumer perceptions. As the global population of companion animals continues to grow, there is an increasing need for high-quality, sustainable pet food options. The recognition of insects as a viable alternative to traditional protein sources such as beef, chicken, and fish is driven by their rich protein content and favorable environmental footprint. This shift responds to the rising costs and environmental concerns associated with conventional livestock farming and aligns with the broader movement towards sustainable food systems [7–10]. The utilization of alternative protein sources, including plant-based proteins and insect-based foods, may present more sustainable options with a reduced environmental impact in comparison to traditional meat production [11,12]. To address these issues, legislative amendments have been introduced over several years, with the objective of facilitating the incorporation of insect protein into nutritional regimes [13–16]. In 2015, a number of insect species were designated as novel foods, and the European Food Safety Authority implemented amendments to the relevant legislation, with the result that they are henceforth referred to as livestock (Figure 1). As a consequence of these modifications, insect protein can be employed in the manufacture of feed for both livestock and companion animals [14,15]. The following insect species have been approved for use in animal feed: the Black solider fly (Hermetia illucens), the Common housefly (Musca domestica), the Mealworm (Tenebrio molitor), the Lesser mealworm (Alphitobius diaperinus), the House cricket (Acheta domesticus), the Banded cricket (Gryllodes sigillatus), and the Jamaican field cricket (Gryllus assimilis).



**Figure 1.** Changes to legislation made since 2015 regarding the use of insects in food based on legal acts [13–16].

There is a growing interest in insects as a source of protein for both humans and animals. This interest is accompanied by a growing body of scientific evidence supporting the use of insects in human and animal food while also providing information on potential risks (Figure 2). One of the principal factors contributing to this phenomenon is the increasing perception that insects are a valuable source of nutrients and a less environmentally damaging alternative to traditional livestock protein sources. This is due to the unique nature of insect farming [17–20]. High protein content, with levels ranging from 30-75 g/100 g DM [11,21–25] depending on the species and stage of development, can be an additional or main protein component in pet foods. This protein may confer benefits to canines with food sensitivities or allergies to the most commonly utilized protein sources in the feed industry, including beef, chicken, and cereals [26]. The distinctive protein profile

of edible insects is compatible with the dietary requirements of canines, while potentially reducing the likelihood of triggering allergic reactions [27]. Furthermore, they constitute a source of unsaturated fatty acids, vitamins, and minerals [17,19,20,25,28,29]. Medium-chain fatty acids (MCFAs), including capric acid, lauric acid, myristic acid, and palmitic acid, are distinctive of certain edible insects. The use of these compounds is indicated during the treatment of pancreatic insufficiency or in individuals with insulin resistance and complex enteropathies, as they do not require the activation of pancreatic enzymes for digestion [30–32]. The content of bioactive compounds in edible insects, including AMPs and quercetin, may contribute to the support of the immune system and overall health [33]. Chitin, a structural component present in insects, fungi, plants, and crustaceans [34,35], may increase the content of indigestible carbohydrates.



**Figure 2.** The factors that are conducive to the increased consumption of edible insects as a foodstuff based on the literature research [7–12,20–23,25,29,34–37].

As pet owners gain a deeper understanding of their dogs' nutritional requirements, the pet food industry is facing the challenge of innovating while adhering to established guidelines [36–38]. It is of great importance to ensure the nutritional adequacy of the product in order to maintain the health of the animal, particularly when one considers that pets are fed a single type of food over an extended period of time. Insufficiently balanced formulations may result in the later detection of abnormalities in nutritional products.

The research hypothesis is that complete pet foods containing edible insects are formulated in such a way as to guarantee coverage of the minimum recommended levels for macronutrients and minerals in the pet food for which the product is intended, in accordance with the FEDIAF nutritional guidelines [36]. The use of insects as the primary protein source may have adverse effects due to the presence of chitin, which may result in higher levels of indigestible carbohydrates than in food containing protein from slaughtered animals. Accordingly, the following objectives were established:

(1) To assess the chemical composition and calculate the energy value based on the results of the analysis;

(2) To verify whether the levels of protein, fat, calcium, and phosphorus meet the minimum recommended levels for these components as defined by the current European nutritional guidelines [36];

(3) To determine the dietary fiber fractions;

(4) To assess the consistency of the results obtained from the chemical analyses and the energy value with the information on the label. Furthermore, the energy value of the products was calculated in order to verify it with the information provided on the product label.

## 2. Materials and Methods

Over-the-counter (OTC) dry dog food (n = 12) and veterinary therapeutic diets (VTD) (n = 2) for adult dogs. The selection of foods for the study consisted of a range of products available from both pet shops and online retailers. A key factor was the presence of edible insects as the main source of animal protein in the food composition. Despite the growing popularity of such products, the range is still limited, and formulations are often duplicated between different brands. Consequently, an effort was made to select products that varied in terms of the percentage of insects in the formulation (between 10% and 42.5%) and the ingredients included in order to provide a greater variety of samples for analysis. Ten foods contained no other animal components in the formulation except insect protein. All products were purchased in original packaging, ranging from 0.1 to 2.5 kg. A representative sample of the foods was taken from each package and ground in a KNIFETEC 1095 laboratory grinder (Foss Tecator, Höganäs, Sweden). The tested foods were assigned the symbols DIF\_1–DIF\_14 (Dog Insect Formula).

## 2.1. Chemical Composition and Ingredient List of Foods

The regulation of the labeling of animal nutrition products is defined by Regulation 767 [38]. On the label of a complete pet food, the manufacturer is obliged to include information such as the moisture content if it is higher than 14% or the dry matter. Furthermore, the label must indicate the level of crude protein, crude fat, and crude fiber. In the event that the crude ash content exceeds 2.2% DM, it is required that this be described. This information is presented on the product label under the heading "Analytical Composition" The minimum recommended level (MRL) is subject to regulation by the FEDIAF nutritional guidelines [36] (Table 1).

