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<b>Authors' contributions</b> Please specify the authors' role using this form.	<p>Conceptualization: Kim KH</p> <p>Data curation: Seo K</p> <p>Formal analysis: Seo K, Kim KH</p> <p>Methodology: Kim KH</p> <p>Validation: Lee SY, Seo K, Kim KH</p> <p>Investigation: Bae IS, Cho HW, Lee MY, So KM, Chun JL</p> <p>Writing - original draft: Lee SY</p> <p>Writing - review &amp; editing: Lee SY, Seo K, Bae IS, Cho HW, Lee MY, So KM, Chun JL, Kim KH</p>
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**(Unstructured) Abstract (up to 350 words)**

9 The protein content of pet food affects its metabolizable energy content and palatability.  
10 Although pork is a high-quality protein source, it is not commonly used in pet food due to the  
11 lack of verification of its potential as a primary protein source. Hence, in this study, the  
12 potential of pork as a protein source in pet food was verified through digestibility testing  
13 involving beagle dogs. A pork-based diet made from pork hind legs and a chicken-based diet  
14 were provided to 12 beagle dogs. The palatability and digestibility of nutrients of the pork-  
15 based diet were compared with those of the chicken-based diet. The results showed that the  
16 palatability and apparent total tract digestibility of nutrients (dry matters, crude fat, crude ash,  
17 nitrogen-free extract, and amino acids) of the pork-based diet were similar to those of the  
18 chicken-based diet. This study suggests that pork hind legs can be used as a protein source in  
19 dog food.  
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21 **Keywords (3 to 6):** dog food; pet food; protein sources; chicken; pork; ATTD  
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## Introduction

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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 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 [9].

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

59 chicken, duck, and beef are relatively high. Consequently, there is a surplus of pork in Korea,  
60 and the inventory of pork parts significantly varies; among the different pork parts, the  
61 inventory of pork hind legs is the highest (35.6 %) (13). If the potential of pork as a protein  
62 source in pet food is verified, less commonly used livestock products, such as pork hind legs,  
63 could be incorporated as ingredients in pet food.

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

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

75

## 76 **Materials and Methods**

### 77 **Preparation of Experimental Diets**

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

91

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

92

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

93

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

96

## 97 **Animals**

98 A total of 12 healthy 4-year-old beagle dogs weighing  $12.70 \pm 0.2$  kg (eight spayed females  
99 and four castrated males) owned by the National Institute of Animal Science (NIAS) were  
100 analyzed in this study. "Approval for the animal experiments performed in this study was  
101 obtained from the Animal Care and Use Committee of the NIAS (NIAS-2022-0585). Each dog  
102 was kept in a separate indoor area measuring  $1.7 \text{ m} \times 2.1 \text{ m}$ , where they were maintained at a  
103 stable temperature of  $22\text{--}24$  °C and subjected to a consistent lighting cycle of 12 hours of light  
104 followed by 12 hours of darkness. During the experimental period, each dog was provided with  
105 free outdoor access for approximately 3 hours daily in an individual outdoor space ( $2.8 \text{ m} \times$   
106  $2.5 \text{ m}$ ) connected to the indoor space. The experimental diets were provided twice daily in  
107 amounts estimated using the maintenance energy requirement (MER) equation (equation 3)  
108 proposed by the AAFCO, and water was provided ad libitum (17).

109

$$MER = 110 \text{ kcal} \times \text{metabolic body weight (mBW, kg; BW}^{0.75}), \quad (3)$$

110

## 111 **Fecal Sampling and Chemical Analysis**

112 The dogs were provided with the CON diet for 15 days, followed by the TRT diet for another  
113 15 days. An adaptation period of 10 days was allowed before each experimental diet was  
114 provided. The diet intake and fecal output were recorded daily, while body weight was recorded  
115 on a weekly basis. The body condition score (BCS) was evaluated weekly according to the  
116 nine-point BCS scale proposed by Laflamme et al. (19). The fecal score was measured daily  
117 using a five-point fecal score scale (1 = dry; 5 = liquid feces) according to the Waltham Fecal  
118 Scoring System and expressed as an average value (20). Fecal samples were gathered five days  
119 before the end of the experiment and preserved at  $-20$  °C until they were analyzed further. The  
120 chemical composition of the experimental diets and fecal samples was assessed following the  
121 standard procedures established by the Association of Official Analytical Chemists (AOAC)  
122 for moisture content (AOAC method 934.01), crude protein (CP; AOAC method 984.13),  
123 crude fat (ether extract, EE; AOAC method 920.39), crude ash (CA; AOAC method 942.05),  
124

125 and crude fiber (CF; AOAC method 978.10) (21). The experimental diets and fecal samples  
126 were subjected to amino acid analysis after they had been hydrolyzed using the  
127 chromatography method described by Llames and Fontaine (22). The apparent total tract  
128 digestibility (ATTD) of nutrients (DM, crude fat, CA, NFE, and amino acids) in the  
129 experimental diets was estimated using the total collection method and calculated using  
130 equation (4):

