RESEARCH ARTICLE

J Anim Sci Technol 2025;67(5):1165-1180
https://doi.org/10.5187/jast.2500227

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Age-related changes in growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry of Beagle dogs

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Abstract
The objective of this study was to evaluate the effects of age on growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry of Beagle dogs. A total of 18 healthy Beagles were allotted into three groups according to age (puppy, under 1 year old; adult, 1–7 years old; senior, over 7 years old). The study lasted for 17 days, with a 7-day adaptation period and 10-day evaluation period. During the evaluation period, individual body weight, length, and food intake were estimated and recorded to calculate growth parameters. Fecal samples were collected from all beagles to evaluate fecal score and nutrient digestibility by visual observation and chemical analyses, respectively. Blood samples were collected from each dog to evaluate hematological and biochemical parameters using an automated hematology analyzer and clinical autoanalyzer, respectively. Puppy group had increased ($p < 0.05$) body weight change during the study compared with adult and senior groups. At the end of the food trial, body condition score of all age groups were ideal. Puppies had higher ($p < 0.05$) fecal moisture content and diarrhea frequency than adult and senior dogs. Senior group had lower ($p < 0.05$) apparent total tract digestibility (ATTD) of crude protein than puppy and adult groups. Puppy group had lower ($p < 0.05$) ATTD of ether extract, crude fiber, and nitrogen-free extract than adult and senior groups. Puppies had higher number of white blood cells and neutrophils at the initial ($p < 0.05$) and final ($p = 0.062$) day of the evaluation period than senior dogs. Senior dogs had lower ($p < 0.05$) levels of serum albumin, glucose, and creatinine during the evaluation than puppies. Puppy group had higher ($p < 0.05$) serum alkaline phosphate level at the initial and final day of the evaluation than adult and senior groups. In conclusion, our study determined the effect of age on growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry of Beagle dogs.

Keywords: Apparent total tract digestibility, Beagles, Biochemical parameters, Complete blood count

Received: Jun 25, 2025
Revised: Jul 14, 2025
Accepted: Jul 15, 2025

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Competing interests
No potential conflict of interest relevant to this article was reported.

Funding sources
This study was supported by the Cooperative Research Program for Agriculture Science and Technology Development (RS-2023-00230754; RS-2024-00398491), Rural Development Administration, Korea.

Acknowledgements
Not applicable.

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

Authors' contributions
Conceptualization: Kang Y, Kim Y, Cho JH, Kim HB, Kim MK, Song M, Kyoung H.
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Ethics approval and consent to participate
The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval #: 202310A-CNU-179).

INTRODUCTION

Companion animals are considered family members who share our lives. Changes in population structure, socioeconomic development, and cultural changes have led to an increase in companion animal ownership worldwide, leading to greater interest in their health and well-being [1–4]. People are increasingly concerned about the quality of the foods for their companion animals, including protein sources, and are investing generously in high-quality and functional products to promote animal health [4,5]. In addition, as the lifespan of companion dogs has increased due to improved veterinary care and quality of canine foods, it is important to understand the physiological changes for nutritional management throughout their life cycle [6,7]. As a result, academic efforts to optimize nutrient utilization and promote health throughout the lifespan of dogs are becoming increasingly important. For this purpose, several organizations such as the National Research Council (NRC), the Association of American Feed Control Officials (AAFCO), and the European Pet Food Industry Federation (FEDIAF) have presented recommendations for nutrient profiles for dogs [8–10]. However, these standards may have limitations in reflecting the diversity of all breeds and ages, and the results of previous studies on nutrient utilization and health are diverse. Thus, it is necessary to investigate age-related physiological changes through various studies.

Nutritional physiology of companion dogs undergoes dynamic changes throughout their life stages. In particular, during the growth period, high nutrient requirements are needed due to tissue development, skeletal formation, and immune system maturation [11–14]. On the other hand, during the aging period, appropriate nutritional management is essential for maintaining health due to decreased digestive efficiency, altered gut microbiota, and reduced immune functions [13,15,16]. Thus, aging is a key variable that influences the digestive function, nutrient metabolism, immune responses, and tissue functions. Previous studies on improving the health of companion dogs have evaluated various indicators such as nutrient digestibility, hematological and biochemical parameters, and gut microbial changes, however these studies focused on one or two age groups [7,17,18]. Therefore, a more comprehensive approach is needed to characterize the age-related physiological changes in dogs fed same diet. The present study hypothesized that there would be changes in growth and fecal characteristics, nutrient digestibility, and hematological and biochemical indices of dogs with age, even when fed same canine foods. Physiological differences are an essential consideration for food formulation and age-specific health management. Therefore, this study aimed to evaluate growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry of three life stages of Beagle dogs fed the same diet.

MATERIALS AND METHODS

Animal ethics

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, South Korea (approval #: 202310A-CNU-179).

