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Nutritional assessment of pork versus chicken as primary protein sources in canine diets

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Abstract

The protein content of pet food affects its metabolizable energy content and palatability. Although pork is a high-quality protein source, it is not commonly used in pet food due to the lack of verification of its potential as a primary protein source. Hence, in this study, the potential of pork as a protein source in pet food was verified through digestibility testing involving beagle dogs. A pork-based diet made from pork hind legs and a chicken-based diet were provided to 12 beagle dogs. The palatability and digestibility of nutrients of the pork-based diet were compared with those of the chicken-based diet. The results showed that the palatability and apparent total tract digestibility of nutrients (dry matters, crude fat, crude ash, nitrogen-free extract, and amino acids) of the pork-based diet were similar to those of the chicken-based diet. This study suggests that pork hind legs can be used as a protein source in dog food.

Keywords: Dog food, Pet food, Protein sources, Chicken, Pork, Apparent total tract digestibility (ATTD)

INTRODUCTION

Companion animals, particularly dogs, have become an important part of people's lives. The growing bond between pets and their owners has led to the rapid development of the pet food industry. Globally, the pet food market reached more than 120 billion U.S. dollars in 2022; thus, it has increased by 20 billion dollars in 3 years since 2019 [1]. According to the 2020 Pet Food Market Status published by Ministry of Agriculture, Food and Rural Affairs, the market size of the pet food industry in South Korea was $753.18 million in 2020, and it was expected to increase to $890.77 million, an 18.3% increment, in 2023. As 83.9% of households with companion animals in South Korea have dogs, the quality of dog food deserves particular attention.

Research has been conducted to explore raw materials, especially diverse protein sources, that can

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Kim KH
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Ethics approval and consent to participate
The animal experiments conducted in this study were approved by the Animal Care and Use Committee of the NIAS (NIAS-2022-0585).

be used as ingredients in pet food. A low amount of protein in pet food may reduce its palatability, especially for carnivorous companion animals, such as cats, that prefer a high-protein diet [2,3]. Despite their high quality, animal-based proteins may cause allergies to companion animals. Chicken, lamb, and beef are the most commonly reported protein sources that cause allergic reactions in dogs, whereas fish and pork are less frequently reported [4,5]. As the populations of humans and companion animals increase, the supply of raw materials as protein sources should be secured amidst the growing demand for food and land use [6].

Proteins obtained through dietary intake provide essential amino acids for the synthesis of structural proteins crucial for the growth and maintenance of the body, and amino acids acquired from a dietary source serve as precursors for the synthesis of non-essential amino acids [7]. Therefore, protein quality represents the ability of proteins to fulfill the metabolic needs of the body through the constant supply of essential amino acids from the regular dietary intake of protein-rich food [8].

Although pork contains vitamin B, minerals, 15%–23% proteins, and high-quality amino acids [8–10], pork is considered an unhealthy protein source because it also contains high levels of cholesterol and fat. Pietruszka et al. [11] revealed that pork contains low amounts of saturated fatty acids, high amounts of unsaturated fatty acids, and biologically active substances, including potent antioxidants for dogs and cats.

Although pork is a potential primary protein source due to its hypoallergenic properties and high nutritional content in pet food, it is not commonly used as a protein source in several countries of the pet food market. According to the Pet Food Production and Ingredient Analysis, pork only accounted for 14,437 tons of the total pet food ingredient volume from slaughter to rendering in the U.S. in 2018, while chicken accounted for 854,988 tons [12]. Similarly, the proportion of pork in pet food products in Korea is relatively low, while those of chicken, duck, and beef are relatively high. Consequently, there is a surplus of pork in Korea, and the inventory of pork parts significantly varies; among the different pork parts, the inventory of pork hind legs is the highest (35.6%) [13]. If the potential of pork as a protein source in pet food is verified, less commonly used livestock products, such as pork hind legs, could be incorporated as ingredients in pet food.

As most dogs rely on pet food to meet their nutrition and energy requirements, consuming a high-quality diet is essential for maintaining their health [14]. As the pet food market evolves, various ingredients are being tested for inclusion in new pet food products [15]. Information on energy and nutrient usability and data on food acceptance and fecal output are important in evaluating pet food quality. Therefore, digestibility testing is essential for assessing ingredient quality and processing technologies [16]. If a raw material demonstrates good performance in digestibility testing, it could be considered a potential ingredient in pet food.

