

Article

Not peer-reviewed version

---

# Chemical Evaluation of an Experimental Dog Cooked Feed

---

[Vasileios V. Paraskeuas](#)\*, [Aikaterini V. Kouvatsou](#), [Evangelos C. Anagnostopoulos](#)

Posted Date: 7 November 2025

doi: 10.20944/preprints202511.0492.v1

Keywords: dog nutrition; cooked feed; balanced diet; chemical evaluation



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

# Chemical Evaluation of an Experimental Dog Cooked Feed

Vasileios V. Paraskeuas \*, Aikaterini V. Kouvatso and Evangelos C. Anagnostopoulos

Laboratory of Nutritional Physiology and Feeding, Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

\* Correspondence: v.paraskeuas@aua.gr; Tel.: +30 2105294460

## Abstract

Balanced nutrition plays a crucial role in maintaining canine health and preventing developmental disorders. Commercially available diets for dogs mainly include dry, wet, and semi-moist formulations. In addition to these, alternative feeding practices such as BARF (Biologically Appropriate Raw Food) and cooked diets are gaining popularity. However, the primary concern with such alternative regimens is ensuring nutritional balance. In this study, we evaluated a cooked chicken-based diet produced on a HACCP-certified production line, formulated for adult, sterilized dogs of approximately 20 kg body weight (no breed specification). A comprehensive chemical evaluation was performed, including analyses of Protein, Fat, Fiber, Dry Matter, Moisture, Ash and Organic Matter, also Vitamins, Fatty Acids and mineral content in the experimental diet. The results revealed that the majority of nutrients met the European Pet Food Industry Federation (FEDIAF) minimum requirements for adult dog maintenance. However, some important micronutrients such as Vitamins A and D were unable to be determined and elevated choline values were observed. These findings indicate that adjustments in formulation are necessary to achieve optimal nutrient composition and ensure that cooked diets can provide a safe and balanced alternative for canine nutrition. Thus, important methods of specific micronutrients determination should be improved.

**Keywords:** dog nutrition; cooked feed; balanced diet; chemical evaluation

## 1. Introduction

Adequate intake of essential macronutrients and micronutrients ensures optimal physiological development, while imbalances may predispose dogs to a variety of metabolic and skeletal disorders, including obesity, osteodystrophy, and cardiovascular dysfunction [1]. As companion animals are increasingly regarded as integral members of the family, nutritional management has become a central aspect of preventive veterinary medicine. This shift underscores the need for scientifically grounded dietary strategies that align with recognized nutritional standards established by organizations such as the Association of American Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF).

The commercial pet food market provides a wide spectrum of dietary options designed to meet the nutritional demands of dogs across different life stages and physiological conditions [2]. Diets are typically available in dry, wet, or semi-moist forms and are further categorized into economy, grocery-mainstream, science-based, low-grain, grain-free, natural, and prescription formulations. Each category differs in nutrient density, ingredient composition, and intended purpose, reflecting both scientific developments in companion animal nutrition and consumer preferences [3]. Advances in formulation technologies and quality control have improved the consistency and safety of commercial diets, ensuring that most dogs receive complete and balanced nutrition when fed according to manufacturer guidelines [4].

Parallel to the development of commercial diets, alternative feeding practices such as the BARF (Biologically Appropriate Raw Food) diet and home-prepared cooked meals have gained substantial

popularity in recent years [5]. Advocates of these regimens often emphasize the perceived benefits of feeding natural, minimally processed ingredients, suggesting improvements in coat condition, digestive health, and vitality. This trend mirrors a broader cultural movement toward “natural” and personalized nutrition within human dietary behavior. However, despite their growing acceptance among pet owners, these diets remain controversial within the veterinary and scientific communities due to concerns about their nutritional adequacy and safety.

The principal challenge associated with non-commercial feeding regimens is the assurance of nutritional balance and microbial safety. Studies have demonstrated that many home-prepared or raw diets fail to meet established nutrient recommendations, often resulting in deficiencies or excesses that can compromise canine health [6]. Therefore, the evaluation of these feeding practices through evidence-based research is essential to determine their efficacy, safety, and long-term effects on canine development and disease prevention. For these reasons, the present study aimed on the design and production of a cooked experimental diet for adult, sterilized dogs of approximately 20 kg body weight (no breed specification) and its analytical chemical evaluation, to confirm the formulation process and to identify the deviations of macro and micro nutrient amounts compared to the respective FEDIAF guidelines in order to ensure the balance of the experimental diet.

## 2. Materials and Methods

The experimental diet was formulated using the Pet Diet Designer software (PetDietDesigner®, version 88.3), which allows precise calculation of nutrient composition based on ingredient selection and target nutritional requirements for canine maintenance. The formulation process aimed to achieve a balanced nutritional profile in accordance with FEDIAF (2025) minimum recommendations for adult dogs’ maintenance. Additionally, a premix (Starmix, Astron Chemicals, Greece) was incorporated into the formulation to ensure proper nutritional balance and adequate levels of vitamins and trace elements in the feed. The complete list of ingredients and their respective proportions is presented in Table 1.

**Table 1.** Ingredients and their proportions of the experimental diet per 100g.

Ingredients	Proportions per 100g
Ground Chicken	38.46
Chicken Gizzards	7.88
Rice Parboiled	15.77
Carrot	9.85
Spinach	7.88
Liver /Hearts	9.85
Apple	7.88
PreMix* <sup>1</sup>	0.98
Yeast	0.59
Fish Oil	0.29
Inulin	0.39
Parsley	0.09
Sunflower Oil	0.09

<sup>1</sup>Premix (Starmix, Astron Chemicals, Greece) provided the following per kg of diet: vitamin D3 (cholecalciferol), 25,000 IU; vitamin E (dl-  $\alpha$ -tocopheryl acetate), 2500 mg; vitamin B1, 50 mg; vitamin B2, 160 mg; vitamin B12 (cobalamine), 1.50 mg; Ferrous, 300 mg; Zinc, 2.090 mg; Copper, 240 mg; Manganese, 200 mg; Iodine, 70 mg; Choline, 31200 mg; Citric acid, 40 mg; BHT (Butylated Hydroxytoluene), 80 mg; N-propyl gallate, 40 mg. Also, Dicalcium Phosphate, Calcium Carbonate, Lignocellulose, Potato Starch, Potassium Chloride, Sodium Chloride, Magnesium Oxide.

