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Abstract

This study was conducted to investigate differences in the apparent total tract digestibility (ATTD) of nutrients and amino acids among different protein sources in companion dogs and to comprehensively evaluate their applicability as pet food ingredients through blood analysis, fecal quality assessment, and palatability evaluation. Twenty Beagle dogs, including eight puppies, eight adult dogs, and four senior dogs, were fed diets containing one of six protein sources: chicken breast meal (CM), beef meal (BM), black soldier fly larvae (BSFL), mealworms (MW), dried Alaska pollock, or isolated soy protein. Following a 7-day adaptation period, total fecal collection was performed for 4 consecutive days to calculate the ATTD. The results showed that dogs fed CM and BM diets exhibited relatively higher digestibility of dry matter, crude protein, metabolizable energy, and most amino acids, whereas lower digestibility was observed in dogs fed BSFL and MW diets. These protein source-dependent digestibility patterns were generally consistent when the data were analyzed separately according to life stage. The results of the blood analysis, fecal quality indices, and palatability assessment based on a multi-bowl test indicated that all experimental diets evaluated in this study were generally acceptable in terms of physiological safety, digestive stability, and palatability. Overall, the findings of this study provide empirical evidence supporting the diversification of protein sources and expanded utilization of alternative ingredients in the companion dog food industry by comparatively characterizing nutrient and amino acid utilization across protein source–based diets.

Keywords: Nutrient; Dog; Protein source; ATTD; Blood parameter; Palatability

Introduction

Proteins are essential nutrients for companion animals and play a critical role in the maintenance of physiological functions, growth, and tissue metabolism; therefore, they are regarded as one of the most important nutrients for sustaining a healthy life [1,2]. The amino acids that constitute proteins perform a wide range of physiological functions, including the maintenance of the musculoskeletal system. Although some amino acids can be synthesized endogenously, an adequate dietary supply of proteins and amino acids is required to maintain physiological homeostasis [3]. For these reasons, both the type and concentration of protein sources in companion dog food are recognized as key considerations by pet food manufacturers and consumers alike [4,5].

Traditionally, animal-derived protein sources such as poultry, chicken, and beef have been widely used as primary protein sources in commercial pet food formulations because of their high protein quality, balanced amino acid profiles, and long history of use in companion animal diets [6-10]. Beef, in particular, has been reported to possess high nutritional value not only as a source of high-quality protein but also because of its rich content of long-chain n−3 fatty acids, vitamins A, B_6, B_{12}, D, and E, and trace minerals such as zinc, copper, and iron in highly bioavailable forms [11]. However, projected global population growth and rising income levels in low- and middle-income countries by 2050 are expected to accelerate worldwide demand for animal-derived proteins, which may intensify competition for protein resources in the pet food industry as well [12,13]. Accordingly, the development of sustainable and nutritionally adequate alternative protein sources has become increasingly important, and rigorous scientific evaluation of their palatability, digestibility, and safety in companion animals is critically required [9]. In this context, insect-based proteins, including black soldier fly larvae (BSFL) and mealworm (MW), have attracted considerable attention because of their high protein and lipid contents, rapid growth rates, and potential for recycling organic waste streams [14,15]. In addition, freeze-dried Alaska pollock, commonly referred to as “Hwangtae” (in Korean), has recently gained attention in the companion animal snack and pet food ingredient markets as a low-fat, high-protein resource, while soy protein (SP), a refined plant-derived protein source obtained by removing non-protein components from soybean raw materials, is valued because major anti-nutritional factors, including trypsin inhibitors, phytic acid, and lectins, are largely eliminated during processing [16,17].

Protein quality cannot be determined solely on the basis of crude protein content. Rather, it is influenced by amino acid composition, digestibility, processing conditions, and the presence of structural or anti-nutritional components that may affect nutrient availability [18-20]. Therefore, apparent total tract digestibility and amino acid digestibility are important indicators for evaluating whether a protein source can supply biologically available nutrients to dogs [21]. In addition, mineral digestibility may provide further information on the overall nutritional contribution of each ingredient, particularly when protein sources differ substantially in origin and composition [22].

Hematological and serum biochemical parameters are commonly used to evaluate overall health status and clinicopathological responses to dietary feeding, and to determine whether animals exhibit clinically relevant adverse effects or maintain values within normal reference ranges [23,24]. In addition, fecal quality is an important practical criterion in companion dog nutrition because it reflects diet acceptability as perceived by pet owners. Since protein source, ingredient composition, and nutrient digestibility can influence fecal characteristics, fecal evaluation should be considered an essential component of dietary assessment [25,26].

Palatability is another critical factor determining the practical value of alternative protein sources in commercial dog foods. Even when ingredients are nutritionally adequate and physiologically safe, their applicability may be limited if they reduce voluntary food intake or feeding preference [21,27]. Because feed acceptability can be influenced by protein source, flavor characteristics, processing-induced physicochemical properties, and ingredient nutritional composition, palatability evaluation is necessary to determine whether alternative protein sources are suitable for inclusion in canine diets under practical feeding conditions [28,29].

Therefore, a rigorous scientific evaluation of the digestibility, physiological safety, fecal quality, and palatability of diverse protein sources in companion dogs is required. The present study aimed to evaluate diets formulated using diverse protein sources, including animal-derived proteins (chicken breast meal; CM, beef meal; BM), insect-based proteins (black soldier fly larvae; BSFL, mealworm; MW), fish-based proteins (dried Alaska pollock; DP), and plant-based proteins (isolated soy protein; SP), in dogs. Specifically, this study compared apparent nutrient and amino acid digestibility, blood parameters, fecal characteristics, and palatability among diets to assess their nutritional value, safety, gastrointestinal tolerance, and practical applicability as protein sources for companion dogs.