Although pork hind legs could be a potential protein source in pet food, its potential has not been verified through digestibility testing involving companion animals. Hence, this study aimed to verify the potential use of pork hind legs as a pet food ingredient by conducting digestibility tests involving dogs.

MATERIALS AND METHODS

Preparation of experimental diets

The primary protein sources for the experimental diets evaluated in this study were fresh commercially available chicken breasts and pork hind legs, with fat tissue removed as much as possible. Except for the protein sources (chicken and pork), both experimental diets contained the same ingredients: calcium monophosphate, guar gum, defatted soybean powder, cellulose, glycerin, hydrolyzed protein powder, propylene glycol, potassium sorbate, clarified chicken fat, a vitamin–mineral premix, and water. The experimental diets were designed to fulfill the nutrient needs outlined in the guidelines set by the Association of American Feed Control Officials (AAFCO) [17]. The chicken-based diet (control, CON; 28.20% crude protein and 4,008 metabolizable energy [ME] kcal/kg dry matter [DM]) and the pork-based diet (treatment, TRT; 28% crude protein and 3,979 ME kcal/kg DM) were prepared following the methods reported by Seo et al. [18] (Table 1). The experimental diets were maintained at –20℃ until feeding. The nitrogen-free extract (NFE) and ME of the diets were calculated using Equations (1) and (2), respectively:

Table 1. Chemical and amino acid compositions of experimental diets

Items	Experimental diets[1)]	
	CON	TRT
Chemical composition, (Analyzed), %		
Moisture	40.02	39.06
Crude protein	28.2	28
Crude fat	10.96	10.33
Crude ash	4.84	4.79
Crude fiber	2.12	1.64
NFE	13.86	16.17
Ca/P ratio	1.07	1.04
Metabolizable energy, kcal/kg	4,008	3,979
Amino acid composition, (Analyzed), % DM		
Alanine	1.52	1.51
Arginine	1.99	1.97
Aspartic acid	2.97	2.98
Cystine	0.46	0.46
Glutamic acid	4.24	4.19
Glycine	1.41	1.42
Histidine	0.74	0.81
Isoleucine	1.26	1.26
Leucine	2.28	2.30
Lysine	2.23	2.23
Methionine	0.71	0.73
Phenylalanine	1.30	1.36
Proline	1.25	1.28
Serine	1.33	1.34
Threonine	1.20	1.21
Tyrosine	0.42	0.41
Valine	1.38	1.40

[1)]The control (CON) diet used chicken as the primary protein source, while the treatment (TRT) diet used pork as the main protein source.

NFE, Nitrogen-free extract; Ca, Calcium; P, Phosphorus.

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$$NFE(\%) = 100 - (\ moisture\ + CP + CF + EE + CA) \times 100, \tag{1}$$

$$ME\ in\ diet \left(\frac{kcal}{kg}\right) = \{(CP \times 3.5) + (EE \times 8.5) + (NFE \times 3.5)\} \times 10, \tag{2}$$

where crude protein (CP), crude fiber (CF), ether extract (EE), and crude ash (CA) stand for crude protein, crude fiber, ether extract, and crude ash, respectively.

Animals

A total of 12 healthy 4-year-old beagle dogs weighing 12.70 ± 0.2 kg (eight spayed females and four castrated males) owned by the National Institute of Animal Science (NIAS) were analyzed in this study. Approval for the animal experiments performed in this study was obtained from the Animal Care and Use Committee of the NIAS (NIAS-2022-0585). Each dog was kept in a separate indoor area measuring 1.7 m × 2.1 m, where they were maintained at a stable temperature of 22℃–24℃ and subjected to a consistent lighting cycle of 12 h of light followed by 12 h of darkness. During the experimental period, each dog was provided with free outdoor access for approximately 3 h daily in an individual outdoor space (2.8 m × 2.5 m) connected to the indoor space. The experimental diets were provided twice daily in amounts estimated using the maintenance energy requirement (MER) equation (Equation (3)) proposed by the AAFCO, and water was provided ad libitum [17].

$$MER = 110\ kcal \times metabolic\ body\ weight\ (\text{mBW, kg; BW0.75}), \tag{3}$$