Experimental design, animals, and diet

Eighteen clinically healthy Beagle dogs were equally divided into three age groups: puppies (n = 6; under 1 year old; average age of 48.2 ± 11.3 wk; mean body weight [BW] = 6.21 ± 0.61 kg; 2 neutered males and 4 neutered females), adults (n =6; 1–7 years old; average age of 4.5 ± 2.1 years; mean BW = 8.16 ± 0.70 kg; 3 neutered males and 3 neutered females), and senior dogs (n = 6; older than 7 years; average age of 9.5 ± 0.8 years; mean BW = 6.95 ± 1.52 kg; 3 neutered males and 3

 https://doi.org/10.5187/jast.2500227

neutered females). Dogs were individually housed in stainless steel cage (0.9 × 0.9 × 1.0 m) with automatically controlled temperature throughout the study (diet adaptation from days –7 to 0; data and sample collection from days 1 to 10). The experimental diet was formulated using hydrolyzed chicken powder, brown rice, and soy protein to meet nutrient profiles for growth and reproduction minimum (crude protein and indispensable amino acids) or adult maintenance minimum (crude fat, calcium [Ca], phosphorus [P], and Ca to P ratio) provided by the AAFCO and manufactured in extruded form (Tables 1 and 2) [19]. The dog food was provided by calculating maintenance energy requirements (MER) for each dog (puppy, 132 × $BW^{0.75}$; adult, 132 × $BW^{0.75}$; senior, 105 × $BW^{0.75}$) based on standard equations [20]. All dogs were fed twice daily (09:00 and 17:00).

Data and sample collection

After the food adaptation period, individual dog BW and body condition score (BCS) were weighed and estimated at the beginning and end of the data and sample collection period and food intake was recorded each day. Body weight change (BWC) and daily food intake (DFI) were calculated for growth parameters of Beagle dogs. The BCS of each dog was measured using a frame

Table 1. Composition of experimental food for Beagle dogs (as-fed basis)

Item	Basal diet
Ingredient, %	
Hydrolyzed chicken powder	35.00
Brown rice	32.65
Soy protein	15.00
Tapioca starch	5.00
Canola oil	3.00
Sweet pumpkin	2.00
Cabbage	2.00
Carrot	1.00
Monocalcium phosphate	1.80
Calcium carbonate	1.60
Vitamin-mineral premix[1)]	0.50
Tocopherol	0.05
Salt	0.40
Total	100.00
Calculated composition	
Dry matter, %	91.09
Metabolizable energy[2)], kcal/kg	3,707
Crude protein, %	40.17
Crude fat, %	6.23
Crude fiber, %	2.72
Calcium, %	0.79
Phosphorus, %	0.66
Crude ash, %	6.55
Nitrogen-free extract, %	35.42

[1)] Vitamin and mineral premix supplied per kg of diets: 3,500 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 $FeSO^4$; 3.8 mg $MnSO^4$; 50 mg $ZnSO^4$; 7.5 mg $CuSO^4$; 0.18 mg Na^2SeO^3; 0.9 mg Ca $(IO^3)^2$.

[2)] Metabolizable energy (ME) was calculated follow equation; ME (kcal/kg) − ([CP × 3.5] + [EE × 8.5] + [NFE × 3.5]) × 10.

Table 2. Comparison of nutritional composition of experimental foods with Association of American Feed Control Officials (AAFCO) guidelines (dry matter-basis)

Item	AAFCO			Basal diet[1)]
	Growth and reproduction minimum	Adult maintenance minimum	Maximum	
Crude protein, %	22.50	18.00		38.82
Arginine. %	1.00	0.51		1.28
Histidine, %	0.44	0.19		0.44
Isoleucine, %	0.71	0.38		0.84
Leucine, %	1.29	0.68		1.41
Lysine, %	0.90	0.63		1.15
Methionine, %	0.35	0.33		0.37
Methionine-cysteine, %	0.70	0.65		0.83
Phenylalanine, %	1.30	0.74		0.87
Threonine, %	1.04	0.48		1.06
Tryptophan, %	0.20	0.16		0.31
Valine, %	0.68	0.49		0.76
Crude fat, %	8.50	5.50		6.65
Calcium, %	1.20	0.50	2.50[2)]	0.78
Phosphorus, %	1.00	0.40	1.60	0.65
Calcium:phosphorus	1:1	1:1	2:1	1.2:1

1) Analyzed composition.