Materials and Methods

Experimental animals

The present study was approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (NIAS-2021-513). The experimental animals consisted of 20 neutered Beagle dogs, including 8 puppies (< 1 year old; 4 females and 4 males), 8 adult dogs (3–4 years old; all females), and 4 senior dogs (10–11 years old; all females).

Experimental diet

The experimental diets were formulated to meet the minimum nutrient requirements for growing dogs, as recommended by the Association of American Feed Control Officials (AAFCO) [30]. Each protein source—CM, BM, BSFL, MW, DP, and SP—was prepared in a powdered form and used as the primary protein source in the experimental diets. The crude protein (CP) content of each diet was standardized to approximately 30%, and the experimental diets were formulated as iso-nutrients among treatments. However, because DP and BSFL contained relatively high calcium levels, formulating diets with these protein sources alone at a CP level of approximately 30% resulted in excessive absolute calcium content and calcium-to-phosphorus ratios exceeding the maximum ratio (2:1) recommended by AAFCO. Therefore, in the present study, the formulation ratios of these protein sources were adjusted to maintain the target CP level while preventing calcium levels from exceeding the maximum allowable limits. Any resulting deficit in dietary protein content was compensated for by supplementation with chicken breast meal powder. The nutrient composition of the primary protein sources is presented in Table 1. The ingredient formulations and chemical compositions of the experimental diets are shown in Tables 2 and 3, respectively. After mixing, all ingredients were heat-treated using steam for 50 min, cut into uniform sizes, and processed into a diet form. The manufactured diets were then dried in a drying oven at 70 °C for 40 min to adjust the moisture content and stored at −20 °C until feeding. Prior to feeding, the diets were thawed at room temperature for 12 h and fed once they reached the ambient temperature.

Feeding protocol

The daily feed allowance was individually restricted based on the metabolizable energy requirement (MER; kcal/day = 132 × $BW^{0.75}$) recommended by the National Research Council (NRC) [31]. For growing puppies, the MER was set at 1.2 times that of adult dogs. The diets were provided twice daily at consistent times (10:00 and 16:00). All dogs were housed individually in pens measuring 170 × 210 cm, with environmental conditions maintained at a constant indoor temperature of 21 ± 1 °C and relative humidity of 60 ± 10%. Fresh water was provided ad libitum throughout the experimental period. Following a 7-day adaptation and acclimation period to the experimental diets, total feces excreted over a 4-day period were collected for apparent total tract digestibility (ATTD) analysis. Fecal samples were collected thrice daily (09:00, 13:00, and 17:00) and stored at −20 °C until analysis. Proximate analyses of the diets and feces were conducted in accordance with the standard methods of the Association of Official Analytical Chemists (AOAC) [32].

Apparent total tract digestibility and correction analysis

Apparent total tract digestibility (ATTD) of nutrients was calculated using the nutrient intake from each experimental diet and the corresponding nutrient output in feces. Correction analyses were additionally performed to estimate the CP digestibility attributable specifically to BSFL and DP in the corresponding diets. Because both the BSFL and DP diets contained CM, rice powder, and egg yolk powder, the proportions of these ingredients and their protein contents were used to calculate the amount of dietary protein derived from BSFL or DP. The CP digestibility of CM, rice powder, and egg yolk powder was assumed to be equivalent to the CP digestibility observed in the CM diet, and this assumption was used as the basis for correcting the CP digestibility values of the BSFL and DP diets.

Blood sampling and analysis

All dogs were fasted for 12 h prior to blood collection. Following fasting, 3 mL of blood was collected from the jugular vein. For complete blood count (CBC) analysis, freshly collected whole blood was immediately transferred to EDTA-treated vacuum tubes (BD Vacutainer, Ref. No. 367861). EDTA-treated whole blood was analyzed immediately after collection using an automated hematology analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA). For serum biochemical analyses, blood samples were transferred to serum separation tubes (BD Vacutainer, Ref. No. 367812) and centrifuged at 2,000 × g for 15 min. After centrifugation, the separated serum was collected and stored at −80 °C until analysis. Serum biochemical analyses were performed using different automated analyzers according to age group. Serum samples from the adult and senior dog groups were analyzed using an automated biochemical analyzer (Hitachi 7180; Hitachi High-Technologies Co., Tokyo, Japan), whereas those from the puppy group were analyzed using an automated biochemical analyzer (Cobas C111; Roche, Basel, Switzerland).

Fecal score and palatability evaluation

Fecal consistency was evaluated using a five-point fecal scoring system based on the Royal Canin Faecal Score Guide [33]. A score of 1 indicated very hard feces, and a score of 5 indicated liquid feces.

Palatability was assessed using the multibowl test. To minimize the influence of noise, odors, and interactions with other dogs, each dog participated individually in the test in a laboratory environment separate from its housing area. The test diets were presented simultaneously at a distance of approximately 2 m from the dog, and the frequency of each diet was recorded based on the first approached, consumed, and completely consumed diets. To minimize positional bias, test diets were randomized for each trial.

Statistical analysis

All statistical analyses were performed using the R software (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). Differences in nutrient digestibility and blood parameters among protein sources were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for post hoc comparisons. For palatability assessment, frequency distributions were analyzed using the chi-square (χ^2) test, and asymptotic significance values were calculated. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Feed intake and body parameters

The average daily feed intake (ADI) did not differ among the dietary treatments because the dogs were fed restricted amounts based on their calculated energy requirements (Table 4). Body weight and body condition score (BCS) also remained similar among treatments throughout the experimental period.

Major nutrient digestibility according to protein source

When data from all dogs were pooled across life stages, significant differences in ATTD among protein sources were observed for all analyzed nutrient components ($p < 0.001$) as summarized in Table 5.

Digestibility of dry matter (DM) and CP levels were significantly higher in dogs fed the CM, BM, DP, and SP diets than in those fed the BSFL and MW diets. In particular, the CM diet had the highest digestibility values for both DM (89.78%) and CP (92.73%). The corrected CP digestibility of the BSFL diet was 81.02%, which was the lowest among all experimental diets.