The production line operated in compliance with the Hazard Analysis and Critical Control Points (HACCP 384Y3EL25PPE) system, ensuring traceability, safety, and quality control throughout all stages of feed preparation and packaging and was held at the premises of NOMA company (NOMA, Fresh meat -Fresh meal, PL, Athens) which specializes in commercial homemade type feed production for pets. The production of freshly cooked pet meals follows a controlled, human-grade process protocol designed to ensure both nutritional integrity and microbiological safety. The procedure consists of the following main stages (Table 2).

**Table 2.** Production Process.

<i>Preparation of Raw Materials:</i>	Fresh fruits, vegetables, and meats are manually inspected and cut into appropriate sizes according to the recipe formulation.
<i>Cooking:</i>	Ingredients are cooked in tilting kettles at approximately 82 °C, ensuring sufficient heat treatment to eliminate potential pathogens while minimizing nutrient degradation.
<i>Rapid Cooling:</i>	Immediately after cooking, the food undergoes rapid chilling to 0–4 °C using a shock freezer, to prevent bacterial growth and preserve product quality.
<i>Fortification:</i>	Once the mixture reaches the target cooling temperature, a premix of vitamins and minerals—formulated to meet FEDIAF nutritional guidelines—is added and thoroughly blended.
<i>Packaging:</i>	The finished product is portioned and sealed in vacuum pouches, maintaining freshness and extending refrigerated shelf life without the use of preservatives.

The proximate composition of the final diet was determined using the Weende analysis (proximate analysis) method, which includes the determination of moisture, crude protein, crude fat, crude fiber, crude ash, and nitrogen-free extract (NFE) contents. All analyses were conducted in triplicate for each of the ten samples (n=10) according to standard AOAC procedures (AOAC, 2019). Crude protein was determined as  $6.25 \times$  Kjeldahl nitrogen using a Kjeltac 2300 analyser unit (Foss Tecator AB, Höganäs, Sweden) according to AOAC 2001.11 and 992.15 [7]. Fat content was determined according to AOAC Official Methods 2007.04 and 960.39 using a Soxhlet extractor. Ash content was measured according to AOAC 942.05 by incinerating the samples at 600 °C for 5 hours in a muffle furnace, while dry matter content was evaluated according to AOAC 934.01 by drying the samples at 100 °C for 24 hours in a drying oven.

For the quantitative and qualitative analysis of biochemical components, validated and standardized high-precision methodologies were employed. Starch content was determined using the Megazyme Starch Assay Kit, following the manufacturer's instructions, based on enzymatic hydrolysis and spectrophotometric detection of the released glucose. Vitamins were analyzed using High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), enabling simultaneous and sensitive determination of both water- and fat-soluble vitamins. Trace elements and minerals were quantified by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) in accordance with the CYS EN 16943:2017 standard,

ensuring high reproducibility and analytical accuracy. Finally, amino acid analysis was performed using an in-house method based on the UPLC Amino Acid Analysis Solution System Guide (71500129702, Revision), which allows for precise detection and quantification of both free and bound amino acids through pre-column derivatization and ultra-performance liquid chromatography.

### Statistical Analysis

The experimental data (n=10 samples from the same diet) were subjected to descriptive statistics to obtain the means and their standard deviations by using the SPSS for Windows Statistical Package Program (SPSS Inc., Chicago, IL). The normality of the experimental data was checked using the Kolmogorov–Smirnov test. and was found to be normally distributed. Statistical significance of the 2-tailed p values was declared at (P< 0.05).

## 3. Results

Descriptive statistics, expressed as mean  $\pm$  standard deviation, along with asymptotic significance values (2-tailed p values < 0.05) are presented in Table 3 for the main macronutrients and in Table 4 about the micronutrients (vitamins and minerals), omega-3 and omega-6 fatty acids and amino acids of the examined feed, respectively. No statistically significant differences were detected between repetitions for any of the measured variables (p > 0.05), indicating that the data were normally distributed. These findings thus provided a robust basis for subsequent analyses. However, Vitamin A and Vitamin D were not detected with HPLC and LC-MS/MS methods which were used for their determination in the present study.

**Table 3.** Main macronutrients values and their statistical analysis in the examined diet.

Ingredients <sup>1</sup>	Mean <sup>2</sup>	St. deviation <sup>2</sup>	p-value <sup>3</sup>
Protein content	35.09	0.193	0.965
Fat	6.45	0.114	0.993
Fiber	3.18	0.180	0.956
Dry Matter	31.00	0.147	0.766
Moisture	69.04	0.557	0.691
Ash	4.42	0.183	0.932
Organic Matter	95.58	0.126	0.874
Starch	17.20	0.077	0.945

\* <sup>1</sup> n=10 feed samples used. <sup>2</sup> Descriptive statistics (mean and standard deviation) <sup>3</sup> Asymptotic Significance 2 Tailed P Value (Test Distribution Is Normal When P<0.05).

**Table 4.** Evaluated micronutrients (vitamins, omega-3 and omega-6 fatty acids, minerals and amino acids) and their statistical analysis in the experimental diet.