Fecal sampling and chemical analysis

The dogs were provided with the CON diet for 15 days, followed by the TRT diet for another 15 d. An adaptation period of 10 days was allowed before each experimental diet was provided. The diet intake and fecal output were recorded daily, while body weight was recorded on a weekly basis. The body condition score (BCS) was evaluated weekly according to the nine-point BCS scale proposed by Laflamme et al. [19]. The fecal score was measured daily using a five-point fecal score scale (1 = dry; 5 = liquid feces) according to the Waltham Fecal Scoring System and expressed as an average value [20]. Fecal samples were gathered five days before the end of the experiment and preserved at –20℃ until they were analyzed further. The chemical composition of the experimental diets and fecal samples was assessed following the standard procedures established by the Association of Official Analytical Chemists (AOAC) for moisture content (AOAC method 934.01), CP (AOAC method 984.13), crude fat (EE; AOAC method 920.39), CA (AOAC method 942.05), and CF (AOAC method 978.10) [21]. The experimental diets and fecal samples were subjected to amino acid analysis after they had been hydrolyzed using the chromatography method described by Llames and Fontaine [22]. The apparent total tract digestibility (ATTD) of nutrients (DM, crude fat, CA, NFE, and amino acids) in the experimental diets was estimated using the total collection method and calculated using Equation (4):

$$\text{ATTD of nutrients (\%)} = \left(\frac{\text{nutrient input(food)} - \text{nutrient output(fecal)}}{\text{nutrient input(food)}}\right) \times 100, \tag{4}$$

At the end of the experimental period, blood samples were obtained from the cephalic vein of each dog and immediately promptly separated into EDTA collection tubes (REF 41.1395.105; Sarstedt) and serum vacutainer tubes (REF 367812, BD Vacutainer). The blood samples contained in the EDTA collection tubes were analyzed for a complete blood count using an automated hematology analyzer (BC-5000 Vet, Mindray), and the serum derived from the blood samples in the serum vacutainer tube was separated by centrifugation (3,000×g, 10 min) and then stored at –80℃ until further analysis. The biochemical parameters of the serum were assessed using an automated clinical chemistry analyzer (Cobas c111, Roche Diagnostics International).

Palatability assessment

After the 30-day feeding period, a two-bowl palatability test was conducted to evaluate the palatability of the experimental diets [23]. The CON and TRT diets were placed in bowls of identical material and shape, and each diet was offered separately to the beagles. To minimize external factors and ensure accurate results, all the beagles were tested individually in their familiar feeding spaces. Time was recorded from the moment the beagles began to consume the food. The order of approach to the diets, initiation of consumption, and completion of consumption was observed, and each action was scored as either one or two points based on the sequence.

Statistical analysis

All statistical analyses conducted in this study were performed using SPSS version 17.0 (SPSS Statistics, 2009). The results of the BCS, fecal score, and palatability test were analyzed using the Chi-square test, while the results of other tests were analyzed using a *t*-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Food intake and body parameters

thTable 2 presents the effects of the pork-based diet on the food intake, body parameters, and fecal scores of the beagle dogs. No statistically significant differences were observed between the TRT and CON groups with respect to body weight, body weight gain, and BCS ($p > 0.05$). Although the TRT group exhibited a tendency towards higher average daily food intake and ME intake than the CON group, these differences were not statistically significant ($p > 0.05$). Both groups maintained

Table 2. Body parameters, diet and ME intake, and fecal score

Items	Experiment groups		SEM	*p*-value
	CON	TRT		
BW, kg				
Initial	12.80	12.70	0.20	0.90
Final	13.10	13.10	0.30	0.96
BWG, g	337	458	52	0.25
BCS				
Initial	5.10	5.10	0.20	1.00
Final	5.20	5.20	0.20	1.00
ADFI, g/day	299	303	4	0.62
MEI, kcal/day	718	735	11	0.44
Fecal score	2.48	2.49	0.08	0.89

A total of twelve-healthy beagle breed dogs were given a CON diet (n=12) containing chicken or a TRT diet (n=12) containing pork for 30 days. BCS were measured weekly using a 9-point scale (1–3, thin; 4–6, idea l; 7–9, overweight or obese). Fecal scores were measured based on a 5-point fecal score scale (1 = hard and dry feces to 5 = liquid diarrhea). A *p*-values for comparisons between CON and TRT group in a same row.