In contrast, the CP digestibility of the DP diet remained at 90.92% even after correction and did not differ significantly from that of the CM and BM diets. Ether extract (EE) digestibility was generally high across all diets, exceeding 97%, regardless of the protein source. However, EE digestibility was significantly higher in dogs fed the CM diet (98.34%) than in those fed the BM, BSFL, or SP diets. In contrast, dogs fed the insect-based BSFL diet exhibited relatively lower EE digestibility (97.14%) than those fed the other diets. Significant differences among the protein sources were also observed in nitrogen-free extract (NFE) and metabolizable energy (ME) digestibility. NFE digestibility was significantly higher in dogs fed the MW diet (95.67%), whereas ME digestibility was the highest in dogs fed animal-derived protein diets, specifically the CM (94.52%) and BM (93.66%) diets. In comparison, dogs fed insect-based protein diets, namely the BSFL (91.29%) and MW diets (91.99%), showed relatively lower ME digestibility. Crude ash (CA) digestibility was relatively high in dogs fed the CM (27.17%) and DP diets (25.93%), whereas extremely low values were observed in dogs fed the BSFL (1.46%) and MW (4.93%) diets. Among the mineral components, phosphorus (P) digestibility was highest in dogs fed the DP diet, reaching 40.24%.

Amino acid digestibility according to protein source

Table 6 presents the ATTD of the amino acids in each experimental diet. Analysis of amino acid digestibility showed that diets formulated with animal-derived protein sources, the CM and BM diets, exhibited generally high digestibility of most amino acids. Dogs fed the DP and SP diets also exhibited generally high amino acid digestibility, with values comparable to those observed for the CM and BM diets. However, in the SP diet, the apparent digestibility of essential amino acids, including lysine (Lys), methionine (Met), threonine (Thr), isoleucine (Ile), and phenylalanine (Phe), was relatively lower than that observed in the CM and BM diets, with values of 92.56%, 92.18%, 91.22%, 93.53%, and 93.39%, respectively. In the DP diet, apparent digestibility values for most amino acids were comparable to those of the animal protein-based diets; however, the digestibility of arginine (Arg) was slightly lower, with a value of 95.36%. In contrast, dogs fed insect-derived protein diets, including BSFL and MW, exhibited consistently lower apparent digestibility values for all amino acids than dogs fed diets based on other protein sources.

Evaluation of the safety of protein sources based on blood parameters

Table 7 presents the results of serum biochemical analyses conducted to evaluate the safety of protein sources as pet food ingredients. Most biochemical parameters were within the established reference ranges, and no clinically meaningful abnormalities were observed throughout the experimental period. Table 8 summarizes the results of the CBC analysis. Among the hematological parameters evaluated, only the MCHC value in the CM diet group slightly exceeded the reference range, with a value of 38.63 g/dL compared with the reference interval of 30–38 g/dL. However, no clinical signs related to anemia or dehydration were observed, and no other hematological abnormalities suggestive of hemolytic changes were detected. Overall, no clinically relevant alterations were observed in major CBC parameters.

Evaluation of fecal score and palatability according to protein sources

Figure 1 presents the results of the fecal consistency evaluation. Based on the five-point fecal scoring system, fecal scores for all protein source-based diets remained within the normal range, with values between 2.5 and 3.0 throughout the experimental period.

Table 9 summarizes the results of palatability assessments. No statistically significant differences were observed among the protein sources for any of the palatability criteria, including the first approached diet, first consumed diet, and first completely consumed diet. However, the trend analysis based on simple frequency distributions indicated relatively higher frequencies for the BM and DP diets in the first approached category, the MW and DP diets in the first consumed category, and the BM and BSFL diets in the first completely consumed category.

Discussion

Nutrient digestibility according to protein source

This study investigated the effects of six protein sources—CM, BM, BSFL, MW, DP, and SP—on nutrient and amino acid digestibility across different life stages (puppy, adult, and senior) in dogs.

In the present study, the effects of protein sources were evaluated across the different life stages of dogs (puppy, adult, and senior). Although physiological differences exist among the life stages, the influence of protein sources showed a consistent pattern across all stages. Therefore, the results are presented using pooled data across life stages, and statistical analyses were conducted using the pooled dataset.

The biological value of dietary proteins is largely determined by their amino acid composition, with greater similarity to body tissue proteins associated with higher utilization efficiency [34,35]. From this perspective, the high nutrient and amino acid digestibility observed for the CM and BM diets in the present study is closely related to the fact that CM and BM represent high-quality protein sources with well-balanced amino acid profiles [36,37]. The superior digestibility of most nutrients and individual amino acids in these diets further suggests that the amino acid composition and structural characteristics of animal-derived muscle proteins are well suited to the canine digestive enzyme system. Accordingly, the consistently high digestibility observed in dogs fed the CM and BM diets reinforces the importance of traditional animal-derived protein sources as key contributors to the high biological availability of nutrients in companion dog nutrition.