131

$$132 \text{ ATTD of nutrients (\%)} = \left( \frac{\text{nutrient input(food)} - \text{nutrient output(fecal)}}{\text{nutrient input(food)}} \right) \times 100, \quad (4)$$

133

134 At the end of the experimental period, blood samples were obtained from the cephalic vein of  
135 each dog and immediately promptly separated into EDTA collection tubes (REF 41.1395.105;  
136 Sarstedt, Nümbrecht, Germany) and serum vacutainer tubes (REF 367812; BD Vacutainer,  
137 Franklin Lakes, NJ, USA). The blood samples contained in the EDTA collection tubes were  
138 analyzed for a complete blood count using an automated hematology analyzer (BC-5000 Vet;  
139 Mindray, Shenzhen, China), and the serum derived from the blood samples in the serum  
140 vacutainer tube was separated by centrifugation ( $3,000 \times g$ , 10 min) and then stored at  $-80^\circ\text{C}$   
141 until further analysis. The biochemical parameters of the serum were assessed using an  
142 automated clinical chemistry analyzer (Cobas c111; Roche Diagnostics International, Risch,  
143 Switzerland).

144

#### 145 **Palatability Assessment**

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

154

#### 155 **Statistical Analysis**

156 All statistical analyses conducted in this study were performed using SPSS version 17.0 (SPSS  
157 Statistics, IL, USA, 2009). The results of the BCS, fecal score, and palatability test were

158 analyzed using the Chi-square test, while the results of other tests were analyzed using a t-test.  
159 Differences were considered statistically significant at  $p < 0.05$ .

160  
161

## 162 **Results**

### 163 **Food Intake and Body Parameters**

164 Table 2 presents the effects of the pork-based diet on the food intake, body parameters, and  
165 fecal scores of the beagle dogs. No statistically significant differences were observed between  
166 the TRT and CON groups with respect to body weight, body weight gain, and BCS ( $p > 0.05$ ).  
167 Although the TRT group exhibited a tendency towards higher average daily food intake and  
168 ME intake than the CON group, these differences were not statistically significant ( $p > 0.05$ ).  
169 Both groups maintained a desirable fecal score (2.48–2.49) throughout the experimental period,  
170 and significant differences were not observed between the groups ( $p > 0.05$ ).

171

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

178

### 179 **Apparent Total Tract Digestibility**

180 The nutrient intake, fecal output, and ATTD of the two experimental diets are shown in Table  
181 4. No statistically significant differences were observed between the CON and TRT groups for  
182 the intake of DM, CP, crude fat, CA, and total amino acids ( $p > 0.05$ ). However, the intake of  
183 NFE and histidine was significantly higher in the TRT group than in the CON group ( $p < 0.05$ ;  
184 Table 4 and Table S1). Similarly, there were no statistically significant differences in the fecal  
185 output of any nutrients between the two groups ( $p > 0.05$ ). Although the ATTD of nutrients  
186 tended to be higher in the TRT group, the difference between the two groups was not  
187 statistically significant ( $p > 0.05$ ).

188

### 189 **Hematological and Biochemical Parameters**

190 Hematological parameters provide information regarding pre-existing anemia, possible  
191 infection, inflammatory responses, immune function, or stress; thus these can be used to assess



192 the impact of chicken- or pork-based diets on canine health (24). Biochemical parameters were  
193 assessed to evaluate the effects of these diets on canine hepatic function, kidney filtration  
194 capacity, other internal organs, and metabolic profiles. The values of all hematological  
195 parameters in both groups were within the reference range, and no significant differences were  
196 observed between the two groups ( $p > 0.05$ ; Table 5).

197

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

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## Discussion (optional)

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### 209 Nutrient Digestibility and Palatability

210 Extensive research has been carried out to explore the nutritional value of pet food in depth.  
211 As dogs have been with humans for a long time, their health should be maintained through  
212 proper nutrition, in the form of balanced diets. A balanced diet contains essential nutrients  
213 (carbohydrates, fats, proteins, vitamins, and minerals) necessary for growth, maintenance, and  
214 reproduction (25). Proteins are polymers of amino acids that not only serve as an energy source  
215 but also as raw materials for synthesizing bones, muscles, and blood proteins, such as those  
216 that transmit cellular messages and transport minerals (26). The body is capable of synthesizing  
217 non-essential amino acids, while it cannot produce essential amino acids. Amino acid  
218 imbalance commonly occurs in dogs fed with commercial plant-based diets because plant  
219 proteins are deficient in some essential amino acids (27). Therefore, an amino acid supply from  
220 animal-sourced proteins such as meat is necessary to maintain dog health.