2) The maximum calcium content level on a dry matter basis for other life stages including non-large size, and for large size dogs (those weighing 70 pounds or more as mature), the maximum is 1.8% [19].

marked with the length of chest and abdomen, and the ratio was calculated to evaluate fatness related to health conditions [21]. The BCS scale ranged from 1 to 9 (under BW to over BW), with an ideal score of 4 to 5, according to the American Animal Hospital Association [22]. Blood samples were collected from each dog using 5 mL tubes (BD Vacutainer Systems) with or without ethylenediaminetetraacetic acid (EDTA) via leg vein on day 1 and 10 after a 12 h fasting period [23]. The collected blood samples in non-EDTA tubes were centrifuged at 3,000 rpm for 15 min at 4°C to obtain serum samples and stored at –80℃ for biochemical analysis [24]. Fecal samples were collected at least twice daily from days 2 to 9 before feeding to evaluate the fecal score and nutrient digestibility using visual observations and total collection method, respectively [25,26]. The fecal score was recorded from 1 to 5 (hard dry to watery diarrhea) with half numerical increments by two independent visual evaluators [27]. The frequency of diarrhea or hard dry feces was calculated by counting cage days with a pen fecal score ≥ 3.5 or < 2.0, respectively. During total fecal collection, feces were collected, weighed, and stored at –80°C with diet until nutrient digestibility analyses.

Nutrient digestibility analyses

Frozen diet and fecal samples were thawed at room temperature, dried in a forced-air oven (FC-PO-91, LabHouse) at 65°C for 72 h, and ground using a coffee grinder (Electric Coffee Grinder, Hamilton Beach) for chemical analyses [28]. The ground samples were analyzed for dry matter, energy (bomb calorimeter; C2000, IKA Works), crude protein (Kjeldahl method; VAPOXX, Gerhardt), ether extract, crude fiber, crude ash, nitrogen-free extract (NFE), calcium, and phosphorus according to the Association of Official Analytical Chemists methods [29]. Amino acid contents were analyzed using high-performance liquid chromatography (Waters Alliance System with a Waters 1525 Binary HPLC Pump, Waters 2707 Autosampler, and Waters 2475 Multi λ Fluorescence Detector) [30]. The analyzed chemical compositions were calculated to determine apparent total tract digestibility (ATTD) according to a previous report [31].

Hematological and biochemical analyses

The blood samples collected in tubes containing EDTA were analyzed for complete blood count (CBC) using an automated hematology analyzer (Mindray BC-5000 auto hematology analyzer, Mindray Medical France Sarl, HyTest). The CBC test included fifteen parameters: number of white blood cells (WBC) and their differentiation counts and proportions (neutrophil, lymphocyte, monocyte, and eosinophil) and red blood cell profile (hematocrit, mean cell volume [MCV], mean cell hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red cell distribution width-coefficient of variation [RDW-CV], red cell distribution width-standard deviation [RDW-SD], platelet, and mean platelet volume [MPV]). Serum samples were analyzed for biochemical parameters using a clinical autoanalyzer (Roche Cobas c 111 chemistry analyzer, Roche Diagnostics). The serum biochemical test included eleven parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), creatinine, calcium, albumin, glucose, total protein, triglyceride, total cholesterol, and blood urea nitrogen (BUN).

Statistical analyses

Data were analyzed using the GLM procedure of SAS software (v. 9.4; SAS Institute) in a completely randomized design. The experimental unit was the Beagle dog. Differences among treatments means were determined using Tukey's multiple range test. Diarrhea and hard dry feces frequency were analyzed using the Chi-square test of SAS. Differences were considered statistically significantat $p < 0.05$ and a trend at $p < 0.10$.

RESULTS

Growth and fecal parameters

Puppies had increased ($p < 0.05$) BWC during the overall experiment compared with adults and senior beagles (Table 3). The DFI values (as-is, DM basis, calorie basis) were highest ($p < 0.05$) in puppies, followed by adults and senior dogs. In addition, daily fecal output (DFO) values (as-is and DM basis) were highest ($p < 0.05$) in puppies, followed by adults and senior dogs. Puppies were higher ($p < 0.05$) calorie-based DFO than adults and senior dogs. However, there was no difference on calorie-based DFO between the adult and senior groups. In fecal characteristics, puppies had higher ($p < 0.05$) fecal moisture content than senior dogs. Additionally, puppies had higher ($p < 0.05$) diarrhea frequency than adults and senior dogs. However, there were no difference on fecal score and hard dry feces frequency among the age groups.

Nutrient digestibility

The ATTD using the total collection method among age groups are shown in Table 4 and 5. Senior dogs had lower ($p < 0.05$) ATTD of crude protein than puppies and adults (Table 4). In addition, senior dogs tended to have lower ($p = 0.082$) crude ash digestibility than puppies. Puppies had lower ($p < 0.05$) ATTD of ether extract, crude fiber, NFE, and phosphorus than adults and senior dogs. The ATTD of calcium was highest ($p < 0.05$) in senior dogs, followed by adults and puppies. However, there were no differences on the ATTD of indispensable and dispensable amino acids among age groups (Table 5).