Nutrient	Unit	MRL	NMaxRL
Crude protein	g	18.00	-
Crude fat	g	5.50	-
Calcium	g	0.50	2.50
Phosphorus	g	0.40	1.60
Ca:P	ratio	1:1	2:1

**Table 1.** Minimum recommended level (MRL) and maximum recommended level (NMaxRL) for selected nutrients in foods with an energy density of 400 kcal ME/100 g DM [36].

The labels of the selected products were organized in descending order, according to the quantity of each food component present. This enabled the researchers to conclude that the components listed first in the formulation by the manufacturer were present in the greatest quantity. The predominant component in each of the foods was insects. The ingredient list of each food is presented in Supplementary Materials (Table S1). The different forms of insects used included whole insects (fresh or dried), dried larvae, and insect meal. In addition to insects, the foods also contained a variety of plant components, including cereals, vegetables, fruits, and herbs.

## 2.2. Proximate Analyses

The chemical composition of the samples was analyzed according to AOAC [39] and included the determination of dry matter (DM), crude protein (CP), crude fat (ether extract, EE), crude fiber (CF), and crude ash (CA). Dry matter was determined by drying the samples at 105 °C to constant weight (method 945.15) and calculated according to Equation (1). Crude protein was determined by the Kjeldahl method (954.01) using a Büchi B-324 distillation apparatus (Büchi Labortechnik AG, Switzerland), as estimated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor: Kp = 6.25. Crude fat was determined by the Soxhlet method using diethyl ether (920.39) and crude ash by combustion in a muffle furnace at 580 °C (920.153). Crude fiber content was determined using the ANKOM<sup>220</sup> Fiber Analyzer (ANKOM Technology, Wayne County,

NY, USA). Nitrogen-free extract (NFE) was calculated by subtracting protein, fat, fiber, and ash from the total dry matter (2) [40]:

$$DM = 100 - \% \text{ moisture}$$
(1)

$$NFE = DM - (CP + EE + CF + CA)$$
(2)

# 2.3. Calcium and Phosphorus

The calcium (Ca) and phosphorus (P) contents were determined by atomic absorption spectrometry using a Thermo Fisher Scientific iCE 3000 Series instrument (Waltham, MA, USA) in triplicate. The samples for phosphorus analysis were mineralized in concentrated sulfuric acid ( $H_2SO_4$ ) and perchloric acid ( $HClO_4$ ), and the phosphorus content was then determined by the Egner–Riehm colorimetric method using a Specol 221 spectrophotometer (Carl Zeiss, Jena, Germany). The accuracy and precision of the analytical method employed were verified through the use of certified reference material. The certified reference material used was IAEA/V-10 Hay (International Atomic Energy Agency, Vienna, Austria). The results were found to be in accordance with the declared values, falling within the 90–99% range. The analyses were conducted using a re-reference material in the form of skimmed milk powder (ERM<sup>®</sup>-BD151). The resulting concentrations of calcium and phosphorus were converted to grams per 100 g of dry matter, and the ratio of calcium to phosphorus was calculated.

## 2.4. Dietary Fiber Fractions

Dietary fiber fractions were determined using the detergent method [41] on an ANKOM<sup>220</sup> (ANKOM Technology, Wayne County, NY, USA) to separate dietary fiber into distinct fractions.

#### 2.4.1. Neutral-Detergent Fiber

The acid-detergent solution, i.e., sodium sulfite and  $\alpha$ -amylase, were used to determine the neutral-detergent fiber (NDF) fraction. The material was subjected to boiling at 100 °C. Following the removal of the solution, distilled water and  $\alpha$ -amylase were added, and the material was then rinsed with distilled water to remove any residual solution components. The material was subjected to degreasing and subsequently placed in a laboratory dryer for a period of four hours at a temperature of 105 °C. Once cooled, the samples were weighed and incinerated in a muffle furnace at 550 °C. The remaining incinerated matter was then reweighed.

## 2.4.2. Acid-Detergent Fiber

The acid-detergent fiber (ADF) content was determined by boiling the material in an acid-detergent solution at 100 °C. Subsequently, the samples were rinsed twice with distilled water. The material was then subjected to a four-hour drying process at 105 °C in a laboratory dryer. Once cooled and weighed, the samples were incinerated in a muffle furnace at 550 °C, after which the remaining incinerated material was reweighed.

#### 2.4.3. Acid-Detergent Lignin

Determination of acid-detergent lignin (ADL) content was performed equally with the ADF fraction until placement in a laboratory dryer for 4 h at 105 °C after cooking in an ANKOM<sup>220</sup> apparatus (ANKOM Technology, Wayne County, NY, USA). After drying and cooling, the samples were placed in 72%  $H_2SO_4$  (VI) for 24 h. Samples were rinsed of residual acid before re-drying. Once a pH of 7 was reached, the samples were dried and subjected to combustion in a muffle furnace at 550 °C, and the residual matter resulting from the combustion was weighed.

# 2.4.4. Hemicellulose and Cellulose

The determination of hemicellulose (HCEL) was based on the calculation of the difference of the NDF and ADF fractions:

$$HCEL = NDF - ADF$$
(3)

The cellulose (CEL) determination was based on calculating the difference from the ADF and ADL fractions:

$$CEL = ADF - ADL$$
(4)

The results of the analysis are presented in grams per 100 g of dry matter (DM) as a mean of three repetitions.

# 2.5. Energy Value

Metabolizable energy is the form used to express the energy density of dog food. Metabolizable energy can be calculated from the results of the chemical analysis of the food; therefore, the energy value expressed as metabolizable energy was estimated according to the current guidelines for complete dry/wet dog and cat food according to a four-step calculation (5)–(8) [36,42].