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

242 Pet food requires higher palatability than livestock feed so that pet owners may gain satisfaction  
243 from their purchase of pet food. In the two-bowl palatability test conducted by Hall et al. (29),  
244 89% of dogs initially selected the bowl containing pet food with four different flavors,  
245 suggesting that olfactory preference is a determining factor for palatability, consistent with the  
246 palatability assessment results of the present study. The significantly higher completion  
247 frequency of intake in the TRT group than in the CON group ( $p = 0.01$ ) indicates that while  
248 there was no difference in olfactory values affecting the initial approach frequency, the taste of  
249 the feed significantly affected the time needed to complete feed intake (Table 3). Therefore,  
250 dogs may find that feed containing pork tastes better than feed containing chicken. Amino acids  
251 have a significant impact on taste compound formation, as taste is generated from the  
252 enzymatic or chemical conversion of amino acids resulting from the breakdown of proteins in  
253 raw materials (30). In the present study, although there was no significant difference in total  
254 amino acid levels between the CON and TRT diets, differences in the ratios of administered  
255 amino acids may have resulted in variations in taste (Table S1).

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

263

## 264 **Safety and Health Parameters**

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

272 There were no significant differences in the hematological chromatography between the two  
273 diets, and all values were within the reference ranges (Table 5). Serum biochemistry results  
274 also showed no significant differences between the two diets; however, LDH levels were lower  
275 in the TRT group than in the CON group ( $p = 0.07$ ). LDH catalyzes the conversion of lactate  
276 to pyruvate during anaerobic glycolysis, and an increase in LDH levels in the blood can occur

277 because of cellular damage during disease (34). However, as the LHD levels in both groups  
278 were within the reference range, it did not seem to be a health problem (Table 6).  
279 No negative clinical signs were observed in the CON and TRT groups during the experimental  
280 period. The present study demonstrated the safety of feed containing pork hind legs. However,  
281 further research considering factors such as duration of intake, breed, and age is necessary for  
282 a more precise assessment of safety.

283

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

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## Tables and Figures

Table 1. Chemical and amino acid compositions of experimental diets.

Items	Experimental diets <sup>1</sup>	
	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

<sup>1</sup>The control (CON) diet used chicken as the primary protein source, while the treatment (TRT) diet used pork as the main protein source. Abbreviations: NFE, Nitrogen-free extract; Ca, Calcium; P, Phosphorus.



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

Items	Experiment groups		SEM	p value
	CON	TRT		
Body weight, 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, ideal; 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). p values for comparisons between CON and TRT group in a same row. Abbreviations: ADFI, average daily food intake; MEI, metabolic energy intake; BW, body weight; BWG, body weight gain; BCS, body condition score; SEM, standard error of the mean.

Table 3. Palatability

Items <sup>1</sup>	Experiment groups		SEM	p value <sup>2</sup>
	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, second	115.5	102.5	26.90	0.78

<sup>1</sup>Scoring: 2 points for first approach, intake and completion, 1 point for second approach, intake and completion, intake completion time (seconds); <sup>2</sup>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. p values for comparisons between CON and TRT group in a same row. Abbreviations: SEM, standard error of the mean.

Table 4. 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 <sup>1</sup> , %				
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

<sup>1</sup>ATTD was calculated as a following equation:  $ATTD = \{(intake - excretion)/intake\} \times 100$ . p values for comparisons between CON and TRT group in a same row. Abbreviations: NFE, Nitrogen-free extract; ATTD, Apparent total tract digestibility; SEM, standard error of the mean.

Table 5. Hematological characteristics

Items	Ref. range (min-max)	Experiment groups		SEM	p value
		CON	TRT		
WBC, ×10 <sup>6</sup> /mL	6.00 – 17.00	7.78	7.59	0.26	0.39
NEU, ×10 <sup>3</sup> /uL	3.62 – 12.30	5.23	5.08	0.23	0.46
LYM, ×10 <sup>3</sup> /uL	0.83 – 4.91	1.96	1.91	0.08	0.85
MONO, ×10 <sup>3</sup> /uL	0.14 – 1.97	0.34	0.34	0.01	0.59
EOS, ×10 <sup>3</sup> /uL	0.04 – 1.62	0.25	0.26	0.02	0.27
BASO, ×10 <sup>3</sup> /uL	0 – 0.12	0.01	0.01	0.00	1.00
RBC, ×10 <sup>6</sup> /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 <sup>3</sup> /U1	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

p values for comparisons between CON and TRT group in a same row. Abbreviations: 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 – 1310	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

p values for comparisons between CON and TRT group in a same row. Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALB, Albumin; ALP, Alkaline Phosphatase; AMYL,  $\alpha$ -Amylase; LDH, Lactate Dehydrogenase; LIP, Lipase; TP, Total protein; BIL, Bilirubin; CHO, Cholesterol; CRE, Creatinine; GLU, glucose; TRIGL, Triglycerides; SEM, standard error of the mean.