Hematological and biochemical profiles

The hematological and biochemical profiles are shown in Tables 6 and 7, respectively. At the

Table 3. Age-related changes in growth and fecal parameters of Beagle dogs[1)]

Item[2)]	Puppy	Adult	Senior	SEM	*p*-value
Body size					
Initial body weight, kg	6.21[b]	8.16[a]	6.95[ab]	0.42	0.016
Final body weight, kg	6.94	8.23	6.91	0.47	0.104
Body weight change, g/d	72.50[a]	7.17[b]	–3.83[b]	11.54	< 0.001
Initial body condition score	5.50	4.50	5.58	0.58	0.369
Final body condition score	5.00	4.67	4.58	0.37	0.711
Food intake					
Daily food intake, g/d (as-is)	210.33[a]	172.33[b]	121.53[c]	6.54	< 0.001
Daily food intake, g/d (DM basis)	191.60[a]	156.99[b]	110.71[c]	5.95	< 0.001
Daily food intake, kcal/d	932.52[a]	764.04[b]	538.81[c]	28.98	< 0.001
Feces					
Daily fecal output, g/d (as-is)	63.75[a]	44.80[b]	31.76[c]	2.16	< 0.001
Daily fecal output, g/d (DM basis)	20.98[a]	16.11[b]	12.32[c]	1.01	< 0.001
Daily fecal output, kcal/d	66.27[a]	49.64[b]	39.82[b]	3.41	< 0.001
Fecal score	3.07	2.73	2.63	0.15	0.123
Fecal moisture, %	67.12[a]	63.97[ab]	61.35[b]	1.11	0.008
Diarrhea frequency, %	27.08[a]	8.33[b]	6.25[b]		0.022
Hard dry feces frequency, %	0.00	0.00	4.17		0.143

[1)]Each value is the mean of 6 replicates (one dog per cage).
[2)]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old; Diarrhea frequency = (number of fecal score ≥ 3.5 / number of cage days) × 100; Hard dry feces frequency = (number of fecal score < 2.0 / number of cage days) × 100.
[a–c]Means with different superscript letters within a row are different ($p < 0.05$).

Table 4. Age-related changes in apparent total tract nutrient digestibility of Beagle dogs[1)]

Item[2)]	Puppy	Adult	Senior	SEM	*p*-value
Dry matter, %	89.07	89.76	88.95	0.39	0.323
Organic matter, %	95.49	96.10	95.27	0.32	0.192
Gross energy, %	92.90	93.51	92.68	0.37	0.289
Crude protein, %	92.39[a]	91.77[a]	89.99[b]	0.35	0.001
Ether extract, %	82.66[b]	85.28[a]	85.53[a]	0.57	0.005
Crude fiber, %	79.82[b]	83.57[a]	83.80[a]	0.60	< 0.001
Crude ash, %	64.58[x]	61.76[xy]	58.87[y]	1.66	0.082
Nitrogen-free extract, %	90.51[b]	92.42[a]	92.52[a]	0.34	0.001
Calcium, %	82.49[c]	84.45[b]	86.47[a]	0.62	0.002
Phosphorus, %	80.27[b]	85.01[a]	85.35[a]	0.64	< 0.001

[1)]Each value is the mean of 6 replicates (one dog per cage).
[2)]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old.
[a–c]Means with different superscript letters within a row are different ($p < 0.05$).
[x,y]Means with different superscript letters within a row are different ($p < 0.10$).

beginning of the food trial, puppies had higher ($p < 0.05$) number of WBC than adults and senior dogs (Table 6). In addition, puppies had higher ($p < 0.05$) number of neutrophils and monocytes than adults and senior dogs. The MCHC level was the highest in the adult group followed by the puppy group and the senior group ($p < 0.05$). On the last day of the study, puppies have tended to higher WBC ($p = 0.062$) and neutrophil ($p = 0.096$) counts than senior dogs. Additionally, the puppy group had higher ($p < 0.05$) lymphocyte counts on day 10 than the adult group. The

 https://doi.org/10.5187/jast.2500227

Table 5. Age-related changes in apparent total tract amino acid digestibility of Beagle dogs[1)]

Item[2)]	Puppy	Adult	Senior	SEM	p-value
Indispensable amino acids, %					
Arginine	97.78	97.93	97.78	0.09	0.462
Histidine	97.63	97.86	97.73	0.11	0.339
Isoleucine	97.68	97.83	97.68	0.09	0.443
Leucine	97.68	97.83	97.67	0.09	0.416
Lysine	97.77	97.91	97.76	0.09	0.456
Methionine	97.69	97.85	97.72	0.13	0.671
Phenylalanine	97.70	97.85	97.71	0.09	0.450
Threonine	97.73	97.88	97.73	0.09	0.428
Tryptophan	97.59	97.75	97.63	0.15	0.750
Valine	97.62	97.77	97.62	0.10	0.467
Dispensable amino acids, %					
Alanine	97.53	97.71	97.55	0.16	0.693
Aspartic acid	97.68	97.83	97.67	0.09	0.386
Cysteine	97.40	97.57	97.41	0.12	0.548
Glutamic acid	97.70	97.85	97.69	0.08	0.357
Glycine	97.67	97.82	97.67	0.09	0.426
Proline	97.74	97.89	97.74	0.09	0.447
Serine	97.68	97.83	97.68	0.11	0.547
Tyrosine	98.08	98.22	98.12	0.11	0.681

[1)]Each value is the mean of 6 replicates (one dog per cage).