Gross energy (GE) was calculated according to the following equation:

$$GE = (5.7 \times CP) + (9.4 \times EE) + 4.1 (NFE + CF)$$
(5)

Energy digestibility (ED) was calculated according to the following equation:

$$ED = 91.2 - (1.43 \times \% CF \text{ in } \% DM)$$
(6)

In the next step, digestible energy was calculated according to the following equation:

$$DE (kcal) = (GE (kcal) \times ED)/100$$
(7)

Metabolizable energy (ME) for dogs, as the final step of calculation, was calculated by the following equation:

$$ME = DE (kcal) - (1.04 \times \% CP)$$
(8)

## 2.6. FEDIAF Nutirtional Guidelines

The European Pet Food Industry Federation's (FEDIAF) European-wide nutritional guidelines provide a framework for the development of pet foods that meet the basic nutritional requirements of dogs and cats at different life stages [36]. Compliance with these guidelines by pet food manufacturers is beneficial in preventing nutritional deficiencies and excesses in healthy animals, which can result in significant health complications. These guidelines establish the minimum recommended levels (MRL) for specific nutrients, including protein, essential amino acids, fats, essential fatty acids, vitamins, and minerals (micro- and macronutrients). The guidelines also define the nutritional maximum recommended levels (NMaxRL) for certain ingredients, which should not be exceeded. For adult animals, the guidelines define MRLs in relation to their maintenance energy requirement (MER). To assess the nutritional adequacy of the study foods, MRLs for individual ingredients were adjusted for dogs with an average physical activity of 1 to 3 h per day (MER = 110 kcal/kg<sup>0.75</sup> body weight). Importantly, the MRLs and NMaxMRLs specified by FEDIAF nutritional guidelines [36] are provided for an energy density of 400 kcal ME/100 g DM (Table 1); the tested foods had different ME, so the values were corrected properly. Based on the manufacturer's label information on crude protein, ether extract, crude fiber, crude ash, and energy value, analytical tolerances were determined in accordance with Regulation 767 [38] and FEDIAF nutritional guidelines [36].

## 2.7. Statistical Analyses

The results of the chemical analyses are presented as mean values derived from three replicates per 100 g dry matter (DM). The statistical analysis was conducted using STATISTICA v13.30 software (TIBCO Software Inc., Palo Alto, CA, USA). A one-way analysis of variance was performed on the results of chemical composition, dietary fiber fraction, and metabolizable energy values, assuming a significance level of  $\alpha$  = 0.05 and a *t*-Tukey test.

# 3. Results

# 3.1. Chemical Composition of Foods

The nutrient content and metabolizable energy level, determined from analyses, showed statistically significant differences (p < 0.05) between the different foods (Table 2). The values from the analyses were calculated to be correct for amounts in foods with an energy density of 400 kcal ME/100 g DM (Table 3). The lowest protein content was observed in the DIF\_2 (23.35 g/100 g DM), while the highest was noted in the DIF\_11 (35.75 g/100 g DM). Each of the foods was found to meet the MRL [36] for protein, which is equal to 18.00 g/100 g DM. However, in contrast to the values indicated on the manufacturer's label, the results of the analysis demonstrated that the DIF\_5, DIF\_9 and DIF\_11 exhibited protein levels that fell below the established analytical tolerance range (Figure 3).

Table 2. Nutrients and energy values derived from analyses of the tested foods.

Item	DM <sup>1</sup>	СР	EE	CF	CA	NFE	ME
	g/100 g			g/100 g DM			kcal/100 g DM
DIF_1	95.92	26.08 <sup>c</sup>	11.75 <sup>d</sup>	5.36 <sup>e</sup>	6.05 <sup>c</sup>	50.77 <sup>b</sup>	381.70 <sup>c</sup>
DIF_2	95.73	23.35 <sup>a</sup>	11.67 <sup>cd</sup>	3.33 <sup>b</sup>	7.43 <sup>e</sup>	54.22 <sup>d</sup>	389.59 <sup>d</sup>
DIF_3	95.39	23.45 <sup>a</sup>	12.04 <sup>e</sup>	5.56 <sup>e</sup>	4.90 <sup>a</sup>	54.05 <sup>d</sup>	384.23 <sup>c</sup>
DIF_4	94.62	26.45 <sup>d</sup>	11.63 <sup>cd</sup>	3.40 <sup>b</sup>	5.85 <sup>bc</sup>	52.67 <sup>c</sup>	403.52 <sup>f</sup>
DIF_5	94.88	24.56 <sup>b</sup>	7.58 <sup>a</sup>	4.03 <sup>bc</sup>	6.11 <sup>c</sup>	57.72 <sup>g</sup>	371.11 <sup>b</sup>
DIF_6	94.00	23.54 <sup>a</sup>	10.43 <sup>b</sup>	4.18 <sup>c</sup>	6.67 <sup>d</sup>	55.18 <sup>e</sup>	380.32 <sup>c</sup>
DIF_7	94.69	29.23 <sup>e</sup>	13.20 <sup>f</sup>	7.16 <sup>g</sup>	6.96 <sup>d</sup>	43.46 <sup>a</sup>	373.10 <sup>b</sup>
DIF_8	94.67	25.68 <sup>c</sup>	10.22 <sup>b</sup>	6.39 <sup>f</sup>	7.48 <sup>e</sup>	50.23 <sup>b</sup>	362.70 <sup>a</sup>
DIF_9	94.41	33.19 <sup>f</sup>	11.40 <sup>c</sup>	4.89 <sup>d</sup>	5.21 <sup>a</sup>	45.32 <sup>a</sup>	388.19 <sup>d</sup>
DIF_10	95.71	24.17 <sup>a</sup>	11.24 <sup>c</sup>	5.65 <sup>e</sup>	7.15 <sup>e</sup>	51.79 <sup>c</sup>	372.82 <sup>b</sup>
DIF_11	95.84	35.75 <sup>g</sup>	11.22 <sup>c</sup>	2.21 <sup>a</sup>	6.69 <sup>d</sup>	44.13 <sup>a</sup>	402.16 <sup>f</sup>
DIF_12	94.73	24.62 <sup>b</sup>	10.45 <sup>b</sup>	3.35 <sup>b</sup>	5.58 <sup>b</sup>	56.00 <sup>f</sup>	391.09 <sup>d</sup>
DIF_13	96.06	25.19 <sup>bc</sup>	11.77 <sup>cd</sup>	3.83 <sup>b</sup>	5.72 <sup>b</sup>	53.50 <sup>c</sup>	393.13 <sup>e</sup>
DIF_14	94.23	26.71 <sup>d</sup>	12.86 <sup>ef</sup>	4.34 <sup>c</sup>	6.79 <sup>d</sup>	49.30 <sup>b</sup>	391.54 <sup>e</sup>