Ingredients <sup>1</sup>	Mean <sup>2</sup>	St. deviation <sup>2</sup>	p-value <sup>3</sup>
<b>Vitamins</b>			
Vitamin A (IU/ 100g D.M.)	ND <sup>4</sup>	-	-
Vitamin D (IU/ 100g D.M.)	ND	-	-
Choline (mg/100 g D.M.)	1020.1	2.17	0.315
<b>Fatty acids</b>			
$\omega$ 3: EPA+DHA (g/100g D.M)	0.45	0.033	0.520
$\omega$ 3: Alpha - Linolenic acid (g/100g D.M)	0.90	0.048	0.994

$\omega$ 6: Linoleic acid (g/100g D.M)	9.33	0.119	0.986
$\omega$ 6: Arachidonic acid (g/100g D.M)	0.64	0.063	0.492
<b>Minerals</b>			
Calcium (Ca) (g/ 100 g D.M.)	0.84	0.061	0.998
Phosphorus (P) (g/ 100 g D.M.)	0.78	0.074	0.888
Magnesium (Mg) (g/ 100 g D.M.)	0.94	0.066	0.802
Potassium (K) (g/ 100 g D.M.)	0.86	0.051	0.884
Sodium (Na) (g/ 100 g D.M.)	0.31	0.025	0.548
<b>Microminerals</b>			
Copper (Cu) (mg/ 100 g D.M.)	0.84	0.053	0.744
Zinc (Zn) (mg/ 100 g D.M.)	12.52	0.066	0.819
Ferrum (Fe) (mg/ 100 g D.M.)	10.50	0.087	0.942
<b>Amino Acids</b>			
Lysine (g/100 g D.M.)	2.48	0.074	0.907
Arginine (g/100 g D.M.)	1.84	0.097	0.716
Tryptophane (g/100 g D.M.)	0.70	0.064	0.847
Methionine (g/100 g D.M.)	1.35	0.099	0.989

\* <sup>1</sup> n=10 feed samples used. <sup>2</sup> Descriptive statistics (mean and standard deviation) <sup>3</sup> Asymptotic Significance 2 Tailed P Value (Test Distribution Is Normal When P<0.05. <sup>4</sup> Not detected.

#### 4. Discussion

The present study evaluates the proximate composition, vitamin and mineral profile, and overall nutritional adequacy of the experimental cooked canine diet in comparison with the reference minimum nutrient requirements of FEDIAF (2025) guidelines for adult dog maintenance. Particular emphasis is placed on the balance of macronutrients (protein, fat, fiber, and starch) and the adequacy of key micronutrients and trace elements, as these parameters collectively determine the diet's suitability for adult maintenance. The findings are interpreted within the context of established nutritional requirements for dogs, aiming to elucidate potential strengths and limitations of the formulation, as well as to provide insights for its optimization in future applications. It has to be highlighted that, although the levels of organic matter, ash, and moisture were determined during the proximate analysis, these parameters are not presented in detail in this discussion, as the FEDIAF (2025) nutritional guidelines do not provide specific maximum reference values for them. However, for all measurements dry matter was used to express all the determined values on dry matter (DM) basis, ensuring that they can be compared in the same unit of measurement with the respective minimum nutrient requirements of FEDIAF guidelines for adult dogs in maintenance nutritional period.

### *Proteins*

Protein is a fundamental macronutrient in canine diets, essential for tissue maintenance, muscle development, enzymatic activity, and overall metabolic balance [8]. In the present analysis, the crude protein content of the experimental diet was 35.09% DM, higher than the FEDIAF (2025) recommended minimum level of 18% DM for adult maintenance diets. This elevated value likely reflects a high inclusion level of animal-derived protein sources, contributing to excellent amino acid availability and supporting lean muscle mass. Most commercially available adult dog foods offer protein levels higher than this minimum, often from 25% to 35% of crude protein on dry matter (D.M.) basis. In the present study nutrient content resulted at 35.90% on DM basis, and was higher enough than the recommended FEDIAF concentration (18%) for adult dog maintenance. This result means that the dog will get more protein than the minimum nutritional requirements. This nutritional handling is explained mainly due to the need to ensure that the dietary protein levels will meet the needs of a wide range of dogs with genetic variation and different activity levels [9]. Moreover, by meeting minimum requirement crude protein levels it is not ensured that all the essential amino acids are provided in the optimum concentrations [10]. For this reason, the high overall percentage of crude protein in dog diets helps to meet all the essential amino acid needs.

### *Fat*

Dietary fat provides a concentrated source of metabolizable energy and supplies essential fatty acids necessary for maintaining skin health, coat quality, cell membrane integrity, and hormonal function [5]. In this study, the crude fat content was 6.45% DM, and it is almost near the minimum FEDIAF requirements for fat (5.5% DM) for adult dog maintenance and below the respective minimum limit for puppies which is 10.25% of DM. Such a lipid level ensures energy density without predisposing to obesity or excessive caloric intake. Considering the relatively high protein content of the formulation, the fat proportion appears balanced, contributing to a favorable protein-to-energy ratio.

### *Fiber*

In recent years, substantial attention has been devoted to understanding the influence of dietary fiber on nutrient utilization and gastrointestinal health and function. Although dogs do not have a specific dietary requirement for fiber, moderate inclusion can improve gastrointestinal motility, fecal consistency, and satiety [11]. The measured crude fiber concentration in the current analysis was 3.18% DM, which remains well and meets the FEDIAF minimum recommendation range from 2 to 5% DM. This value indicates a moderate fiber content, likely derived from vegetable ingredients or grains incorporated into the formulation. Such a level supports digestive health without compromising nutrient digestibility, making it appropriate for adult dogs in maintenance. Excessively high fiber inclusion could reduce nutrient absorption, whereas very low fiber diets might affect stool quality—thus, the present value reflects a well-balanced approach.