[2)]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old.

neutrophil proportion was higher ($p < 0.05$) on day 10 in adult dogs than in senior dogs. In the red blood cell profile, the puppy group had higher ($p < 0.05$) MCV level on day 10 than the adult and senior groups. The MCH level was higher ($p < 0.05$) in puppies than in senior dogs. The adult group tended to have a higher ($p = 0.080$) MCHC level than the senior group. The RDW-CV and RDW-SD values were higher ($p < 0.05$) in senior dogs than in adult dogs. Puppies had lower ($p < 0.05$) MPV level on day 10 than the adults and senior groups.

In the serum biochemistry, adult dogs had lower ($p < 0.05$) albumin, glucose, and creatinine levels than puppies or senior dogs at the start of the study (Table 7). Senior dogs tended to have higher ($p = 0.085$) serum triglyceride level than puppies. Adult dogs tended to have a higher ($p = 0.072$) the BUN level than puppies. The puppy group had a higher ($p < 0.05$) level of ALP in serum than the adult and senior groups. On the last day of the food trial, senior dogs had lower ($p < 0.05$) serum albumin, glucose, and creatinine levels than puppies and adults. However, levels of serum BUN, calcium, and ALP were higher ($p < 0.05$) in the puppy group than the adult and senior groups.

DISCUSSION

Our study demonstrated that Beagles fed the same diet showed age-related differences in growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry. These findings reflect physiological and metabolic differences in nutrient utilization according to age and suggest the need for age-related feeding programs in canine food design. During the pre-adult period (< 1 year of age; post-weaning and growing period), MER is high due to high nutritional metabolic demands for growth and body tissue synthesis [20]. On the other hand, during adult and senior periods, food intake is relatively low compared to puppies because it is recommended to meet the

Table 6. Age-related changes in hematological profile of Beagle dogs[1)]

Item[2)]	Puppy	Adult	Senior	SEM	*p*-value
Day 1					
White blood cell, ×10³/μL	10.70[a]	7.56[b]	7.60[b]	0.83	0.026
Neutrophil, ×10³/μL	7.56[a]	5.14[b]	5.07[b]	0.70	0.040
Lymphocyte, ×10³/μL	2.01	1.70	1.64	0.19	0.354
Monocyte, ×10³/μL	0.51[a]	0.31[b]	0.27[b]	0.03	< 0.001
Eosinophil, ×10³/μL	0.53	0.39	0.56	0.12	0.531
Neutrophil, %	69.77	67.81	66.46	2.29	0.599
Lymphocyte, %	19.22	22.49	21.78	1.78	0.415
Monocyte, %	4.94	4.25	3.85	0.53	0.360
Eosinophil, %	5.14	5.15	7.12	1.15	0.400
Red blood cell, ×10⁶/μL	6.24	6.84	6.13	0.26	0.157
Hemoglobin, g/dL	14.78	16.07	13.93	0.67	0.109
Hematocrit, %	41.37	44.12	39.72	1.79	0.245
MCV fL	66.37	64.55	64.72	0.99	0.381
MCH, pg	23.67	23.53	22.67	0.41	0.202
MCHC, g/dL	35.68[b]	36.43[a]	35.05[c]	0.20	0.001
RDW-CV, %	13.32	12.87	13.37	0.28	0.413
RDW-SD, fL	34.55	32.48	33.42	0.63	0.102
Platelet, ×10³/μL	268.83	325.17	132.67	64.59	0.130
MPV, fL	9.50	9.98	8.97	0.47	0.336
Day 10					
White blood cell, ×10³/μL	8.71[x]	7.62[xy]	5.89[y]	0.78	0.062
Neutrophil, ×10³/μL	6.29[x]	5.97[xy]	4.12[y]	0.71	0.096
Lymphocyte, ×10³/μL	1.76[a]	1.12[b]	1.32[ab]	0.17	0.049
Monocyte, ×10³/μL	0.50	0.40	0.34	0.06	0.213
Eosinophil, ×10³/μL	0.08	0.10	0.09	0.02	0.755
Neutrophil, %	72.10[xy]	77.58[x]	69.62[y]	2.31	0.074
Lymphocyte, %	20.24	15.55	22.69	2.30	0.117
Monocyte, %	5.80	5.19	5.99	0.71	0.715
Eosinophil, %	0.87	1.29	1.47	0.27	0.289
Red blood cells, ×10⁶/μL	6.63	7.00	6.64	0.19	0.308
Hemoglobin, g/dL	16.07	16.28	15.10	0.39	0.103
Hematocrit, %	45.58	45.75	43.43	1.19	0.334
MCV fL	68.80[a]	65.48[b]	65.40[b]	0.99	0.046
MCH, pg	24.27[a]	23.32[ab]	22.72[b]	0.34	0.019
MCHC, g/dL	35.35[xy]	35.60[x]	34.73[y]	0.26	0.080
RDW-CV, %	13.08[b]	12.90[b]	14.23[a]	0.33	0.024
RDW-SD, fL	34.92[ab]	32.92[b]	35.87[a]	0.70	0.027
Platelet, ×10³/μL	218.50	169.00	146.33	46.74	0.550
MPV, fL	9.10[b]	10.40[a]	10.18[a]	0.31	0.020