CON, control; TRT, treatment; ADFI, average daily food intake; ME, metabolizable energy; MEI, metabolic energy intake; BW, body weight; BWG, body weight gain; BCS, body condition score; SEM, standard error of the mean.

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a desirable fecal score (2.48–2.49) throughout the experimental period, and significant differences were not observed between the groups ($p > 0.05$).

The results of the palatability test revealed no significant differences in the order or frequency by which the diets were approached and consumed by the dogs ($p > 0.05$). However, the CON diet was approached first and more frequently than the TRT diet. The completion frequency of intake was significantly higher in the TRT diet ($p < 0.05$). The TRT diet was consumed faster than the CON diet; however, the difference between the two diets was not statistically significant ($p > 0.05$).

Apparent total track digestibility

the nutrient intake, fecal output, and ATTD of the two experimental diets are shown in Table 3. No statistically significant differences were observed between the CON and TRT groups for the intake of DM, CP, crude fat, CA, and total amino acids ($p > 0.05$). However, the intake of NFE and histidine was significantly higher in the TRT group than in the CON group ($p < 0.05$; Table 3 and 4). Similarly, there were no statistically significant differences in the fecal output of any nutrients between the two groups ($p > 0.05$). Although the ATTD of nutrients tended to be higher in the

Table 3 . Nutrients intake, excretion, and apparent total tract digestibility (ATTD)

Items	Experiment groups		SEM	*p*-value
	CON	TRT		
Average daily nutrients intake, g/day				
Dry matter	179.00	185.00	3.00	0.31
Crude protein	84.20	84.90	1.20	0.78
Crude fat	32.70	31.30	0.50	0.16
Crude ash	14.40	14.50	0.20	0.84
NFE	41.40	49.00	1.00	< 0.01
Total amino acid	79.70	81.40	1.20	0.48
Average daily nutrients excretion (Fecal), g/day				
Dry matter	37.40	34.30	1.80	0.39
Crude protein	8.68	7.91	0.50	0.45
Crude fat	2.25	1.99	0.21	0.55
Crude ash	11.80	10.20	0.60	0.20
NFE	8.75	7.10	0.96	0.41
Total amino acid	6.00	6.39	0.49	0.70
ATTD[1)], %				
Dry matter	79.20	81.40	0.90	0.23
Crude protein	89.70	90.60	0.61	0.46
Crude fat	93.10	93.60	0.70	0.73
Crude ash	18.40	30.00	4.30	0.19
NFE	79.10	85.70	2.26	0.14
Total amino acid	92.50	92.10	0.63	0.77

[1)]ATTD was calculated as a following equation: ATTD = {(intake – excretion)/intake} × 100. A *p*-values for comparisons between CON and TRT group in a same row.

CON, control; TRT, treatment; NFE, Nitrogen-free extract; ATTD, Apparent total tract digestibility; SEM, standard error of the mean.

Table 4. Amino acids intake, excretion, and apparent total tract digestibility (ATTD)

Items	Experiment groups		SEM	*p*-value
	CON	TRT		
Average daily nutrients intake, g/day				
Alanine	4.55	4.59	0.07	0.77
Arginine	5.95	5.96	0.09	0.95
Aspartic acid	8.87	9.03	0.13	0.57
Cystine	1.37	1.41	0.02	0.46
Glutamic acid	12.66	12.71	0.19	0.91
Glycine	4.20	4.30	0.06	0.47
Histidine	2.21	2.45	0.04	0.003
Isoleucine	3.75	3.81	0.06	0.62
Leucine	6.80	6.99	0.10	0.38
Lysine	6.65	6.75	0.10	0.62
Methionine	2.12	2.22	0.03	0.13
Phenylalanine	3.89	4.12	0.06	0.07
Proline	3.73	3.89	0.06	0.18
Serine	3.96	4.05	0.06	0.46
Threonine	3.57	3.67	0.05	0.39
Tyrosine	1.25	1.25	0.02	0.98
Valine	4.13	4.24	0.06	0.42
Average daily nutrients excretion (Feacal), g/day				
Alanine	0.41	0.44	0.04	0.65
Arginine	0.24	0.26	0.02	0.68
Aspartic acid	0.67	0.70	0.05	0.78
Cystine	0.25	0.25	0.01	0.90
Glutamic acid	0.77	0.81	0.07	0.77
Glycine	0.36	0.39	0.03	0.60
Histidine	0.14	0.15	0.01	0.68
Isoleucine	0.29	0.31	0.02	0.59
Leucine	0.47	0.49	0.04	0.80
Lysine	0.44	0.49	0.04	0.53
Methionine	0.13	0.14	0.01	0.56
Phenylalanine	0.28	0.31	0.02	0.64
Proline	0.31	0.32	0.02	0.81
Serine	0.36	0.36	0.03	0.97
Threonine	0.33	0.35	0.03	0.76
Tyrosine	0.18	0.22	0.02	0.31
Valine	0.35	0.38	0.03	0.64
ATTD[1)], %				
Alanine	91.02	90.25	0.86	0.66
Arginine	95.90	95.54	0.40	0.67
Aspartic acid	92.44	92.19	0.61	0.84
Cystine	81.99	81.98	0.92	0.99
Glutamic acid	93.89	93.55	0.56	0.77
Glycine	91.48	90.90	0.68	0.68
Histidine	93.78	93.90	0.57	0.92
Isoleucine	92.32	91.70	0.63	0.64