<sup>1</sup> DM—dry matter; CP—crude protein; EE—ether extract; CF—crude fiber; CA—crude ash; NFE—nitrogen free extract; ME—metabolizable energy; a, b, c, ...—averages marked with different letters are statistically significantly different according to the *t*-Tukey test at a significance level of p = 0.05 (for all columns separately).

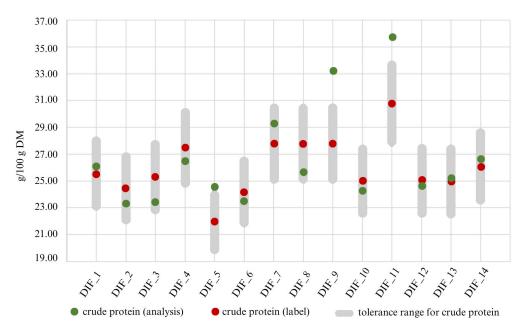
**Table 3.** Corrected protein and fat contents for an energy density of 400 kcal according to FEDIAF nutritional guidelines [36].

Item	СР	EE		
icin	g/100 g DM <sup>1</sup>			
DIF_1	27.33	12.31		
DIF_2	23.97	11.98		
DIF_3	24.41	12.54		
DIF_4	26.22	11.53		
DIF_5	26.47	8.17		
DIF_6	24.76	10.97		
DIF_7	31.34	14.15		
DIF_8	28.32	11.28		
DIF_9	34.20	11.74		

Item	СР	EE	
item	g/100 g DM <sup>1</sup>		
DIF_10	25.94	12.06	
DIF_11	35.56	11.16	
DIF_12	25.18	10.69	
DIF_13	25.63	11.97	
DIF_14	27.29	13.14	
FEDIAF <sub>MRL</sub>	18.00	5.50	
FEDIAFNMaxRL	ND <sup>2</sup>	ND	

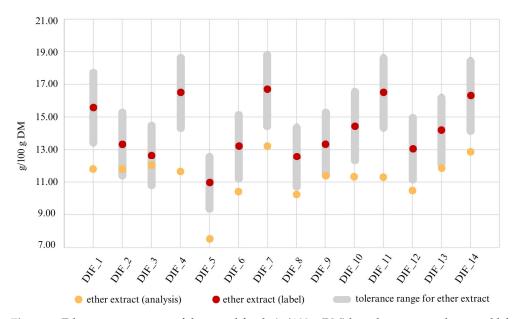
Table 3. Cont.

<sup>1</sup> at an energy density 400 kcal in 100 g dry matter (DM); <sup>2</sup> ND—not defined.

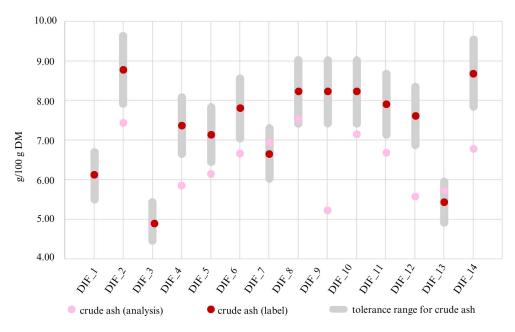


**Figure 3.** Protein content of tested foods (g/100 g DM) based on own analyses and labeled values (g/100 g DM) with analytical tolerance range calculated according to [38].

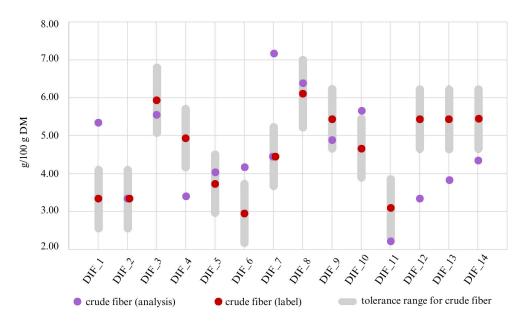
The lowest fat content was observed in the DIF\_5 (7.58 g/100 g DM), while the highest was noted in the DIF\_7 (13.20 g/100 g DM). The MRL for fat, as defined by FEDIAF nutritional guidelines [36], is 5.50 g, a value that was also met in each of the foods. With regard to this ingredient, twelve foods exhibited ether extract (EE) levels that fell below the established analytical tolerance range, as indicated on the packaging (Figure 4). The lowest level of crude ash, representing the mineral content that does not break down during combustion, even at high temperatures, was observed in the DIF\_3 (4.90 g/100 g DM), while the highest level was found in the DIF\_7 (7.48 g/100 g DM). Crude ash levels exhibited a deviation from the analytical tolerance range in nine foods, with a value below the expected value (Figure 5). The lowest crude fiber content was determined in DIF\_11 (2.21 g/100 g DM) and the highest in DIF\_7 (7.16 g/100 g DM). Relative to the expected value from the product label, three foods had CF levels above the analytical tolerance range, and four were below this value (Figure 6). In the tested foods, nitrogen-free extract content was lowest in DIF\_7 (43.46 g/100 g DM) and highest in DIF\_12 (56.00 g/100 g DM). The metabolizable energy levels of the tested foods ranged from the lowest 362.70 kcal/100 g DM in DIF\_8 to 403.52 kcal/100 g DM in DIF\_4. Relative to the manufacturer's specified content, four foods had this value below the analytical tolerance range (Figure 7).