When digestibility data were analyzed across all life stages in the present study, the BSFL and MW diets consistently exhibited lower CP and amino acid digestibility than diets formulated with conventional animal-derived protein sources. These lower digestibility values may be partly explained by the presence of chitin, a structural component of the insect exoskeleton that can form complexes with proteins and act as a physical barrier limiting the accessibility of digestive enzymes and inhibiting enzymatic hydrolysis [14,38]. Chitin is a nitrogen-containing polysaccharide, and its presence may lead to an overestimation of the CP content during proximate analysis, thereby representing a critical limitation in evaluating the true biological utilization of insect-derived proteins [39-41]. In this regard, the present results are consistent with previous reports indicating that insect-based proteins generally exhibit lower digestibility than conventional livestock-derived protein sources [42-44]. Nevertheless, given that CP and major amino acid digestibility values remained above 80% [31], a level generally considered commercially acceptable, the feasibility of insect proteins as ingredients in companion dog food was also supported. This suggests that although additional processing technologies aimed at improving insect protein digestibility may be theoretically effective, such interventions may not necessarily represent an indispensable prerequisite when cost–benefit considerations are considered. However, the digestibility evaluation of the BSFL diet in the present study was conducted using experimental diets in which CM was included as a supplementary protein source. Therefore, the observed digestibility values may have been partially overestimated, owing to the contribution of animal-derived proteins. After correcting for this contribution, the apparent CP digestibility of BSFL-derived proteins tended to decrease. In particular, digestibility in puppies was below 80% (77.64%; data not shown), which may be associated with physiological characteristics during the growth stages, such as incomplete development of the digestive system and insufficient secretion of digestive enzymes. These findings suggest potential limitations in protein utilization when BSFL is used as the sole protein source and highlight the need for complementary formulation strategies, such as combining insect protein with other animal-derived protein sources, to improve the amino acid balance and overall nutrient digestibility in future puppy diet formulations.

DP is rich in essential amino acids, including lysine, methionine, threonine, and valine, and previous studies have suggested that freeze-drying may increase amino acid concentrations per unit weight due to substantial moisture reduction [45]. In addition, DP contains relatively high levels of minerals, such as Ca, P, potassium, magnesium, and zinc, supporting its potential use as a functional protein ingredient [46]. In the present study, the fish-based protein source DP exhibited CP digestibility in the adult dog group comparable to that observed for the CM, BM, and SP diets, demonstrating that the digestibility of the DP diet indicates that it is a high-quality protein source that is nutritionally competitive with both animal- and plant-derived proteins. Although correction analyses were conducted to account for the potential overestimation of digestibility owing to the inclusion of CM in the DP diet, CP digestibility remained comparable to that of the CM and BM diets, further supporting the inherently high protein utilization of DP itself. Nevertheless, the overall amino acid digestibility observed for the DP diet showed trends comparable to those of animal protein-based diets, suggesting that DP may be competitive not only in terms of quantitative protein utilization, but also in terms of qualitative protein utilization. The high digestibility of Ca and P observed in the DP diet warrants particular attention. Previous studies have reported that the inclusion of fish protein substrates in the diets of senior dogs results in protein digestibility comparable to that of poultry by-product meal [47]. Similarly, a study conducted in Beagle dogs demonstrated that diets containing fish meal exhibited higher ATTD of gross energy and organic matter [48]. These findings support the notion that fish-derived proteins can provide high digestibility and nutritional value to canine diets. Although studies directly evaluating the digestibility of fish-derived minerals in dogs are limited, P from fish-based ingredients exists primarily in the form of inorganic P or hydroxyapatite, which may exhibit relatively high bioavailability in animals. Indeed, studies conducted in pigs have reported relatively high P digestibility of fish meal, showing a trend similar to the findings of the present study [49,50]. Taken together, these results indicate that DP possesses favorable characteristics not only in terms of protein digestibility but also in terms of mineral bioavailability, supporting its potential value as a multifunctional ingredient in companion dog nutrition.

SP has been widely recognized as a high-quality plant-based protein source with relatively high digestibility and adequate levels of essential amino acids [51]. In the present study, the SP diet exhibited generally high CP digestibility, supporting its nutritional suitability as a refined soybean-derived protein ingredient for canine diets. This finding is further supported by a previous study reporting high apparent CP digestibility in Beagle dogs fed soybean protein isolate-containing diets [52]. However, lower digestibility was observed for certain individual amino acids compared with diets formulated with animal-derived protein sources. Particularly, the digestibility of several essential amino acids, including lysine, isoleucine, leucine, and methionine, was lower in the SP diet than in the CM diet. A closer examination of age-specific data indicated that this trend was primarily driven by the relatively reduced amino acid digestibility in growing dogs fed the SP diet. Thus, the amino acid utilization limitations observed at specific life stages may have contributed to the overall digestibility patterns when the data were pooled across ages. One possible explanation for these findings is the structural characteristics of plant-derived proteins. Protein structure plays a critical role in determining enzymatic accessibility and degradation patterns within the gastrointestinal tract, with secondary and tertiary structures being the key determinants of susceptibility to digestive enzymes. Plant proteins have been reported to generally contain a higher proportion of β-sheet structures and a lower proportion of α-helix structures than animal-derived proteins [53-55]. β-sheet-rich structures are stabilized by intermolecular hydrogen bonding, which may increase resistance to proteolytic enzymes in the gastrointestinal environment, thereby promoting protein aggregation and limiting enzymatic hydrolysis [53,54]. Such structural features may restrict the release and absorption of specific amino acids, ultimately leading to the reduced digestibility of individual amino acids [56,57]. In addition, plant protein sources often coexist with non-starch polysaccharides and dietary fiber components, which can physically hinder the access of digestive enzymes to protein substrates [58]. This limitation in substrate accessibility may slow protein degradation, potentially exacerbating the reduction in amino acid utilization, particularly in growing dogs with relatively high essential amino acid requirements. Taken together, these results suggest that although SP can serve as a highly digestible plant-based protein source in adult and senior dog diets, its application in puppy diets may require complementary formulation strategies. Specifically, the combined use of SP and animal-derived protein sources may be necessary to compensate for limitations in the digestibility of specific amino acids and ensure adequate amino acid availability during growth.

The consistently high EE digestibility, exceeding 97% across all protein sources, indicated that differences among protein ingredients had limited influence on lipid emulsification and absorption mechanisms. Although no statistically significant differences were observed, the highest fat digestibility in dogs fed the CM diet may be attributed to the fatty acid composition or physical structure of CM, which could confer favorable interactions with lipase activity. ME digestibility is closely associated with DM and CP digestibility. The lower ME digestibility observed in dogs fed insect-based protein diets may be explained by the increased excretion of indigestible protein fractions, accompanied by endogenous nitrogen loss during fecal elimination.