[1)]Each value is the mean of 6 replicates (one dog per cage).

[2)]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old; MCV, mean cell 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; MPV, mean platelet volume.

[a–c]Means with different superscript letters within a row are different ($p < 0.05$).

[x,y]Means with different superscript letters within a row are different ($p < 0.10$).

 https://doi.org/10.5187/jast.2500227

Table 7. Age-related changes in serum biochemical profile of Beagle dogs[1)]

Item[2)]	Puppy	Adult	Senior	SEM	p-value
Day 1					
Total protein, g/dL	5.15	5.15	5.17	0.03	0.923
Albumin, g/dL	3.57[a]	3.38[a]	2.82[b]	0.17	0.017
Glucose, mg/dL	108.50[a]	101.00[a]	94.17[b]	3.50	0.036
Triglyceride, mg/dL	35.50[y]	36.00[xy]	46.00[x]	3.47	0.085
Total cholesterol, mg/dL	142.50	174.83	165.67	14.75	0.308
Creatinine, mg/dL	0.34[a]	0.34[a]	0.20[b]	0.03	0.007
Blood urea nitrogen, mg/dL	16.77[y]	25.44[x]	21.22[xy]	2.44	0.072
Calcium, mg/dL	9.71	8.79	8.48	0.45	0.164
Alkaline phosphatase, U/L	95.33[a]	34.00[b]	47.50[b]	15.44	0.032
Aspartate aminotransferase, U/L	50.00	28.50	33.00	9.35	0.261
Alanine aminotransferase, U/L	37.40	32.83	26.00	3.41	0.108
Day 10					
Total protein, g/dL	5.83	4.35	5.73	0.99	0.513
Albumin, g/dL	4.13[a]	3.92[a]	3.42[b]	0.09	< 0.001
Glucose, mg/dL	109.00[a]	103.50[a]	93.17[b]	3.17	0.010
Triglyceride, mg/dL	40.50	41.33	41.17	3.45	0.984
Total cholesterol, mg/dL	124.50	162.17	148.83	13.07	0.152
Creatinine, mg/dL	0.30[a]	0.33[a]	0.23[b]	0.02	0.005
Blood urea nitrogen, mg/dL	40.18[a]	30.98[b]	26.12[b]	2.00	< 0.001
Calcium, mg/dL	10.76[a]	9.64[b]	9.72[b]	0.15	< 0.001
Alkaline phosphatase, U/L	70.50[a]	26.50[b]	36.00[b]	4.54	< 0.001
Aspartate aminotransferase, U/L	36.83	40.83	41.67	5.37	0.796
Alanine aminotransferase, U/L	33.80	34.33	39.00	3.49	0.590

[1)]Each value is the mean of 6 replicates (one dog per cage).

[2)]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old.

[a,b]Means with different superscript letters within a row are different ($p < 0.05$).

[x,y]Means with different superscript letters within a row are different ($p < 0.10$).

MER for maintenance metabolism. Accordingly, the food supply amount in our study was set based on the NRC, and DFI differed among the age groups based on the as-is, DM basis, and calorie basis. In addition, puppies showed the highest BWC, indicating that energy utilization through dog food was effective during the period when energy for growth was required, as mentioned above. Adult and senior dogs with low BWC would have relatively low energy utilization due to a lower food supply compared with puppies, as their nutritional metabolism is focused on maintenance. In particular, the decreased BWC of the senior group indicates that the efficiency of energy metabolism may decrease with age. However, the BCS remained within the ideal range (score of 4–5) [22] in all age groups throughout the study, suggesting that the feeding amount of experimental canine food was appropriately adjusted to the NRC-based recommendations.