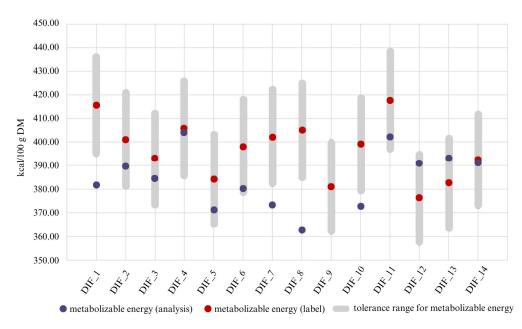
**Figure 4.** Ether extract content of the tested foods (g/100 g DM) based on own analyses and labeled values (g/100 g DM) with analytical tolerance range calculated according to [38].



**Figure 5.** Crude ash content of the tested foods (g/100 g DM) based on own analyses and labeled values (g/100 g DM) with analytical tolerance range calculated according to [38].



**Figure 6.** Crude fiber content of the tested foods (g/100 g DM) based on own analyses and labeled values (g/100 g DM) with analytical tolerance range calculated according to [38].



**Figure 7.** Metabolizable energy content of the tested foods (g/100 g DM) based on own analyses and labeled values (g/100 g DM) with analytical tolerance range calculated according to [38].

# 3.2. Calcium and Phosphorus

The calcium and phosphorus levels showed statistically significant differences (p < 0.05) between the different analyzed foods (Table 4). The lowest calcium content was found in the DIF\_9, with a value of 0.38 g/100 g DM. The highest calcium content among the tested foods was observed in the DIF\_1, with a concentration of 2.53 g/100 g DM. The lowest phosphorus content was observed in the DIF\_1 (0.66 g/100 g DM), while the highest phosphorus content was observed in the DIF\_11 (1.26 g/100 g DM). The calcium/phosphorus ratio should be between 1:1 and at maximum 2:1 [36]—only two of the foods (DIF\_2 and DIF\_8) were found to meet the recommendations. The ratio of DIF\_1 was the highest at 3.82:1, while the ratio of DIF\_9 was the lowest at 0.43:1. Compared to the FEDIAF nutritional guidelines [36] defining MRL and NMaxRL in foods, the content of ingredients for which these levels were

set was converted to content in g/100 g DM at an energy density of 400 kcal/100 g DM (Table 4).

Item	Ca <sup>1</sup>	Р	Ca	Р	Ca:P
	g/100 g DM <sup>2</sup>		g/100 g DM <sup>3</sup>		Ratio
DIF_1	2.53 <sup>f</sup>	0.66 <sup>a</sup>	2.65	0.69	3.82
DIF_2	1.56 <sup>e</sup>	0.78 <sup>b</sup>	1.60	0.80	1.99
DIF_3	0.78 <sup>c</sup>	1.16 <sup>e</sup>	0.81	1.21	0.68
DIF_4	0.88 <sup>c</sup>	1.03 <sup>d</sup>	0.87	1.02	0.86
DIF_5	0.87 <sup>c</sup>	1.16 <sup>e</sup>	0.94	1.25	0.74
DIF_6	0.84 <sup>c</sup>	1.02 <sup>d</sup>	0.88	1.07	0.83
DIF_7	0.68 <sup>b</sup>	0.76 <sup>b</sup>	0.73	0.81	0.89
DIF_8	1.07 <sup>d</sup>	0.90 <sup>c</sup>	1.18	0.99	1.19
DIF_9	0.38 <sup>a</sup>	0.87 <sup>bc</sup>	0.39	0.90	0.43
DIF_10	0.95 <sup>d</sup>	1.05 <sup>d</sup>	1.02	1.13	0.90
DIF_11	1.15 <sup>d</sup>	1.26 <sup>e</sup>	1.14	1.25	0.91
DIF_12	0.52 <sup>b</sup>	1.01 <sup>d</sup>	0.53	1.03	0.52
DIF_13	0.56 <sup>b</sup>	1.09 <sup>d</sup>	0.57	1.11	0.51
DIF_14	0.90 <sup>cd</sup>	1.25 <sup>e</sup>	0.92	1.28	0.72
FEDIAF MRL	ND <sup>4</sup>	ND	0.50	0.40	1:1
FEDIAF <sub>NMaxRL</sub>	ND	ND	2.50	1.60	2:1

Table 4. Calcium and phosphorus content and their ratio in the tested foods.

<sup>1</sup> Ca—calcium; P—phosphorus; <sup>2</sup> g per/100 g dry matter (DM) of analyzed food; <sup>3</sup> at an energy density 400 kcal of analyzed food; a, b, c, . . . —averages marked with different letters are statistically significantly different according to the *t*-Tukey test at a significance level of p = 0.05 (for all columns separately); <sup>4</sup> ND—not defined.

In DIF\_9, the Ca content found was 22% below the MRL. The NMaxRL was exceeded in one (DIF\_1) by 6%. Phosphorus in the tested foods was between the required nutritional minimum and the designated maximum level.

#### 3.3. Dietary Fibee Fractions (NDF, ADF, ADL, HCEL, and CEL)

There was a statistically significant difference (p < 0.05) between the foods tested with regard to the content of individual fiber fractions (Table 5). The lowest content of the NDF fraction was found in DIF\_5 (6.72 g/100 g DM) and the highest in DIF\_3 (24.77 g/100 g DM). The ADF fraction, which is part of the NDF, was found to be lowest in DIF\_2 (6.00 g/100 g DM) and highest in DIF\_8 (12.27 g/100 g DM). The ADL fraction, consisting solely of lignin, was lowest in DIF\_4 (1.02 g/100 g DM) and highest in the DIF\_3 (4.62 g/100 g DM). The hemicellulose content ranged from the lowest in DIF\_5 (0.12 g/100 g DM) to the highest in DIF\_3 (14.24 g/100 g DM). In contrast, the lowest cellulose content was recorded in DIF\_2 (3.49 g/100 g DM) and the highest in DIF\_9 (10.20 g/100 g DM).