Evaluation of the safety of protein sources based on blood parameters

CBC analysis revealed a slight tendency toward increased mean corpuscular hemoglobin (MCH) and MCHC in dogs fed the CM diet compared to those fed other dietary treatments. However, these changes should be interpreted with caution, as partial hemolysis of blood samples can artificially elevate MCHC values during analysis [59], and the observed variations were not indicative of clinically relevant abnormalities. Neutrophil counts (Neu#) in dogs fed the CM diet remained within the established reference range for all individuals but tended to be slightly higher than those observed in dogs fed diets based on other protein sources. Nevertheless, because the values remained within the reference range, this difference does not appear to indicate overt clinical inflammation or immune dysfunction. Rather, it may reflect normal individual variation or diet-related modulation of immune status. Previous studies in mice have demonstrated that the type of dietary protein can modulate immune cell activity and the expression of immune-related proteins, even under comparable physiological conditions [60]. Such immunological changes have been reported to be regulated not only through direct effects on the host but also indirectly via alterations in the gut microbial environment [61]. Accordingly, the minor differences in Neu# observed in the present study may reflect the protein source–dependent modulation of immune responsiveness rather than pathological immune activation. All serum biochemical parameters remained within the established reference ranges, and no clinically relevant abnormalities were detected throughout the experimental period. Blood biochemical parameters are widely recognized as representative indicators of systemic physiological status, including protein metabolism and hepatic and renal functions [62,63]. In this context, the present findings suggest that the evaluated protein sources did not adversely affect the physiological homeostasis when fed to companion dogs.

Evaluation of fecal score and palatability according to protein sources

Fecal scores were stably maintained within the range of 2.5 to 3.0 throughout the experimental period, and no clinical signs of digestive disturbance, such as diarrhea or loose stools, were observed. This indicates that the overall fecal characteristics and digestive status remained within normal physiological ranges, collectively supporting the notion that all protein sources evaluated in the present study were acceptable in terms of fecal quality and digestive stability. Furthermore, the palatability assessment revealed no statistically significant differences among the evaluated protein sources. Although the trend analysis based on simple frequency distributions indicated relatively higher frequencies for certain protein sources, these trends did not reach statistical significance. Therefore, the observed patterns should be interpreted with caution as they do not provide sufficient evidence to conclude that any specific protein source exhibited superior or inferior palatability.

Conclusion

In conclusion, the present study showed that protein source influenced the digestibility of diets, while all evaluated protein sources were generally acceptable in terms of safety, fecal quality, and palatability in companion dogs. CM and BM exhibited consistently high nutrient and amino acid digestibility, confirming their value as highly digestible conventional animal-derived protein sources. DP showed favorable protein, amino acid, and mineral digestibility, suggesting its potential as a high-quality fish-based protein ingredient. SP demonstrated high overall CP digestibility, although reduced digestibility of certain essential amino acids in growing dogs indicates that its use in puppy diets may require complementary formulation strategies. Insect-based protein sources, particularly BSFL, showed relatively lower CP and amino acid digestibility than conventional animal-derived protein sources, suggesting the need for careful formulation or processing to improve protein utilization. Nevertheless, all evaluated protein sources were generally well tolerated, as blood parameters remained within clinically acceptable ranges, fecal quality was stable, and no significant differences in palatability were observed. These findings suggest that diverse protein sources may be strategically utilized in companion dog food formulations when their digestibility, physiological safety, fecal quality, and palatability are comprehensively evaluated.

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Tables and Figures

Table 1. Chemical composition of protein sources used in the experimental diets

Chemical composition (%)	CM	BM	BSFL	MW	DP	SP
Moisture	6.06	3.13	3.39	4.16	8.72	5.55
CP	89.73	84.91	55.71	66.98	58.98	84.30
EE	3.10	10.36	8.00	11.84	3.30	0.05
CF	0.31	1.10	9.52	8.07	0.02	0.12
CA	2.90	5.04	18.01	4.80	28.47	5.02
Ca	0.02	0.01	5.85	0.11	9.33	0.39
P	0.50	0.41	0.95	0.85	5.33	0.84
GE (kcal/g)	5.426	5.827	4.491	5.546	3.579	5.049
Lys	7.72	7.82	3.08	3.80	4.44	5.30
Met	2.60	2.18	0.79	0.87	1.55	1.06
Arg	5.62	5.66	2.60	3.60	3.71	6.42
His	2.50	2.76	1.64	2.18	1.09	2.24
Thr	4.01	4.22	2.15	2.78	2.59	3.33
Val	4.45	4.52	3.24	4.16	2.73	4.22
Ile	4.27	4.29	2.22	2.85	2.29	4.03
Leu	7.07	7.36	3.57	4.79	4.09	6.84
Phe	3.49	3.62	2.12	2.50	2.15	4.50
Asp	8.40	8.64	4.85	5.73	5.90	9.82
Glu	12.79	13.47	6.59	7.99	8.32	15.99
Ala	4.95	5.17	3.58	4.79	3.75	3.61
Tyr	2.97	3.15	3.40	5.18	1.69	3.21
Cys_2	1.01	0.88	0.43	0.61	0.56	1.06

CM, chicken breast meal; BM, beef meal; BSFL, black soldier fly larvae; MW, mealworm; DP, dried Alaska pollock; SP, isolated soy protein; CP, crude protein; EE, ether extract; CF, crude fiber; CA, crude ash; Ca, calcium; P, phosphorus; GE, gross energy; Lys, lysine; Met, methionine; Arg, arginine; His, histidine; Thr, threonine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Asp, aspartic acid; Glu, glutamic acid; Ala, alanine; Tyr, tyrosine; Cys_2, cystine.