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Table 4. Continued

Items	Experiment groups		SEM	p-value
	CON	TRT		
Leucine	93.08	92.91	0.60	0.89
Lysine	93.33	92.68	0.57	0.58
Methionine	93.88	93.52	0.51	0.73
Phenylalanine	92.70	92.50	0.62	0.87
Proline	91.70	91.68	0.66	0.99
Serine	90.94	91.14	0.75	0.90
Threonine	90.65	90.35	0.82	0.86
Tyrosine	85.37	82.56	1.35	0.31
Valine	91.44	90.88	0.75	0.72

[1)] ATTD was calculated as a following equation: ATTD = {(intake – excretion)/intake} ×100. A p-value for comparisons between CON and TRT group in a same row.

CON, control; TRT, treatment; ATTD, Apparent total tract digestibility; SEM, standard error of the mean.

TRT group, the difference between the two groups was not statistically significant ($p > 0.05$).

Hematological and biochemical parameters

Hematological parameters provide information regarding pre-existing anemia, possible infection, inflammatory responses, immune function, or stress; thus these can be used to assess the impact of chicken- or pork-based diets on canine health [24]. Biochemical parameters were assessed to evaluate the effects of these diets on canine hepatic function, kidney filtration capacity, other internal organs, and metabolic profiles. The values of all hematological parameters in both groups were within the reference range, and no significant differences were observed between the two groups ($p > 0.05$; Table 5).

The results of serum biochemical parameters revealed that the levels of alanine aminotransferase, aspartate aminotransferase, albumin, alkaline phosphatase, α-amylase, lipase, total protein, bilirubin, cholesterol, creatinine, glucose, and triglycerides were within the reference range in both groups, with no significant differences between the CON and TRT groups ($p > 0.05$). Although the concentration of lactate dehydrogenase (LDH) was significantly lower in the TRT group (56 U/L) than in the CON group (78 U/L) ($p < 0.05$; Table 6), it remained within the reference range (24–388 U/L) in both groups.

DISCUSSION

Nutrient digestibility and palatability

extensive research has been carried out to explore the nutritional value of pet food in depth. As dogs have been with humans for a long time, their health should be maintained through proper nutrition, in the form of balanced diets. A balanced diet contains essential nutrients (carbohydrates, fats, proteins, vitamins, and minerals) necessary for growth, maintenance, and reproduction [25]. Proteins are polymers of amino acids that not only serve as an energy source but also as raw materials for synthesizing bones, muscles, and blood proteins, such as those that transmit cellular messages and transport minerals [26]. The body is capable of synthesizing non-essential amino acids, while it cannot produce essential amino acids. Amino acid imbalance commonly occurs in dogs fed with commercial plant-based diets because plant proteins are deficient in some essential