### *Starch*

Starch serves as the principal carbohydrate and an important energy source in canine nutrition. When properly processed (gelatinized), it is efficiently digested, but excessive inclusion may alter glycemic response or displace essential macronutrients [12]. In this study, the starch content was 17.2% DM, which is well below the FEDIAF limit of 30–40% DM. This indicates a low-to-moderate carbohydrate level, consistent with a protein-rich formulation emphasizing animal-based ingredients. Such a composition may enhance metabolic stability, reduce postprandial glycemia, and improve nutrient digestibility, aligning with nutritional strategies aimed at optimizing canine health and maintaining ideal body condition.

**Table 5.** Proximate composition of the experimental canine diet compared with FEDIAF adult dog maintenance reference values.

Ingredients	Measured Values	Reference minimum Values (FEDIAF)
Proteins	35.09% D.M.	18% D.M.
Fat	6.45% D.M.	5.5% D.M.
Fiber	3.18% D.M.	2-5% D.M.
Starch	17.2% D.M.	30-40% D.M.
Dry Matter	31.0%	-
Moisture	69.0%	-
Ash	4.42% D.M.	-
Organic Matter	95.58% D.M.	-

#### *Vitamin A*

In recent years, many researchers have emphasized the pivotal role of vitamins. Among them, Vitamin A is considered by many animal nutritionists to be the most vital vitamin for cats and dogs, as dogs possess only a limited capacity to convert plant carotenoids such as  $\beta$ -carotene into vitamin A, while cats lack this ability entirely, in contrast to other animals such as pigs, poultry, ruminants, and wild species [13]. Vitamin A is involved in vision, has an immunoregulatory role and also participates in cellular differentiation and animal reproduction [14]. In dogs' diets, feed sources such as liver, eggs, and fish oil, and plant-based sources like carrots, sweet potatoes, pumpkin, and spinach, or dietary vitamin premixes provide the competent and necessary amounts of Vitamin A for meeting their nutritional requirements and ensuring animal health and wellbeing [15]. In this work, Vitamin A amounts were not detected with HPLC and LC-MS/MS methods due to the fact that these methods, as it is reported by FEDIAF guidelines, measure free retinol which is a form of Vitamin A, while in cooked food, Vitamin A is found as retinyl esters. The retinyl esters are formed when a fatty acyl group is esterified to the hydroxyl terminus of retinol that is a storage form of retinol [15]. Considering the above, more research is needed in order to determine the total amounts of Vitamin A in the experimental diet.

#### *Vitamin D*

Vitamin D, is well known for involvement in calcium and phosphorus body absorption and has an essential role on skeletal health. The amount of Vitamin D depends on the levels included in the Vitamin Premix or the endogenous Vitamin D content of feed ingredients and as a result, their variation in the feed analysis might be large between recipes [16]. Moreover, the optimum ratio of Vitamin A to Vitamin D levels (10:1) is vital for the immune response and skeletal growth and as result for the maintenance of dog's general health [17]. In the present study Vitamin D amounts were also not detected by the aforementioned methods for Vitamin A. In FEDIAF guidelines it has been reported that Vitamin D analysis of pet foods is difficult and unreliable. That is because, the detection limit for HPLC methods is approximately 300 to 500 IU/100 g on a DM basis and the FEDIAF minimum nutrient guidelines for adult maintenance dogs are 55.2 IU – 63.9 IU 100 g DM (Table 6). It is highlighted by FEDIAF guidelines that chemical analysis is not required if supplementation is practiced. In the experimental diet despite of Vitamin D presence in dietary ingredients (i.e. carrots), vitamin D was also added in the form of cholecalciferol in Premix at the concentration of 25,000 IU (Table 1). However, more research has to be occurred in order to evaluate the accurate amounts of Vitamin D in diet.

### Choline

Choline is an essential nutrient for dogs, involved in metabolism of phospholipids as a precursor of acetylcholine and also plays a key role in lipid metabolism due to its ability to hinder the abnormal accumulation of fatty acids in liver tissues resulting in prevention of hepatic diseases such as steatosis [18]. The dietary supplementation of choline in dog diets is commonly in the form of choline chloride although due to some of its properties this compound is highly hygroscopic and it is difficult to remain stable in pets feed production. In order to ensure its absorption, other sources of choline in dogs' diets are commonly used such as dairy products, meat and eggs [19]. The nutritional requirements of choline for adult dogs range from 164 to 189 mg/100 g of DM [20]. In the present study the high percentage of meat in the recipe (56.19%, ground chicken, gizzards, liver and hearts) and especially choline chloride high levels (31.200 mg) in the Vitamins and Minerals Premix resulted in higher level of choline (1020.1 mg) in the diet compared to FEDIAF's nutrient requirements limitations. The experimental diet must be corrected mainly by reducing choline chloride in the Vitamins and Minerals Premix which is important due to the negative effects of choline excess which are vomiting, low blood pressure, and mainly liver fat issues [21].

### Omega 3 and Omega 6

In the past years, omega-3 fatty acids have been supplemented mainly in veterinary pet medicine for pets to control immune response linked with pruritic disease which is a disease associated with skin irritation and itchy sensation that increases the young and aged dogs the desire to scratch [22]. Omega-3 fatty acids addition in pet nutrition, triggers the production of eicosanoids with lower inflammatory potential than those produced by omega-6 fatty acids and as a result reduce the negative effects on skin by pruritic disease [22]. A possible way to control this mechanism is the regulation with nutritional interventions of omega-6:3 fatty acids ratio in pet diets. In bibliography there are experimental indices which have shown that feeding dogs with a diet with omega 6:3 ratios is optimum at 5:1 due to its positive effects on inflammatory response by neutrophils and skin of the dogs compared to four diets with 10:1, 25:1, 50:1 and 100:1 omega-6:3 ratios [23]. In the present study, chemical analysis showed (Table 6), that omega-6:3 ratio has been measured closely to 7:1 (7.23:1) in order to reach as close, it could be the optimum 5:1 ratio to avoid the aforementioned possible skin problems.