Table 2. Ingredient composition of the experimental diets

Ingredient (%)	CM	BM	BSFL	MW	DP	SP
White rice powder	29.8	30.4	26.3	26.8	28.8	29.7
Chicken breast powder	13.0	0.0	5.4	5.4	5.4	
Beef powder	-	13.0	-	-	-	-
Black soldier fly larvae power (defatted)	-	-	11.9	-	-	-
Mealworm powder (defatted)	-	-	-	17.3	-	-
Dried Alaska pollock powder	-	-	-	0.0	10.8	-
Isolated soy protein powder	-	-	-	0.0	0.0	13.0
Calcium citrate	0.9	0.9	0.9	0.9	0.9	0.9
Egg yolk powder	13.0	13.0	13.0	13.0	13.0	13.0
Pork fat	1.6	0.9	2.2	0.5	2.6	2.2
Vitamin and mineral premix	0.4	0.4	0.4	0.4	0.4	0.4
Salt	0.1	0.1	0.1	0.1	0.1	0.1
Cabbage powder	1.1	1.1	1.1	1.1	1.1	1.1
Monocalcium phosphate	1.8	2.0	2.0	1.5	0.0	1.5
Calcium carbonate	1.6	1.6	0.1	1.7	0.2	1.5
Seaweed powder	1.6	1.6	1.6	1.6	1.6	1.6
Water	35.0	35.0	35.0	35.0	35.0	35.0
Total	100	100	100	100	100	100

Vitamin and mineral premix supplied per kg of diets: 3500 IU vitamin A; 250 IU vitamin D3; 25 mg vitamin E; 0.052 mg vitamin K; 2.8 mg vitamin B1(thiamine); 2.6 mg vitamin B2 (riboflavin); 2 mg vitamin B6 (pyridoxine); 0.014 mg vitamin B12; 6 mg Cal-d-pantothenate; 30 mg niacin; 0.4 mg folic acid; 0.036 mg biotin; 1,000 mg taurine; 44 mg FeSO4; 3.8 mg MnSO4; 50 mg ZnSO4; 7.5 mg CuSO4; 0.18 mg Na2SeO3; 0.9 mg Ca(IO3)2

Table 3. Chemical composition of the experimental diets

Chemical composition (%[DM BASIS], Analyzed)	CM	BM	BSFL	MW	DP	SP
CP	30.08	30.67	30.95	31.34	31.34	32.26
EE	13.08	14.87	15.81	14.70	14.70	14.38
CF	0.64	0.43	1.64	0.22	0.22	2.13
CA	7.68	7.24	9.33	7.83	7.83	8.08
NFE	48.52	46.79	42.26	45.92	45.92	43.16
OM	92.32	92.76	90.67	92.17	92.17	91.92
Ca	1.63	1.62	2.20	1.37	1.46	1.55
P	1.21	1.23	1.59	1.16	1.07	1.14
Ca/P	1.35	1.32	1.38	1.17	1.35	1.37
ME(kcal/kg)	3862.93	3975.35	3906.94	3953.31	3953.31	3861.86

CP, crude protein; EE, ether extract; CF, crude fiber; CA, crude ash; NFE, nitrogen-free extract; Ca, calcium; P, phosphorus; Ca/P, calcium to phosphorus ratio. NFE was calculated using the following equation: NFE = Dry matter - (Crude protein + Ether extract + Crude fiber + Crude ash); ME was calculated using the following equation: ME (kcal/kg) = (Crude protein × 3.5) + (Ether extract × 8.5) + (Nitrogen free extract × 3.5)

Table 4. Average daily feed intake, body weight, and body condition score of dogs fed experimental diets

	CM	BM	BSFL	MW	DP	SP	SEM
ADI (g)	284.62	293.66	285.80	282.38	286.93	297.73	14.96
BW (kg)							
Initial	11.00	12.01	11.63	11.47	11.68	11.94	0.99
Final	12.01	11.63	11.47	11.68	11.94	12.10	1.00
BCS	2.77	2.73	2.72	2.73	2.92	2.69	0.21

Values are expressed as the means; CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet; SEM, standard error of mean; ADI, average daily feed intake; BW, body weight; BCS, body condition score.

Table 5. Apparent total tract digestibility of major nutrients

ATTD (%)	CM	BM	BSFL	MW	DP	SP	p-value
DM	89.78^{a}	88.79^{a}	84.35^{b}	86.28^{b}	89.45^{a}	88.86^{a}	<0.001
CP	92.73^{a}	92.37^{a}	88.38^{b}	87.12^{b}	91.88^{a}	92.12^{a}	<0.001
CP (correction)	92.73^{a}	92.37^{a}	81.02^{b}	87.12^{c}	90.92^{a}	92.12^{a}	<0.001
EE	98.34^{a}	97.24^{b}	97.14^{b}	97.89^{ab}	97.60^{ab}	97.27^{a}	<0.001
NFE	94.90^{ab}	94.40^{a}	93.19^{a}	95.67^{b}	94.78^{ab}	93.75^{a}	<0.001
Ca	27.17^{a}	13.43^{ab}	1.46^{b}	4.93^{b}	25.93^{a}	18.10^{ab}	<0.001
P	33.65^{ab}	29.16^{a}	28.92^{a}	33.24^{a}	40.24^{b}	37.28^{ab}	<0.001
ME	94.52^{a}	93.66^{a}	91.29^{b}	91.99^{b}	93.12^{ab}	93.00^{ab}	<0.001

Values are expressed as the means; a-b Different letters above the number within the same row indicate significant differences between means ($p < 0.05$). If the same letters, such as a and ab or ab and b, are present, it indicates an insignificant difference. Additionally, if the ANOVA was not statistically significant, no letters were displayed in the table. CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet; DM, dry matter; CP, crude protein; EE, ether extract; NFE, nitrogen-free extract; Ca, calcium; P, phosphorus; ME, metabolizable energy; NFE was calculated using the following equation: NFE = Dry matter - (Crude protein + Ether extract + Crude fiber + Crude ash) ME was calculated using the following equation: ME (kcal/kg) = (Crude protein × 3.5) + (Ether extract × 8.5) + (Nitrogen free extract × 3.5)