**Table 5.** Content of dietary fiber fraction  $(g/100 \text{ g DM}^{1})$  in the tested foods.

Item	NDF <sup>2</sup>	ADF	ADL	HCEL	CEL
DIF_1	14.03 <sup>b</sup>	9.86 <sup>a</sup>	2.26 <sup>b</sup>	4.17 <sup>c</sup>	7.60 <sup>b</sup>
DIF_2	9.57 <sup>e</sup>	6.00 <sup>c</sup>	2.50 <sup>b</sup>	3.57 <sup>c</sup>	3.49 <sup>f</sup>
DIF_3	24.77 <sup>a</sup>	10.53 <sup>a</sup>	4.62 <sup>a</sup>	14.24 <sup>a</sup>	5.92 <sup>d</sup>
DIF_4	13.71 <sup>bc</sup>	8.58 <sup>b</sup>	1.02 <sup>c</sup>	5.13 <sup>c</sup>	7.56 <sup>b</sup>
DIF_5	6.72 <sup>f</sup>	6.60 <sup>c</sup>	1.66 <sup>b</sup>	0.12 <sup>e</sup>	4.95 <sup>e</sup>
DIF_6	7.41 <sup>f</sup>	6.03 <sup>c</sup>	1.24 <sup>c</sup>	1.38 <sup>d</sup>	4.80 <sup>e</sup>
DIF_7	12.19 <sup>c</sup>	9.11 <sup>a</sup>	2.11 <sup>b</sup>	3.08 <sup>c</sup>	7.00 <sup>b</sup>
DIF_8	20.70 <sup>a</sup>	12.27 <sup>a</sup>	2.74 <sup>b</sup>	8.43 <sup>b</sup>	9.54 <sup>a</sup>
DIF_9	16.34 <sup>b</sup>	11.83 <sup>a</sup>	1.63 <sup>b</sup>	4.51 <sup>c</sup>	10.20 <sup>a</sup>
DIF_10	10.79 <sup>d</sup>	8.58 <sup>b</sup>	1.91 <sup>b</sup>	2.22 <sup>d</sup>	6.66 <sup>bc</sup>
DIF_11	11.12 <sup>d</sup>	7.71 <sup>b</sup>	1.50 <sup>c</sup>	3.41 <sup>c</sup>	6.21 <sup>c</sup>

Item	NDF <sup>2</sup>	ADF	ADL	HCEL	CEL
DIF_12	11.57 <sup>d</sup>	7.75 <sup>b</sup>	2.16 <sup>b</sup>	3.82 <sup>c</sup>	5.59 <sup>d</sup>
DIF_13	12.04 <sup>c</sup>	8.83 <sup>b</sup>	1.52 <sup>b</sup>	3.21 <sup>cd</sup>	7.31 <sup>b</sup>
DIF_14	10.75 <sup>d</sup>	6.52 <sup>c</sup>	1.50 <sup>b</sup>	4.22 <sup>c</sup>	5.02 <sup>e</sup>

Table 5. Cont.

<sup>1</sup> DM—dry matter; <sup>2</sup> NDF—neutral-detergent fiber; ADF—acid-detergent fiber; ADL—acid-detergent lignin; HCEL—hemicellulose; CEL—cellulose; a, b, c, . . . —averages marked with different letters are statistically significantly different according to the *t*-Tukey test at a significance level of p = 0.05 (for all columns separately).

## 4. Discussion

The nutritional value of pet food is becoming an increasingly important consideration for pet owners [3]. Moreover, the pet food market is undergoing a transformation due to the growing popularity of functional foods, which aim to provide health benefits that extend beyond basic nutrition. Such products frequently comprise probiotics and prebiotics, polyunsaturated fatty acids, herbs, and extracts, which are designed to enhance immune function, digestive health, or skin and coat condition [43]. The function of complete pet food is to provide the requisite quantity of essential nutrients for the maintenance of optimal animal health. In order to fulfil this function, manufacturers of this type of food must adhere to the FEDIAF nutritional guidelines [36], which define the minimum recommended levels (MRLs) for key nutrients and represent best practice in food labeling [37].

The nutritional value of insect-based dog food is supported by scientific evidence indicating that insects provide essential amino acids and other nutrients that are beneficial to canine health. As evidenced by the findings of Bosch et al. [44], the quality of insect protein can be on par with or even exceed that of traditional protein sources such as fish meal. Additionally, the digestibility of organic matter derived from insects has been shown to reach as high as 90% [45].

The incorporation of insects into dog food not only fulfils their protein requirements, which vary depending on life stage and activity level, but also provides a balanced alternative to conventional meat sources [46]. This is particularly important in the context of growing concerns about the environmental impact of conventional animal husbandry, which contributes significantly to greenhouse gas emissions [47,48]. The quality, quantity, and source of protein in a dog's diet can affect digestibility and assimilability [49–51]. Plant-based protein sources often do not provide adequate levels of some exogenous amine acids, requiring additional supplementation to meet the basic nutritional needs of dogs [52]. A deficiency or excess in this nutrient is deleterious to health. While the FEDIAF nutritional guidelines [36] do not specify an upper limit for this component in the diet, studies indicate that consuming high levels of protein with an incomplete amino acid profile may pose health risks [53]. These risks include structural protein damage, impaired neurotransmission, and reduced coat quality. The protein derived from insects approved for use in animal feeds is a valuable dietary component for animals due to its adequate content of essential amino acids for humans [54]. The protein content of the products under examination in this study is consistent with that observed in other extruded dry foods [55,56]. The conversion factor most commonly used for protein determination in this study was found to overestimate the protein content of insects. This method is based on the estimation of the total nitrogen (N) concentration, which is converted into protein by multiplying it by the nitrogen-to-protein conversion factor (N-factor) for meat, which is typically set at 6.25. Other authors have highlighted that the prior removal of non-protein fractions rich in nitrogen can influence the final result, thereby providing a more accurate representation of the actual protein level [57–59]. Alternative ratios, such as 4.76 or 5.60, have been proposed [60], but the most widely accepted method for calculating protein content in feeds and pet food remains the use of a conversion factor of 6.25.