**Table 6.** Detailed nutrient profile of the experimental canine diet and its comparison with FEDIAF minimum adult dog maintenance nutritional guidelines.

Ingredients	Measured Values (units, on a dry matter basis)	Reference minimum Values (FEDIAF)
<b>Vitamins</b>		
Vitamin A	N. D.	606 IU-702 IU /100 g D.M.
Vitamin D	N. D.	55.2 IU – 63.9 IU 100 g D.M.
Choline	1020.1 mg/100 g D.M.	164 - 189 mg/100g D.M.
<b>Fatty Acids</b>		
ω3: EPA+DHA	0.45 g/100 g D.M.	- no limits for adult dogs
ω3: Alpha - Linolenic acid	0.90 g/100g D.M.	-
ω6: Linoleic acid	9.33 g/100g D.M.	-
ω6: Arachidonic acid	0.64 g/100 g D.M.	-
<b>Minerals</b>		
Calcium (Ca)	0.84 g/ 100 g D.M.	0.50 - 0.58 g/100g D.M.
Phosphorus (P)	0.78 g/ 100 g D.M.	0.40- 0.46 g/100g D.M.
Magnesium (Mg)	0.94 g/ 100g D.M.	0.7 – 0.8 g/100g D.M.
Potassium (K)	0.86 g/100g D.M.	0.50 – 0.58 g/100g D.M.
Sodium (Na)	0.31 g/ 100g D.M.	0.10 – 0.12 g/100g D.M.

<b>Microminerals</b>		
Copper (Cu)	0.84 mg/ 100 g D.M.	0.72– 0.83 mg/ 100g D.M.
Zinc (Zn)	12.52 mg/ 100 g D.M.	7.2 – 8.34 mg/ 100g D.M.
Ferrum (Fe)	10.50 mg/100g D.M.	3.6 – 4.17 mg/ 100g D.M.
<b>Amino acids</b>		
Lysine	2.48 g/100 g D.M.	0.42 – 0.46 g/100g D.M.
Arginine	1.84 g/100 g D.M.	0.52 - 0.60 g/100g D.M.
Tryptophan	0.70 g/100 g D.M.	0.17 - 0.20 g/100g D.M.
Methionine	1.35 g/100 g D.M.	0.40 - 0.46 g/100g D.M.

### *Minerals*

#### Calcium

Calcium is a vital micromineral in canine nutrition, essential for bone and teeth formation, muscle function, nerve transmission, and blood clotting. Adequate calcium intake is crucial for maintaining these physiological processes and overall health in dogs. With calcium at 0.84 g/100 g DM (vs. FEDIAF recommended adult maintenance minimum 0.50–0.58 g/100 g DM), the formulation provides an elevated but acceptable calcium supply for canine nutrition. Such a level can be justified when formulating for growth, gestation/lactation or when using raw materials with lower calcium bioavailability [24]. should be interpreted alongside dietary phosphorus and the Ca:P ratio to ensure overall balance.

#### *Phosphorus*

Phosphorus is an essential micromineral in canine nutrition, playing a fundamental role in energy metabolism (ATP formation), bone and teeth mineralization, acid–base balance, and cellular signaling [9][25]. Adequate phosphorus intake is necessary for maintaining skeletal health and supporting various metabolic functions. The dietary phosphorus concentration (0.78 g/100 g DM) is above the FEDIAF minimum adult guideline range (0.40–0.46 g/100 g DM). This could be attributed to the limited inclusion of animal-based ingredients, which are rich and highly bioavailable sources of phosphorus, and the predominance of plant-derived components (e.g., cereals, vegetables) that contain phytic acid, known to form insoluble phytate complexes that reduce phosphorus absorption [24]. However, the Ca:P ratio ( $\approx 1.1:1$ ) remained close to the recommended range, suggesting a balanced mineral proportion that may help maintain normal physiological mineral interactions and skeletal homeostasis [20].

#### *Magnesium*

Magnesium is an essential micromineral required for neuromuscular function, enzyme activation, energy metabolism, and bone formation. In canine nutrition, it plays a critical role as a cofactor for over 300 enzymatic reactions and in maintaining electrolyte balance [25]. The dietary magnesium concentration (0.94 g/100 g DM) is approximately 17–18% above the FEDIAF minimum adult guideline limit (0.70–0.80 g/100 g DM). Although this level is unlikely to cause toxicity in healthy adult dogs, it warrants justification in the formulation dossier [9]. On the other hand, elevated dietary magnesium levels, particularly in combination with alkaline urine pH, may predispose to struvite urolith formation [24].

#### *Potassium*

Potassium is a major intracellular cation that plays a critical role in acid–base regulation, osmotic balance, nerve transmission, and muscle contraction [25]. It also supports cardiac function and enzymatic activity, making it an essential micromineral in canine nutrition. The dietary potassium concentration in our study (0.86 g/100 g DM) exceeds the FEDIAF minimum reference range for adult

dogs (0.50–0.58 g/100 g DM). This moderate elevation remains within physiologically safe limits and may reflect the natural mineral contribution of raw materials rich in potassium (e.g., sweet potatoes, carrots, and spinach), or to processing losses during cooking and dehydration [26]. Such levels can support neuromuscular function and electrolyte balance, provided that sodium and chloride contents are appropriately adjusted. However, the obtained level still provides a nutritionally acceptable contribution to maintain electrolyte and cellular homeostasis, especially when sodium and chloride concentrations remain within their normal ranges. Furthermore, the balance between potassium, sodium, and magnesium is essential for maintaining neuromuscular and cardiac stability [24].