In fecal parameters, the DFO based on the as-is and DM basis followed the order of the puppy, adult, and senior groups can be seen as the result of "high input, high output" in proportion to the amount of food supply and intake, but there may be differences in energy utilization efficiency among age groups. This is because the DFO based on the as-is and DM basis in the senior group was lower than in the adult group, but there was no difference in calorie-based DFO between the two groups. The fecal moisture content and frequency of diarrhea were higher in puppies. The high diarrhea rate in puppies may be due to underdeveloped gut health, such as an immature digestive system and/or an imbalance in the gut microbial environment at this phase, or may be due to a short gut transit time following high intake. Previous studies have reported that controlling the crude protein content in the feed of weaned piglets can prevent diarrhea caused by protein fermentation [32,33]. In this study, the canine food followed the minimum amount of crude protein for growth and reproduction, including amino acids, and the minimum amount of crude fat, calcium, and phosphorus for adult maintenance. Although the experimental food contained a high level of crude protein compared with the minimum level, which may be associated with diarrhea in puppies with an immature gut system, the growth parameters confirmed that the diet supported a high MER during this period. These findings emphasize the need to provide an appropriate nutritional profile considering age of dogs.

Nutrient digestibility is a dynamic physiological response influenced by the utilization of nutrients from food, and the overall health and wellness of companion dogs, and varies according to the age of dogs. From birth to the adult period, the secretion and activity of digestive enzymes develop rapidly [34]. In addition, nutrient digestibility differs with breed size [35]. Previous studies have reported that nutrient digestibility was stable or even increased with age [7,13,36,37]. In addition to age, factors that influence nutrient digestibility of dogs include nutrient composition (dietary fiber, starch, and bioactive substances) and ingredients (plant and meat) of the food and the technical manufacturing process (heating, cooking, extrusion, and particle size) [38,39]. For instance, the ATTD of dry matter and main nutrients (crude protein, ether extract, starch, and carbohydrates) in cooked food, including meat (lamb meal, poultry meal, or fish meal) and plant ingredients (wheat and corn-based) is 79%–99% [40]. On the other hand, uncooked, unextruded, or overheated ingredients can reduce nutrient digestibility of dogs, especially protein and amino acids [2,41]. Pet food regulatory agencies, including AAFCO and FEDIAF, base minimum nutrient requirements on assumed digestibility of at least 70% for dry matter and 80% for protein [42]. Additionally, canine nutritionists generally consider 80% to be an appropriate minimum target for digestibility, with 80-90% being the ideal value for nutrients [42]. In this study, the ATTD of dry matter, organic matter, and energy was 88.95%–89.76%, 95.27%–96.10%, and 92.68%–93.51%, respectively, confirming the appropriate basic digestibility among age groups.

However, some nutrients showed clear age-related differences in digestibility. The minimum recommendation for crude protein is higher during the growth and reproductive stages than during the maintenance period in adults [19]. These differences reflect fundamental biological differences between life stages and are supported by the results of the ATTD of crude protein. Additionally, the decreased ATTD of crude protein in the senior group is considered to be due to complex factors such as reduced gut functions and digestive enzyme secretion and activity following aging. On the other hand, it has been reported that the activities of pepsin, trypsin, and chymotrypsin, which are involved in protein digestion, develop rapidly during puppies and stabilize in adult dogs [34]. In addition, no difference on the digestibility of crude protein was confirmed between weanling or adults and senior dogs [7,17,36]. However, the decrease in the number of proliferative cells in the intestinal epithelium with age implies a decrease in cellular turnover and may lead to a low adaptability to changes in the digestive environment [43]. Additionally, increased fiber fermentation by gut microbiota can increase microbial nitrogen excretion, which may decrease the ATTD of crude protein [36,44].

In this study, the ATTD of crude fiber was lower in puppies than in adults and senior dogs. Since fiber digestion occurs primarily through microbial fermentation in the large intestine, the difference may be related to the development of the gut microbial ecosystem. Microbial diversity is a complex interaction involving genetics, breed, birth type, and diet, which collectively compose the microbial community [15]. While previous studies have shown inconsistent results in age-related changes

 https://doi.org/10.5187/jast.2500227

on microbial diversity [45–47], it is generally accepted that the composition of gut microbiota undergoes rapid restructuring during early life and reaches a relatively stable state by one year of age [46,48]. Thus, this microbial development in adult and senior dogs may contribute to more efficient fermentation and subsequent short-chain fatty acids (SCFA) production from dietary fiber. Furthermore, the similar ATTD of crude fiber between adult and senior groups suggests that fiber digestibility is well maintained with age. This may be due to the low non-starch polysaccharides and anti-nutritional factor contents of brown rice, which was used as the main source in the experimental diet [49] and is supported by the results of improved digestibility reported in a previous study [50,51].