Table 6. Apparent total tract digestibility of amino acids

ATTD (%)	CM	BM	BSFL	MW	DP	SP	p-value
Lys	95.07^{a}	94.23^{a}	91.29^{b}	90.41^{b}	93.70^{ac}	92.56^{bc}	<0.001
Met	95.48^{a}	95.25^{a}	91.69^{bc}	90.29^{c}	94.09^{ab}	92.18^{bc}	<0.001
Arg	96.54^{a}	96.37^{a}	94.11^{b}	94.81^{b}	95.36^{bc}	95.99^{ac}	<0.001
His	94.41^{a}	94.02^{ab}	90.15^{c}	91.57^{cd}	93.33^{ab}	93.12^{bd}	<0.001
Thr	93.86^{a}	93.71^{a}	90.59^{b}	90.63^{b}	93.12^{a}	91.22^{b}	<0.001
Val	94.51^{a}	93.95^{ab}	87.31^{cd}	86.26^{c}	93.81^{ab}	92.28^{bd}	<0.001
Ile	95.56^{a}	94.91^{a}	91.65^{b}	92.41^{b}	94.72^{ac}	93.53^{bc}	<0.001
Leu	95.75^{a}	95.52^{a}	92.21^{b}	93.32^{b}	95.12^{a}	93.39^{b}	<0.001
Phe	95.10^{a}	94.67^{ab}	91.82^{cd}	92.26^{c}	94.24^{ab}	93.74^{bd}	<0.001
Asp	93.71^{a}	93.04^{ab}	89.58^{c}	90.57^{c}	92.45^{b}	92.71^{ab}	<0.001
Glu	95.01^{a}	94.61^{ab}	91.23^{c}	92.04^{c}	94.00^{b}	94.32^{ab}	<0.001
Gly	92.88^{a}	92.19^{ab}	85.71^{c}	87.54^{cd}	91.96^{ab}	90.51^{bd}	<0.001
Ala	94.51^{a}	94.04^{a}	89.96^{b}	91.87^{bc}	93.49^{ac}	91.07^{b}	<0.001
Pro	94.27^{a}	94.00^{a}	90.50^{b}	92.41^{bc}	92.64^{bc}	93.37^{ac}	<0.001
Ser	86.69^{a}	86.06^{a}	83.45^{b}	84.58^{b}	86.86^{a}	87.15^{a}	<0.001
Tyr	94.12^{a}	94.15^{a}	90.86^{b}	93.54^{ac}	92.47^{bc}	92.71^{abc}	<0.001
Cys$_2$	87.11^{a}	85.07^{a}	78.73^{b}	82.99^{b}	84.52^{ab}	86.63^{a}	<0.001

Values are expressed as the means; a-d Different letters above the number within the same row indicate significant differences between means ($p < 0.05$). If the same letters, such as a and ab or ab and b, are present, it indicates an insignificant difference. Additionally, if the ANOVA was not statistically significant, no letters were displayed in the table. CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet; Lys, lysine; Met, methionine; Arg, arginine; His, histidine; Thr, threonine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ala, alanine; Pro, proline; Ser, serine; Tyr, tyrosine; Cys$_2$, cystine.

Table 7. Serum biochemical parameters of dogs fed experimental diets

	Ref. range	Unit	CM	BM	BSFL	MW	DP	SP	SEM
ALT	17-95	U/L	56.92	46.10	59.98	57.16	62.54	52.21	27.31
AST	18-56	U/L	30.59	28.89	28.75	29.64	33.16	31.09	2.58
ALB	2.7-4.4	U/L	3.19	2.98	3.10	3.04	2.93	3.18	0.59
ALP	7-115	U/L	74.75	65.33	51.86	66.40	67.13	71.63	27.20
AMY	322-1310	g/dL	1203.91	1218.75	1230.73	1266.97	1166.41	1133.95	592.17
Ca	9.4-11.1	U/L	10.54	9.94	10.39	10.16	9.93	10.25	0.63
CK-MB	-	U/L	187.42	156.61	170.39	144.81	185.93	142.12	53.84
LDHI	24-388	mg/dL	57.71	59.27	57.01	48.45	57.32	39.63	12.89
LIP	15-228	-	19.01	23.01	21.06	28.92	25.77	23.00	8.18
TP	5.4-7.5	g/dL	6.26	5.86	5.41	4.92	5.70	6.15	1.18
CHOL	136-392	g/dL	284.42	269.72	303.29	274.99	258.35	252.34	38.09
CRE	0.6-1.4	mg/dL	0.71	0.63	0.67	0.62	0.70	0.72	0.12
GLU	68-104	mg/dL	99.04	92.18	97.49	98.08	92.4	99.33	6.42
TRIG	23-102	U/L	77.13	100.00	73.11	87.99	68.40	69.21	38.58
BUN	9-26	mg/dL	18.86	16.31	16.51	18.38	19.66	20.49	6.77

CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet; SEM, standard error of mean; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; ALP, alkaline phosphatase; AMY, amylase; Ca, calcium; CK-MB, creatine kinase–MB; LDHI, lactate dehydrogenase isoenzyme I; LIP, lipase; TP, total protein; CHOL, cholesterol; CRE, creatinine; GLU, glucose; TRIG, triglycerides; BUN, blood urea nitrogen.