### *Sodium*

Sodium is an essential electrolyte that plays a fundamental role in maintaining osmotic pressure, acid–base balance, nerve impulse transmission, and muscle function [25]. It also regulates extracellular fluid volume and contributes to the transport of nutrients across cell membranes. According to the FEDIAF Nutritional Guidelines (2025), the minimum sodium requirement for adult maintenance diets is 0.1–0.12 g/100 g DM. Sodium at 0.31 g/100 g DM is higher than this FEDIAF reference range but remains acceptable within the tolerance limits for canine nutrition. The elevated level likely reflects ingredient composition and contributes to maintaining a balanced Na:K ratio (~1:2.7), supporting hydration and electrolyte stability [26]. Despite being slightly higher the target value, this level is unlikely to cause deficiencies, provided that potassium (0.86 g/100 g DM) and chloride concentrations remain within recommended limits, ensuring proper electrolyte and osmotic balance. Moreover, maintaining a balanced Na:K ratio is crucial for physiological homeostasis, neuromuscular function, and hydration. The ratio observed in this study (approximately 1:3) falls within the acceptable range described by the NRC (2006) and supports adequate electrolyte equilibrium.

### *Microminerals*

#### *Copper*

Copper is an essential trace mineral in canine nutrition, acting as a cofactor in numerous enzymatic systems involved in Ferrum metabolism, connective-tissue formation, pigmentation, and antioxidant defense. Although copper deficiency is uncommon in dogs consuming complete and balanced diets, insufficient levels may lead to anemia, coat depigmentation, bone malformations, and impaired immune responses [10].

Conversely, excessive copper accumulation in the liver can result in copper-associated hepatopathy (CAH), a condition particularly observed in predisposed breeds such as Bedlington Terriers, Doberman Pinschers, and Labrador Retrievers [26]. Diet composition, ingredient selection, and the form of supplemental copper (organic vs inorganic) strongly influence hepatic copper storage and bioavailability. According to FEDIAF's Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs published in July 2025, the minimum recommended level for copper in complete dog foods is defined on a dry matter (DM) basis and manufacturers are also advised not to exceed 0.72–0.83 mg/100g DM (EU legal maximum for additives equivalent) when copper is added as an additive (EU legal maximum for additives equivalent). In this work, Copper (Cu) amount was 0.84 mg/100 g D.M. This result could be explained by the presence of liver, carrots, spinach in the experimental diet and also due to the amount of Cooper in the vitamins and mineral premix.

#### *Zinc*

Zinc is an essential trace mineral in canine nutrition, playing a critical role in numerous biological processes, including protein synthesis, immune system function, wound healing, and the maintenance of healthy skin and coat. Zinc deficiency can lead to dermatological disorders, such as zinc-responsive dermatosis, characterized by hyperkeratosis, ulcers, alopecia, and skin lesions [21].

Excessive zinc intake can cause toxicity, presenting with vomiting, diarrhea, anemia, and liver damage. High zinc levels may also interfere with the absorption of other trace minerals, such as copper, potentially leading to imbalances and hepatic accumulation [27,28]. In this work, Zinc (Zn) amount was 12.52 mg/ 100 g D.M. while the FEDIAF's minimum recommended amount is 7.2 – 8.34 mg/100g D.M. Although the zinc content in the experimental diet exceeds the FEDIAF recommended amount, this can be justified by the dietary copper level, which maintains a Zn/Cu ratio within the safe and physiologically acceptable range. Maintaining a copper-to-zinc ratio close to 1:15 helps prevent trace mineral interactions that could impair copper absorption, while providing sufficient zinc to support skin integrity, enzyme activity, or hepatic accumulation and overall health [27]. The copper-to-zinc ratio (1:14.8) in this formulation falls within the recommended range for canine nutrition according to the FEDIAF (2025) guidelines, ensuring adequate trace mineral balance. Therefore, despite the apparently higher zinc content, the diet composition allows for effective mineral utilization without compromising canine health.

#### *Ferrum*

Ferrum is a vital trace mineral in canine nutrition, essential for oxygen transport via hemoglobin, energy metabolism, and immune function. Adequate Ferrum intake is crucial for maintaining these physiological processes and overall health in dogs. Although the Ferrum content (10.50 mg/100 g DM) is higher than the FEDIAF guideline minimum requirements (3.6–4.17 mg/100 g DM), it falls within acceptable limits for canine nutrition. This concentration supports sufficient Ferrum reserves and contributes to maintaining normal hematological parameters without posing a toxicity risk [24].

#### *Amino Acids*

##### Lysine

The measured lysine concentration (2.48 g/100 g DM) is considerably higher than the FEDIAF (2025) minimum recommended requirements for adult dog maintenance (0.42–0.46 g/100 g DM). This elevation likely reflects the use of animal-based protein sources such as meat meals, fish, or poultry by-products, which are naturally rich in essential amino acids, particularly lysine. Elevated lysine content is generally not detrimental; on the contrary, lysine is a key limiting amino acid in many formulations and plays a crucial role in maintaining nitrogen balance, supporting muscle protein synthesis, and ensuring adequate growth and tissue repair [10]. Moreover, excess lysine is typically catabolized without adverse metabolic effects when the diet maintains adequate arginine levels and balanced amino acid ratios [24]. Therefore, while the observed lysine value exceeds guideline recommendations, it can be considered physiologically acceptable and even beneficial for ensuring amino acid adequacy, provided that the overall protein quality and amino acid balance meet FEDIAF specifications for canine nutrition.

##### *Arginine*

The arginine concentration (1.84 g/100 g DM) exceeds the FEDIAF (2025) minimum recommendation for adult dogs' maintenance (0.52–0.60 g/100 g DM). This elevation is likely due to the use of animal-derived protein sources naturally rich in arginine. Elevated arginine levels are not considered detrimental and may, in fact, be beneficial, as arginine plays a critical role in the urea cycle, nitric oxide synthesis, and immune modulation. The lysine-to-arginine ratio (2.48:1.84  $\approx$  1.35:1) remains within the optimal physiological range (1.2–1.4:1), indicating balanced amino acid composition. Therefore, despite exceeding FEDIAF reference values, the dietary arginine content can be regarded as physiologically appropriate and supportive of metabolic and muscular functions [24].