The ATTD of ether extract and NFE was lower in puppies than in adults and senior beagles. These results suggest that immaturity of digestive enzyme function in early life may contribute to reduced nutrient utilization, which is consistent with previous findings indicating that digestibility values stabilize or increase with age [7,13,36,37]. In addition, although the results of calorie-based DFO predicted a difference in energy utilization between adult and senior dogs, the digestibility of energy-related nutrients did not differ between the two groups. This suggests that there may be no difference in energy utilization for maintenance between adult and senior dogs under normal physiological conditions. Compared with the ATTD of crude ash, the ATTD of calcium and phosphorus showed opposite results with age. This suggests that the metabolic utilization of minerals may differ across the life cycle of beagles. Ash is a broad indicator of minerals and may indicate increased utilization with growth and development. On the other hand, the improved or maintained ATTD of calcium and phosphorus with age may be related to morphological and functional changes in the intestine of dogs [43,52]. In addition, it can be assumed that passive utilization is enhanced due to an increase in mineral solubility by changing gut pH through the production of SCFA following dietary fiber fermentation in the gut. Furthermore, since the calcium content in the food affects the digestibility of phosphorus [53] formulation of food reflecting nutritional metabolic characteristics should be considered. The ATTD of amino acids did not differ between age groups, which may have induced changes in the amino acid profile due to contributions from the gut microbiota [38]. Therefore, the evaluation of apparent ileal digestibility is also considered to evaluate the bioavailability of amino acids [2,54]. Collectively, our nutrient digestibility results revealed differences by age, suggesting the need for appropriate nutrient supply through diet composition according to developmental stage.

The CBC and serum biochemical profiling can objectively and specifically evaluate physiological status, and screening or monitoring through the test is important in health management [55]. WBC play an important role in response to inflammation, disease, and infection, and changes in their numbers indicate health status. In this study, age-related differences were observed in the numbers of WBC, including neutrophil, lymphocyte, and monocyte, suggesting dynamic changes in the immune system across life stages. The results of WBC differentiation by age are similar to those of previous studies [16,18,56], indicating changes in immunosenescence characteristics due to aging. It is possible that aging was accompanied by inflammaging, a state of low-grade, chronic, and systemic inflammation [6,18]. In addition, changes in WBC indices in senior beagles suggest a possible disruption of immune balance, which may also be related to decreased oxidative stress response ability and susceptibility to infection. These immunological changes are important considerations when developing health management and nutritional intervention strategies for older dogs. Regarding red blood cell indices, MCHC level was higher in adult beagles than in puppies or senior beagles. On the other hand, MCV and MCH were higher in the puppy group than in the adult or senior groups, but RDW and MPV were higher in the senior group than in the puppy or adult groups. These results suggest an increase in erythrocyte heterogeneity due to age-related development of immature red blood cells combined with the effects of inflammation or iron deficiency [16,57]. Therefore, it is necessary to understand the physiological changes related to bone marrow production and hematopoiesis in the life cycle of dogs and to provide appropriate nutritional management, especially in senior dogs, since these changes can lead to chronic inflammation or malnutrition.

In serum biochemistry, age-related differences were observed in indicators reflecting nutritional metabolism and tissue functions. Throughout the study, senior beagles consistently exhibited lower concentrations of albumin, glucose, and creatinine in serum than puppies and adult beagles. On the other hand, puppies had higher serum ALP level than adults and senior beagles. These findings suggest changes in systemic metabolism associated with aging, involving the liver, kidney, and skeletal muscle functions. The decline in serum albumin level with age may be due to reduced hepatic synthetic capacity as well as renal and/or intestinal losses, as previously reported in aging dogs [58,59]. In addition, the decrease in serum glucose level has been reported to be due to decreased hepatic glycogen storage function or decreased functional liver mass [16,60]. Serum creatinine level may indicate changes in muscle mass or renal function [61,62]. In addition, creatinine, a byproduct of muscle metabolism, may be present at lower concentrations in young animals due to lower muscle mass [7]. In contrast, a previous study revealed a positive correlation between serum creatinine and lean body mass [63]. It has been reported that an increase in serum ALP level is an enzyme associated with the physiological response to bone growth, and that levels were high during the bone growth period and decreased with age [7,62]. In this regard, puppies also have higher serum calcium levels, reflecting active regulation of calcium metabolism during the growth process. At the end of the experiment, puppies showed higher level of BUN than adults and senior dogs. Because BUN is a product of protein metabolism, including hepatic urea cycle and renal excretion [64], elevated level in puppies may reflect higher dietary protein intake in puppies than adults and old dogs. Taken together, these results suggest that age-related changes in basal metabolic rate and nutritional metabolic functions with aging, as well as differences in food intake, should be considered when formulating diets to support healthy aging.

CONCLUSION

Our findings highlight the importance of considering age as a major factor influencing nutritional requirements, digestive efficiency, and systemic health physiology in Beagle dogs. Overall, optimal nutritional feeding is required during the growth period to support development with high nutrient availability, and with advancing age to address changes in digestive capacity, nutrient metabolism, and systemic health. In addition, this study suggests the need for age-specific food formulations and health screening or monitoring strategies to optimize the health maintenance of dogs throughout their lifespan. Collectively, the current study is expected to contribute to the development of age-specific dietary strategies and long-term health management of companion dogs.

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