Insects allowed for consumption by pets can not only provide high levels of valuable protein but also fat. The quality of fat from *Alphitobius diaperinus*, estimated using the AI (atherogenic index) and TI (thrombogenic index), indicates values of 1 [61]. The consequences of an abnormal fat diet in dogs can be complex. Dogs can tolerate a fairly wide range of dietary fat content, from 5% to as high as 66% by dry weight [62], without causing diarrhea. The foods tested for fat content fit within this range. The type of fat contained in the food is also of key importance. Deficiencies in exogenous fatty acids for puppies (linoleic, arachidonic,  $\alpha$ -linolenic, eicosapentaenoic, and docosahexaenoic acids) or adult dogs (linoleic acid) can lead to skin problems and poorer coat quality, impaired learning ability, or susceptibility to infection [63–65]. However, insects have a specific fat profile consisting of capric, lauric, myristic, and palmitic acids, among others [23,61,66]. A more comprehensive analysis in this direction could show whether the use of insects in different forms (larvae or meal) is reflected in the levels of these acids in the product. The advantage of the fat profile of insects is that it can be regulated through the use of an appropriate nutrient solution [67], so insects can be a valuable source of n - 3 and n - 6 essential fatty acids.

The digestibility of diets containing insects may be a cause for concern due to the presence of chitin, a biopolymer that forms a structural component in the exoskeletons of arthropods, such as insects and crustaceans, as well as the cell walls of fungi. It is a long-chain polymer known for its elasticity. One of the primary concerns regarding the ingestion of chitin by canines is its digestibility. Dogs, being primarily carnivores, lack the enzymes required for the breakdown of components such as chitin in their digestive tract. The active enzyme (chitinase) that breaks down chitin is primarily present in omnivorous animals [68]. This indicates that dogs may encounter difficulties in digesting it effectively. Given its insolubility in water, chitin may serve as a fermentable component for the resident gastrointestinal microbiota, potentially leading to alterations at the bacterial genus level [69,70]. The diversity of the microbiota that inhabits the gastrointestinal tract is determined by a number of factors, including the animal's health status, its living environment, and its level of stress. However, the most significant factor influencing this diversity is the type and quality of nutrients provided in the diet [71,72]. The content of ingredients in the diet that are resistant to digestion and can be broken down by microorganisms represents a significant factor influencing dietary variation. The term "crude fiber" is used on nutrition labels to describe the portion of ingredients that are not completely broken down by the dog's digestive enzymes. Such components may include plant matter and, in some cases, insects that contain chitin. However, the method of determining crude fiber content does not provide comprehensive information regarding the content of indigestible carbohydrates [39]. It is notable that food products that claim an approximate level of crude fiber on their packaging may in fact have significantly different dietary fiber contents. This is evidenced by the results of the analyses conducted in this study, which revealed a discrepancy of up to 19.21 g between the NDF fraction (24.77 g/100 g DM) and the crude fiber content (5.56 g/100 g DM) in one particular food (DIF\_3). This study shows that limiting the chemical analysis to the basic composition, which shows the amount of crude fiber and not the dietary fiber fraction, can lead to misinformation about the content of indigestible ingredients. In particular, products containing edible insects may not only contribute high quality protein and fatty acids to the diet but may also contribute to reduced digestibility of the diet through the presence of chitin.

Canine diets deficient in indigestible carbohydrates may predispose animals to gastrointestinal disorders, including constipation. Conversely, excessive intake of these carbohydrates can precipitate diarrhea and increased gut fermentation [73]. However, the specific effects of dietary fiber fractions on the gastrointestinal tract are contingent upon their inherent composition and the proportion of each fraction present in the diet. In order to more accurately estimate the effect of resistant ingredients on digestion, it is necessary to conduct a more detailed analysis of the dietary fiber fractions in order to determine their content and type with greater precision. The fraction with the highest content is NDF, which is a combination of hemicellulose, cellulose, and lignin, among other components [41]. The ADF fraction enables the determination of the cellulose and lignin contents as well as the amount of lignin present in the ADF fraction. Hemicellulose is a complex polysaccharide that is more readily fermented by intestinal bacteria than cellulose [74]. The incorporation of hemicellulose into the diet of canines may confer a number of advantages, including a prebiotic effect that stimulates the growth of a beneficial gut microbiota. Subsequently, the production of short-chain fatty acids (SCFAs), which serve as an energy source for colonocytes, occurs as a result of the fermentation process [75].

The insoluble but fermentable fractions like cellulose and hemicellulose can serve as substrates for bacterial fermentation in the intestines and may also facilitate the restoration of the intestinal mucosa in dogs [74,76]. Due to its ability to retain water within the feces, cellulose has the potential to affect fecal consistency, thereby preventing constipation. However, an excess of this component in the diet can lead to increased gas production, resulting in bloating and diarrhea. Individuals who have previously consumed predominantly low-carbohydrate foods may experience digestive discomfort as a result of the high levels of indigestible carbohydrates present in their current diet [77]. This results in an inefficient fermentation process due to a mismatch in the gut microbiota. Lignin, which is not a fermentable fraction, has been demonstrated to facilitate fecal formation and accelerate the passage of contents through the gastrointestinal tract [76]. The digestible carbohydrates (NFE) present in extruded foods typically reach levels of around 40-60 g/100 gDM [78,79], which is consistent with the results obtained, where the average NFE content was 51.33 g/100 g DM. These carbohydrates serve as a source of readily available energy. The elevated level is attributable to the necessity of incorporating plant-based components that ensure the product possesses optimal organoleptic characteristics. The long-term consumption of a diet with a high proportion of digestible carbohydrates has been linked to insulin resistance and an elevated risk of obesity in canines, as evidenced by studies [80–82].