### *Tryptophan*

The measured arginine content (0.70 g/100 g DM) is markedly above the FEDIAF (2025) reference minimum guidelines for adult dogs' maintenance (0.17–0.20 g/100 g DM). This elevation is most likely attributed to the inclusion of animal-derived protein sources naturally rich in arginine, or to the targeted use of amino acid fortification for metabolic or immune support. Elevated arginine levels are not considered detrimental, as this amino acid plays a pivotal role in the urea cycle, nitric oxide synthesis, and immune modulation. Provided that the lysine-to-arginine ratio remains balanced, the observed concentration can be considered physiologically appropriate and beneficial for maintaining metabolic and vascular health [24].

### *Methionine*

The measured methionine concentration (1.35 g/100 g DM) substantially exceeds the FEDIAF (2025) adult maintenance reference minimum recommended levels (0.40–0.46 g/100 g DM). This elevation is most plausibly explained by the use of animal-derived protein ingredients or targeted supplementation with methionine (or methionine-rich ingredients) during formulation, since methionine is typically abundant in meat and fish sources. Sulfur amino acids (methionine + cystine) are essential for protein synthesis, methylation reactions and for supporting endogenous cysteine and taurine synthesis; therefore, an elevated methionine content can be nutritionally advantageous to ensure adequacy and to spare cysteine under certain formulation scenarios. Excess dietary methionine is not generally associated with acute toxicity in healthy adult dogs, but it increases the dietary acid load (sulfuric acid excretion) and can lower urinary pH — an effect exploited therapeutically (DL-methionine) to reduce struvite formation [24].

In conclusion, the present analysis of the experimental cooked canine diet highlights that while the formulation meets most of the FEDIAF's minimum nutrient requirements for adult dogs' maintenance, some components require adjustment to optimize overall nutritional balance. Protein, fat, fiber, and key micronutrients such as minerals and amino acids were largely within or close to minimum FEDIAF recommended proportions, although choline exceeded the upper limit. Moreover, there was a difficulty in the chemical determination of the fat-soluble vitamins A and D. The  $\omega$ 6:  $\omega$ 3 fatty acid ratio, measured at approximately 7.23:1, approaches the optimal ratio of 5:1 suggested for reducing inflammatory responses and supporting skin health, indicating a largely favorable lipid profile, though fine-tuning could further enhance anti-inflammatory benefits.

These findings indicate the need for further refinement of chemical evaluation methods and experimental formulation, particularly through adjustments in the premix composition mainly for choline proportion to achieve precise nutrient balance. Given the critical role of a well-balanced diet in maintaining canine health, metabolism, and longevity, optimizing this experimental recipe is essential before it can be considered suitable for long-term feeding trials or commercial application. Overall, this study emphasizes the critical importance of a balanced diet in maintaining canine health, supporting optimal metabolic function, immune competence, and skin integrity, and preventing potential long-term deficiencies or excesses associated with suboptimal nutrient composition.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data analyzed in this study are available from the corresponding author on reasonable request.