Table 5. Hematological characteristics

Items	Ref. range (min-max)	Experiment groups		SEM	*p*-value
		CON	TRT		
WBC, ×10^6/mL	6.00–17.00	7.78	7.59	0.26	0.39
NEU, ×10^3/uL	3.62–12.30	5.23	5.08	0.23	0.46
LYM, ×10^3/uL	0.83–4.91	1.96	1.91	0.08	0.85
MONO, ×10^3/uL	0.14–1.97	0.34	0.34	0.01	0.59
EOS, ×10^3/uL	0.04–1.62	0.25	0.26	0.02	0.27
BASO, ×10^3/uL	0–0.12	0.01	0.01	0.00	1.00
RBC, ×10^6/uL	5.10–8.50	6.85	6.85	0.13	0.67
HGB, g/dL	11–19	16.30	16.20	0.31	0.69
HCT, %	33–56	44.80	44.60	0.80	0.65
MCV, fL	60–76	65.40	65.20	0.48	0.89
MCH, pg	20–27	23.80	23.70	0.17	0.96
MCHC, g/dL	30–38	36.30	36.30	0.15	0.92
RDW-CV, %	12.5–17.2	13.20	13.20	0.10	0.97
RDW-SD, fL	33.2–46.3	34.20	34.00	0.30	1.00
PLT, 10^3/Ul	117–490	305	308	20.00	0.67
MPV, fL	8–14.1	9.60	9.40	0.21	0.73
PCT, mL/L	0.9–5.80	2.87	2.84	0.16	0.68

A *p*-values for comparisons between CON and TRT group in a same row.

CON, control; TRT, treatment; WBC, white blood cell; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, Red blood cell distribution width-coefficient of variation; RDW-SD, Red blood cell distribution width-standard deviation; PLT, platelet; MPV, Mean platelet volume; PCT, plateletcrit; SEM, standard error of the mean.

Table 6. Serum biochemistry

Items	Ref. range (min-max)	Experiment groups		SEM	*p*-value
		CON	TRT		
ALT, U/L	17–95	37.20	38.30	3.60	0.88
AST, U/L	18–56	30.80	30.10	2.00	0.87
ALB, g/dL	3.2–4.1	4.11	4.11	0.09	1.00
ALP, U/L	7–115	40.60	62.90	6.80	0.10
AMYL, U/L	322–1,310	629.00	627.00	41.00	0.99
LDH, U/L	24–388	78.00	56.30	6.00	0.07
LIP, U/L	15–228	44.00	55.70	4.60	0.21
TP, g/dL	5.5–7.2	6.58	6.46	0.17	0.75
BIL, mg/dL	0–0.2	0.13	0.13	0.01	0.79
CHO, mg/dL	136–392	219.00	209.00	5.00	0.30
CRE, mg/dL	0.6–1.4	0.62	0.63	0.02	0.80
GLU, mg/dL	68–104	91.80	95.60	1.70	0.26
TRI, mg/dL	23–102	71.20	66.40	7.00	0.74

A *p*-values for comparisons between CON and TRT group in a same row.

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALB, Albumin; ALP, Alkaline Phosphatase; AMYL, α-Amylase; LDH, Lactate Dehydrogenase; LIP, Lipase; TP, Total protein; BIL, Bilirubin; CHO, Cholesterol; CON, control; CRE, Creatinine; GLU, glucose; TRIGL, Triglycerides; TRT, treatment; SEM, standard error of the mean.

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amino acids [27]. Therefore, an amino acid supply from animal-sourced proteins such as meat is necessary to maintain dog health.

In this study, the chemical properties and amino acid composition of the feed prepared using pork hind legs as the primary protein source were measured to confirm its nutritional components for dogs. The results revealed that the TRT diet had a higher NFE value than the CON diet; however, the amino acid composition of the two diets did not significantly differ (Table 1). Similar results were observed for the average daily nutrient intake, as the NFE intake in the TRT group was significantly higher than that in the CON group. However, the two diets did not significantly differ in terms of the average daily nutrient excretion and ATTD, suggesting that the actual digestibility of the TRT diet was maintained even when the NFE value was high (Table 3). There were no significant differences in the intake, excretion, and ATTD of other nutrients (DM, CP, crude fat, CA, and total amino acids) between the CON and TRT groups. Furthermore, the protein and amino acid digestibility of pork hind legs (Table 3) was similar to that of chicken. These results indicate that pork has sufficient nutritional value as a protein source for dogs. Oba et al. [28] used a cecectomized rooster model to assess the amino acid digestibility of chicken meal and reported that the amino acid digestibility of chicken exceeded 80%. Based on its CP content, essential amino acid concentration, and digestibility, chicken can be considered a moderate- to high-quality protein source according to the National Research Council, European Pet Food Industry Federation, and AAFCO guidelines. In the present study, the amino acid digestibility of the CON diet exceeded 80%, similar to that reported by Oba et al. [28]. As the TRT diet did not significantly differ from the CON group in terms of amino acid digestibility, our findings suggest that pork could also be a potential protein source for dog food (Table 4).