The presence of crude ash confirms the mineral content present in the product, as it is the inorganic residue from the complete combustion of organic matter. Foods with a high ash content can lead to urinary tract problems in animals due to the crystallization of the minerals contained [83]. In pet foods containing protein from slaughter animals, the PS level in 100 g DM averages about 8 g of ash [84], and the foods analyzed had an average of 6.33 g/100 g DM. Components derived from animals other than insects, such as animal by-products, were not used in the tested foods, a definition that includes bones, which can be a rich source of minerals. Their addition may be justified in order to balance the calcium or phosphorus content. Both of these elements in pet foods are sometimes incorrectly balanced in terms of content and/or ratio [85,86]. A calcium deficiency in the diet can translate into skeletal problems by drawing calcium from its largest stores, the bones and teeth. As a result, their structure is weakened, but its short-term low levels in the diet do not result in a decrease in serum calcium in adult dogs [87].

It is recommended that the calcium/phosphorus ratio should not exceed 2:1, although a ratio closer to 1:1 is considered to be optimal [36]. The balance between calcium and phosphorus is regulated by hormones such as parathormone and vitamin D. It is essential to maintain a proper balance between these two minerals in order to prevent conditions such as secondary hyperparathyroidism, which can occur when dietary phosphorus levels exceed calcium levels [88]. The majority of the foods analyzed exhibited a disturbed ratio, with the majority having a ratio of less than 1:1.

The label appearing on the packaging of a nutritional product performs several key functions, which are essential for consumers to make informed decisions about the feeding of their animals. First and foremost, labels provide detailed information on ingredients and nutrient content [37]. The appearance of information conveyed by the label is defined by a series of guidelines, which emphasize that the message should be clear to the consumer and not misleading and that the information provided must be reliable, truthful, and honest. By information of the nutrient content, it is possible to confront the actual content with the composition communicated to the consumer, taking into account the tolerance range for the content of individual ingredients as defined by Regulation 767/2009 [38]. In the tested foods, not all nutrients were within the specified tolerance range. Particularly, often out of the tolerance range was fat, which was below the tolerance range in twelve out of fourteen foods.

Unfortunately, despite the growing popularity of insect protein-based diets, there are currently few comprehensive studies to verify their nutritional value with the FEDIAF nutritional guidelines [36] and label claims. In addition, it would be worthwhile to test such products for the presence of DNA from other animals due to the possible contamination associated with adjacent production lines in processing factories as well as the greater availability of typical animal proteins, which form the basis of the ingredient composition of most diets on the market. The issue of the presence of undeclared foreign proteins in animal feed is being addressed, but there is a lack of research on relatively new products such as insect feed.

#### 5. Conclusions

The use of dog foods with protein derived from insects can provide an alternative to foods with traditional animal proteins. In both cases, however, it is crucial to balance the food in terms of essential nutrients. According to the recommendations, adult dogs should receive a minimum of 18 g of protein per 100 g of dry matter. All analyzed foods contained more than 18 g protein, but in three foods, this level exceeded the tolerance range for the label value calculated according to Regulation 767/2009 [38]. It is of the utmost importance to maintain protein levels in accordance with the specifications outlined on the product label in order to comply with the regulatory framework set forth in Regulation 767/2009 [38]. Protein content consistent with the tolerance range can be achieved through the implementation of frequent quality control assessments.

In contrast, the minimum recommended amount of fat is 5.5 g per 100 g dry weight of the food. Although all foods met this requirement, in eleven of them, the fat content was below the permitted tolerance. It would be beneficial to consider additional sources of fat, such as insect-derived fats, to fully realize the potential of these alternative sources of nutrients. The crude fiber content could only be compared with the data on the label, as there are no recommended amounts for this ingredient. In three cases, the crude fiber level was above the permitted tolerance, and in five cases, it was below the range. The crude ash content was out of tolerance in nine foods. The calcium/phosphorus ratio was abnormal in twelve foods. In addition, in four foods, the metabolizable energy value was below the tolerance range for the product label value. The highest digestion-resistant carbohydrate content was almost 25 g per 100 g dry weight of food.

The analysis shows that, although insect-protein dog foods can be an interesting alternative to traditional products, they require a careful balance of nutrients. The addition of insect protein, despite its potential gastrointestinal benefits (due to chitin), also carries the risk of higher indigestible carbohydrate content, which may not be fully detected with current crude fiber determination methods. In addition, although the protein and fat in all foods met the minimum dietary recommendations, non-compliance with the tolerance range indicates the need for better quality control. An abnormal calcium/phosphorus ratio, which is crucial for bone health and hormonal balance, is also a problem. Although insect proteins can be a valuable source of nutrients, it is necessary to take into account the indigestible components present in them, and a more detailed chemical analysis of the products is needed to better understand the health effects that a high supply of digestion-resistant components can cause.

Insect-based proteins show promise as a sustainable protein source for pet foods, but they require better quality control and tailored nutrient adjustments. Further comprehensive chemical analyses are recommended to enhance understanding of the health effects of digestion-resistant components and to optimize the nutritional profile for canine health. This study highlights the need for further refinement in formulation and testing practices to fully realize the potential of insect-based pet foods as balanced dietary options.

**Supplementary Materials:** The following supporting information can be downloaded at https://www. mdpi.com/article/10.3390/app142210258/s1, Table S1. The ingredient list of foods as given on the product label. Author Contributions: Conceptualization, W.J. and W.B.; methodology, W.B. and K.Z.; software, W.B.; validation, W.J., W.B. and K.Z.; formal analysis, W.J. and W.B.; investigation, W.J. and K.Z.; resources, W.B.; data curation, W.B.; writing—original draft preparation, W.J. and W.B.; writing—review and editing, W.J. and W.B.; visualization, W.J.; supervision, W.B.; project administration, W.J.; funding acquisition, W.B. and K.Z. All authors have read and agreed to the published version of the manuscript.

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