Table 8. Complete blood count of dogs fed experimental diets

	UNIT	Ref Ranges	CM	BM	BSFL	MW	DP	SP	SEM
RBC	10^6/uL	5.10-8.50	6.91	6.99	7.03	6.92	7.09	7.23	0.09
HGB	g/dL	11-19	16.91	16.23	16.23	15.96	16.31	16.68	0.20
HCT	%	33-56	43.92	44.89	45.28	44.50	45.96	46.74	0.48
MCV	Fl	60-76	63.76	64.37	64.53	64.41	64.95	64.76	0.20
MCH	Pg	20-27	24.61	23.26	23.11	23.08	23.03	23.13	0.17
MCHC	g/dL	30-38	38.63	36.13	35.81	35.84	35.48	35.71	0.29
RDW-CV	%	12.5-17.2	13.46	12.92	13.33	13.25	13.25	13.14	0.05
RDW-SD	fL	33.2-46.3	34.49	33.40	34.52	34.20	34.26	33.84	0.15
PLT	10^3/uL	117-490	363.33	367.32	345.76	349.22	356.21	321.67	9.65
MPV	fL	8-14.1	10.15	10.09	10.33	10.35	10.09	10.47	0.08
PDW	fL	12-17.5	15.37	15.39	15.44	15.38	15.40	15.50	0.04
PCT	mL/L	0.9-5.80	3.66	3.69	3.57	3.60	3.60	3.34	0.12
WBC	10^3/uL	6-17	11.08	7.83	7.71	7.37	7.81	7.24	0.28
Neu#	10^3/uL	3.62-12.30	7.66	4.96	4.79	4.49	4.91	4.47	0.21
Lym#	10^3/uL	0.83-4.91	2.70	2.21	2.26	2.21	2.33	2.19	0.08
Mon#	10^3/uL	0.14-1.97	0.52	0.40	0.37	0.38	0.32	0.36	0.02
Eos#	10^3/uL	0.04-1.62	0.20	0.25	0.27	0.27	0.23	0.19	0.02
Bas#	10^3/uL	0-0.12	0.01	0.02	0.02	0.02	0.02	0.02	0.00
Neu%	%	52-81	67.76	62.97	61.63	61.42	61.82	60.97	0.52
Lym%	%	12-33	25.78	28.55	29.56	30.79	30.71	31.01	0.61
Mon%	%	2-13	4.50	5.07	4.82	5.21	4.22	5.09	0.19
Eos%	%	0.5-10	1.92	3.18	3.68	3.52	2.95	2.59	0.26
Bas%	%	0-1.3	0.03	0.24	0.31	0.27	0.30	0.34	0.03

CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet; SEM, standard error of mean; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width–coefficient of variation; RDW-SD, red cell distribution width–standard deviation; PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit; WBC, white blood cell count; Neu#, neutrophil count; Lym#, lymphocyte count; Mon#, monocyte count; Eos#, eosinophil count; Bas#, basophil count; Neu%, neutrophil percentage; Lym%, lymphocyte percentage; Mon%, monocyte percentage; Eos%, eosinophil percentage; Bas%, basophil percentage.

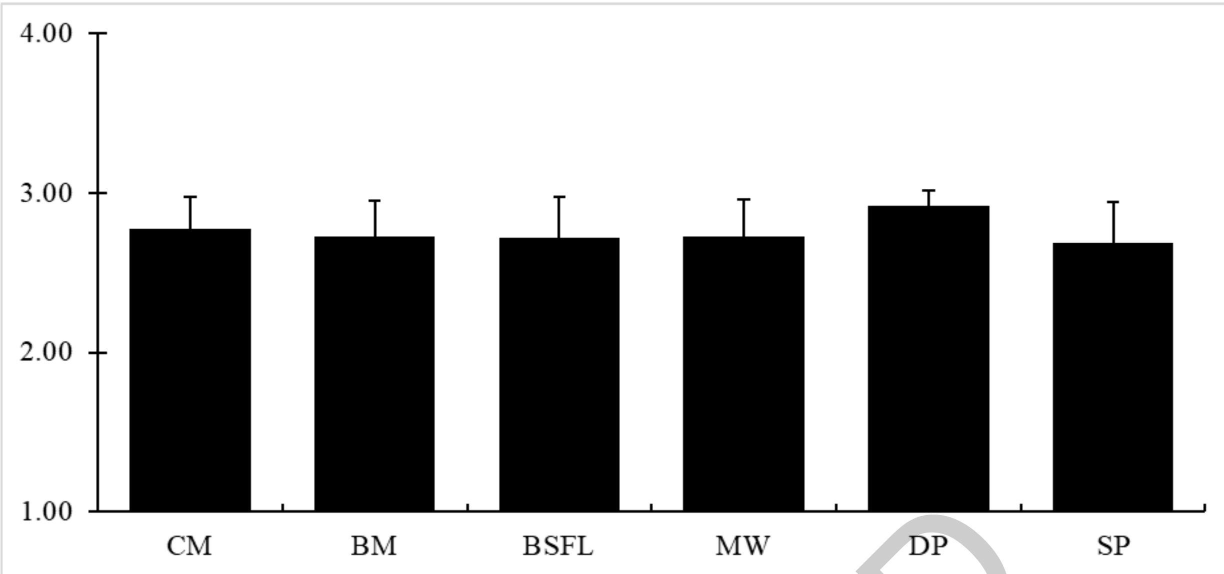

Figure 1. Fecal scores of dogs fed experimental diets
Values are presented as mean ± SEM; CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet. Fecal scores were assessed using a 5-point scale (1 = very hard and dry; 5 = watery diarrhea).

Table 9. Palatability assessment of experimental diets in dogs

Selection frequency (%)	CM	BM	BSFL	MW	DP	SP	χ^2	Asymp. Sig.
AD	18.75	25.00	12.50	6.25	25.00	12.50	2.75	0.738
CD	12.50	12.50	12.50	25.00	25.00	12.50	2.00	0.849
CCD	12.50	31.25	25.00	6.25	12.50	12.50	4.25	0.514

CM, chicken breast meal diet; BM, beef meal diet; BSFL, black soldier fly larvae diet; MW, mealworm diet; DP, dried Alaska pollock diet; SP, isolated soy protein diet; χ^2, chi-square test; Asymp. Sig., asymptotic significance; AD, first approached diet; CD, first consumed diet; CCD, first completely consumed diet.