**Acknowledgments:** The authors would like to thank NOMA company (NOMA, Fresh meat -Fresh meal, PL, Athens) for provision of the premix and the preparation of the experimental diet.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. S. Costa, A. Pereira, E. Passos, J. Alves, C. Garcia, and R. Araujo, 'Evaluation of the Chemical Composition of Dry Feeds for Dogs and Cats', *J. Braz. Chem. Soc.*, 2018, doi: 10.21577/0103-5053.20180142.
2. L. N. Johnson, D. E. Linder, C. R. Heinze, R. L. Kehs, and L. M. Freeman, 'Evaluation of owner experiences and adherence to home-cooked diet recipes for dogs', *J. Small Anim. Pract.*, vol. 57, no. 1, pp. 23–27, Jan. 2016, doi: 10.1111/jsap.12412.
3. J. M. Dust *et al.*, 'Chemical composition, protein quality, palatability, and digestibility of alternative protein sources for dogs', *J. Anim. Sci.*, vol. 83, no. 10, pp. 2414–2422, Oct. 2005, doi: 10.2527/2005.83102414x.
4. J. Choi, B. Kong, B. C. Bowker, H. Zhuang, and W. K. Kim, 'Nutritional Strategies to Improve Meat Quality and Composition in the Challenging Conditions of Broiler Production: A Review', *Animals*, vol. 13, no. 8, p. 1386, Apr. 2023, doi: 10.3390/ani13081386.
5. L. M. Freeman, M. L. Chandler, B. A. Hamper, and L. P. Weeth, 'Current knowledge about the risks and benefits of raw meat-based diets for dogs and cats', *J. Am. Vet. Med. Assoc.*, vol. 243, no. 11, pp. 1549–1558, Dec. 2013, doi: 10.2460/javma.243.11.1549.
6. N. Dillitzer, N. Becker, and E. Kienzle, 'Intake of minerals, trace elements and vitamins in bone and raw food rations in adult dogs', *Br. J. Nutr.*, vol. 106, no. S1, pp. S53–S56, Oct. 2011, doi: 10.1017/S0007114511002765.
7. L. Arendt-Nielsen, G.-L. Jiang, R. DeGryse, and C. Turkel, 'Intra-articular onabotulinumtoxinA in osteoarthritis knee pain: effect on human mechanistic pain biomarkers and clinical pain', *Scand. J. Rheumatol.*, vol. 46, no. 4, pp. 303–316, Jul. 2017, doi: 10.1080/03009742.2016.1203988.
8. S. L. Sanderson *et al.*, 'Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets', *Am. J. Vet. Res.*, vol. 62, no. 10, pp. 1616–1623, Oct. 2001, doi: 10.2460/ajvr.2001.62.1616.
9. C. Daumas *et al.*, 'Evaluation of eight commercial dog diets', *J. Nutr. Sci.*, vol. 3, p. e63, 2014, doi: 10.1017/jns.2014.65.
10. J. B. Van Goudoever, H. Vlaardingerbroek, C. H. Van Den Akker, F. De Groof, and S. R. D. Van Der Schoor, 'Amino Acids and Proteins', in *World Review of Nutrition and Dietetics*, vol. 110, B. Koletzko, B. Poindexter, and R. Uauy, Eds., S. Karger AG, 2014, pp. 49–63. doi: 10.1159/000358458.
11. J. Silvio, D. L. Harmon, K. L. Gross, and K. R. McLeod, 'Influence of fiber fermentability on nutrient digestion in the dog', *Nutrition*, vol. 16, no. 4, pp. 289–295, Apr. 2000, doi: 10.1016/S0899-9007(99)00298-1.
12. A. N. Beloshapka, L. G. Alexander, P. R. Buff, and K. S. Swanson, 'The effects of feeding resistant starch on apparent total tract macronutrient digestibility, faecal characteristics and faecal fermentative end-products in healthy adult dogs', *J. Nutr. Sci.*, vol. 3, p. e38, 2014, doi: 10.1017/jns.2014.28.
13. Y. Shastak and W. Pelletier, 'Pet Wellness and Vitamin A: A Narrative Overview', *Animals*, vol. 14, no. 7, p. 1000, Mar. 2024, doi: 10.3390/ani14071000.
14. Y. Shastak and W. Pelletier, 'The role of vitamin A in non-ruminant immunology', *Front. Anim. Sci.*, vol. 4, p. 1197802, Jun. 2023, doi: 10.3389/fanim.2023.1197802.
15. P. J. Morris *et al.*, 'Safety evaluation of vitamin A in growing dogs', *Br. J. Nutr.*, vol. 108, no. 10, pp. 1800–1809, Nov. 2012, doi: 10.1017/S0007114512000128.
16. N. Weidner and A. Verbrugghe, 'Current knowledge of vitamin D in dogs', *Crit. Rev. Food Sci. Nutr.*, vol. 57, no. 18, pp. 3850–3859, Dec. 2017, doi: 10.1080/10408398.2016.1171202.
17. D. E. Jewell and K. S. Panickar, 'Increased dietary vitamin D was associated with increased circulating vitamin D with no observable adverse effects in adult dogs', *Front. Vet. Sci.*, vol. 10, p. 1242851, Aug. 2023, doi: 10.3389/fvets.2023.1242851.
18. R. C. Do Nascimento, C. M. M. Souza, T. S. Bastos, G. C. B. Kaelle, S. G. De Oliveira, and A. P. Félix, 'Effects of an Herbal Source of Choline on Diet Digestibility and Palatability, Blood Lipid Profile, Liver Morphology, and Cardiac Function in Dogs', *Animals*, vol. 12, no. 19, p. 2658, Oct. 2022, doi: 10.3390/ani12192658.
19. J. K. Virtanen, T.-P. Tuomainen, and S. Voutilainen, 'Dietary intake of choline and phosphatidylcholine and risk of type 2 diabetes in men: The Kuopio Ischaemic Heart Disease Risk Factor Study', *Eur. J. Nutr.*, vol. 59, no. 8, pp. 3857–3861, Dec. 2020, doi: 10.1007/s00394-020-02223-2.

20. European Pet Food Federation. (2025). Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs. Brussels, Belgium: European Pet Food Federation.
21. T. V. Ullal, S. Lakin, B. Gallagher, N. Sbardellati, Z. Abdo, and D. C. Twedt, 'Demographic and histopathologic features of dogs with abnormally high concentrations of hepatic copper', *J. Vet. Intern. Med.*, vol. 36, no. 6, pp. 2016–2027, Nov. 2022, doi: 10.1111/jvim.16580.
22. R. J. Kearns *et al.*, 'Effect of age, breed and dietary omega-6 (n-6):omega-3 (n-3) fatty acid ratio on immune function, eicosanoid production, and lipid peroxidation in young and aged dogs', *Vet. Immunol. Immunopathol.*, vol. 69, no. 2–4, pp. 165–183, Aug. 1999, doi: 10.1016/S0165-2427(99)00052-5.
23. D. M. Vaughn *et al.*, 'Evaluation of Effects of Dietary n-6 to n-3 Fatty Acid Ratios on Leukotriene B Synthesis in Dog Skin and Neutrophils', *Vet. Dermatol.*, vol. 5, no. 4, pp. 163–173, Dec. 1994, doi: 10.1111/j.1365-3164.1994.tb00028.x.
24. Case, L. P., Daristotle, L., Hayek, M. G., & Raasch, M. F. (2010). Canine and feline nutrition: a resource for companion animal professionals. Elsevier Health Sciences.
25. National Research Council. (2006). Nutrient Requirements of Dogs and Cats. Washington, DC: The National Academies Press. <https://doi.org/10.17226/10668>
26. J. Kępińska-Pacelik, W. Biel, R. Witkiewicz, and C. Podsiadło, 'Mineral and heavy metal content in dry dog foods with different main animal components', *Sci. Rep.*, vol. 13, no. 1, p. 6082, Apr. 2023, doi: 10.1038/s41598-023-33224-w.
27. S. A. Center, K. P. Richter, D. C. Twedt, J. J. Wakshlag, P. J. Watson, and C. R. L. Webster, 'Is it time to reconsider current guidelines for copper content in commercial dog foods?', *J. Am. Vet. Med. Assoc.*, vol. 258, no. 4, pp. 357–364, Feb. 2021, doi: 10.2460/javma.258.4.357.
28. S. D. White *et al.*, 'Zinc-responsive dermatosis in dogs: 41 cases and literature review', *Vet. Dermatol.*, vol. 12, no. 2, pp. 101–109, Apr. 2001, doi: 10.1046/j.1365-3164.2001.00233.x.

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.