Pet food requires higher palatability than livestock feed so that pet owners may gain satisfaction from their purchase of pet food. In the two-bowl palatability test conducted by Hall et al. [29], 89% of dogs initially selected the bowl containing pet food with four different flavors, suggesting that olfactory preference is a determining factor for palatability, consistent with the palatability assessment results of the present study. The significantly higher completion frequency of intake in the TRT group than in the CON group ($p = 0.01$) indicates that while there was no difference in olfactory values affecting the initial approach frequency, the taste of the feed significantly affected the time needed to complete feed intake (Table 7). Therefore, dogs may find that feed containing pork tastes better than feed containing chicken. Amino acids have a significant impact on taste

Table 7. Palatability

Items[1)]	Experiment groups		SEM	*p*-value[2)]
	CON	TRT		
First approach order, score	1.5	1.5	0.15	-
First approach frequency, % (n/total n)	50 (6/12)	50 (6/12)	-	1.00
First intake order, score	1.58	1.42	0.15	-
First intake frequency, % (n/total n)	58 (7/12)	42 (5/12)	-	0.41
Completion order of intake, score	1.25	1.75	0.13	-
Completion frequency of intake, % (n/total n)	25 (3/9)	75 (6/9)	-	0.01
Time to completion of intake, seconds	115.5	102.5	26.90	0.78

[1)]Scoring: 2 points for first approach, intake and completion, 1 point for second approach, intake and completion, intake completion time (seconds).

[2)]Except for the time to completion of intake, which was analyzed using a t-test, statistical analyses of all items were conducted using frequency analysis using Pearson chi-square. A *p*-values for comparisons between CON and TRT group in a same row.

CON, control; SEM, standard error of the mean; TRT, treatment.

compound formation, as taste is generated from the enzymatic or chemical conversion of amino acids resulting from the breakdown of proteins in raw materials [30]. In the present study, although there was no significant difference in total amino acid levels between the CON and TRT diets, differences in the ratios of administered amino acids may have resulted in variations in taste (Table 4).

The present study demonstrated that pork has a similar ATTD of nutrients and palatability to chicken as an ingredient in dog food. However, the palatability results were limited to a relative assessment of a chicken-based diet. Each protein source has its unique taste and aroma, which can lead to varying preferences among the dogs. Therefore, it is essential to compare a pork-based diet not only with a chicken-based diet but also with other protein sources such as duck and beef-based diets to clearly assess the dogs' preference for pork. Therefore, further research is needed to include a wider variety of protein sources in dog food.

Safety and health parameters

The present study assessed the body weight, BCS, average daily food intake, metabolic energy intake, and fecal scores of all experimental animals. No negative changes were observed in any of the animals, and no significant differences were observed between the CON and TRT diets (Table 2). Recent studies have verified that the nutritional and health-promoting values of pork have greatly improved [31,32]. As meat quality is important for the health, safety, and availability of meat, the possibility of pork being a protein source in feed could increase in the future [33].

There were no significant differences in the hematological chromatography between the two diets, and all values were within the reference ranges (Table 5). Serum biochemistry results also showed no significant differences between the two diets; however, LDH levels were lower in the TRT group than in the CON group ($p = 0.07$). LDH catalyzes the conversion of lactate to pyruvate during anaerobic glycolysis, and an increase in LDH levels in the blood can occur because of cellular damage during disease [34]. However, as the LHD levels in both groups were within the reference range, it did not seem to be a health problem (Table 6).

No negative clinical signs were observed in the CON and TRT groups during the experimental period. The present study demonstrated the safety of feed containing pork hind legs. However, further research considering factors such as duration of intake, breed, and age is necessary for a more precise assessment of safety.

The present study confirms the potential of pork as a protein source in dog food because its digestibility, palatability, and safety were comparable to those of chicken. To the best of our knowledge, this is the first study to evaluate the value of dog food with pork as the primary protein source. The results of this study are expected to contribute to the diversification of dog food ingredients. However, further research is required to evaluate a wider variety of protein sources in dog food.

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