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# Evaluation of nutritional adequacy after investigating amino acid and mineral content in pet food distributed in South Korea

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**Abstract:** Among the nutrients in feed, amino acids and minerals are important for the growth and development of pets. In particular, interest in nutritional components related to the health of pets is increasing as pet-raising households and pet food markets have recently grown. Therefore, in this study, 55 pet food products distributed in South Korea were purchased, and the content of 3 essential and conditionally essential amino acids (taurine, lysine, arginine) and 4 minerals (Ca, P, Na, K) was investigated. Among the three amino acids, arginine was found to have the highest content, and the average content was 1.79 and 1.37 % in cat and dog foods, respectively. On the other hand, the taurine content was the lowest, but it was found to be higher than the minimum requirement of 0.10 % for cats set by the American Association for Feed Control (AAFCO) and the European Federation of Pet Food Industries (FEDIAF). As a result of the four-component analysis of minerals, the content of Ca was found to be the highest, and the average content was confirmed to be 1.64 and 1.25 % in cat and dog food, respectively. On the other hand, Na was the lowest, but it was higher than the AAFCO minimum requirement and FEDIAF minimum requirement for young cat and dog food. Among all 55 samples examined, the content of the three amino acids and the four inorganic components was confirmed to be suitable for the recommended minimum intake and maximum allowable intake presented by AAFCO and FEDIAF.

**Key words:** amino acid, mineral, pet food, investigation, nutrient

## 1. Introduction

Recently, as the number of households raising dogs and cats increases in Korea, the pet food industry is also growing. These pet-raising homes and feed markets are growing together worldwide. The growth potential of pet food is great due to the growth of the pet market, but the demand for quality control is constantly increasing due to the increase in small businesses. In addition, owners' continuing demand for better quality feed products means that the pet food sector is recognizing the importance of providing

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nutritious food, especially for the health and well-being of animals. In the case of the United States or Europe, where the pet food market has grown and settled earlier than in South Korea, recommendations for nutritional components of pet food are made and provided. In this regard, the European Pet Food Industry Federation (FEDIAF) has established nutritional guidelines based on the recommendations of the National Research Council (NRC) and scientific research. FEDIAF also works closely with American Feed Control Officials.[1] AAFCO or FEDIAF establishes the minimum required amounts of amino acids and maximum and minimum safe levels of various minerals supplied in pet food.[2]

Amino acids are the building blocks of proteins and are essential for the growth and maintenance of physiological functions in animals. There are about 20 kinds of amino acids that make up proteins, and they are classified into essential and non-essential amino acids. It is classified as nutritionally essential and non-essential according to the animal's growth or nitrogen balance. Essential amino acids are amino acids that must be supplied from outside to maintain animal life and include lysine, taurine, leucine, methionine, phenylalanine, threonine, and tryptophan.[3-5] The main function of lysine in animal life is to act as one of 20 types of building blocks for the synthesis of body proteins and peptides, which are essential organic compounds that participate in almost all biochemical reactions and physiological activities of all living organisms.[6] Lysine also exerts many physiological functions for monogastric animals. Lysine can affect animal metabolism of other nutrients, hormone production, and immunity.[7,8] Essential amino acids are generally found in animal feed. Animal feed contains taurine, an important antioxidant, which participates in energy metabolism, especially in tissues such as the brain, skeletal muscle, heart, and gonads of animals.[9] In particular, in the case of cats, taurine is classified as an essential amino acid because it cannot be biosynthesized and must be supplied through food. The minimum taurine content required for adult cats in cat food is set at 0.25 g/1000 kcal for dry food and 0.5 g/1000 kcal for wet food.[10] Dietary requirements for amino acids depend on factors such as species, developmental stage, physiological state, and pathological conditions. Therefore, some of the amino acids synthesized by animals are classified as conditionally essential because the utilization rate is higher than the synthesis rate under special conditions such as lactation, pregnancy, and injury. One such amino acid is arginine.[11,12] Arginine is a conditionally essential amino acid present in living organisms. To remove ammonia from the body in the liver, the synthesis of urea takes place, and at this time, arginine is decomposed into urea in the urea cycle. Arginine is necessary for epithelial cells, brain neurons, and nitric oxide production. Arginine has been reported to be an essential amino acid for the optimal growth and maintenance of newborn piglets and nitrogen balance.[13] It has been reported to be essential for optimal growth in young rodents.[14] Arginine also has a wide range of biological functions, making it an important raw material for protein synthesis, as well as being a precursor for substances such as polyamines and nitric oxide in the body.[15]

Minerals are required for cell function, nerve conduction, regulation of various cell channels, regulation of blood pressure, and regulation of acid-base conditions in the blood. Minerals can be grouped into three categories according to the relative amounts needed by the body: macro, micro, and trace minerals. Macro minerals include calcium, phosphorus, magnesium, sodium, potassium, and chloride. Microminerals include chromium and boron. Calcium, phosphorus, and magnesium are involved in the development and maintenance of bones and teeth, as well as are required for the proper functioning of the dog's muscular and nervous systems.[16] Potassium and sodium are responsible for maintaining electrolyte balance, regulating body fluids, and transporting nutrients in the blood.[17] Except trace minerals, AAFCO has determined the maximum and minimum safe levels of various minerals supplied in pet food.[10] According to the AAFCO established standards, the minimum and maximum calcium requirements for adult dogs are 0.6 % and 2.5 %, respectively. Understanding the physiological function of amino acids and minerals is very necessary

to manage the health of pets. In this regard, it can be said that content management in feeds eaten by pets is also very important to provide appropriate amounts of amino acids and minerals.

In this study, 55 pieces of pet food distributed in South Korea were collected to manage amino acids and minerals in pet food. Then, the content of major amino acids (lysine, arginine, and taurine) and minerals (calcium, phosphorus, sodium, and potassium) in the samples was investigated. In addition, the quality of distributed products was reviewed by comparing the results of the investigation of the content of amino acids and minerals with the recommended usage amount provided by FEDIAF and AAFCO.

## 2. Experimental

### 2.1. Chemicals and reagents

Amino acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and mineral standards were purchased from Accustandard (New Haven, CT, USA). Silver nitrate (69-71 %) was purchased from Dawon Science (Suwon, Gyeonggi-do), and distilled water was prepared using a Milli-Q Direct 8 model manufactured by Merck Millipore (MA, USA). Methanol was purchased from Merck (Darmstadt, Germany) in HPLC grade. Formic acid (98% ) and ammonium acetate ($NH_4CH_3CO_2$) were purchased from Thermo Fisher Scientific (Waltham, USA). A syringe filter (Whatman, Maidstone, UK) for sample filtration was made of polytetrafluoroethylene (PTFE) and had a size of 0.2 μm. Hydrochloric acid was purchased in EP-S grade from Chemitop (Korea), and ethanol was purchased in HPLC grade from Merck (Darmstadt, Germany). The mobile phase used for amino acid analysis was purchased from KANTO Chemical (Tokyo, Japan), and reaction reagents were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan).

### 2.2. Samples

For the monitoring of amino acids and minerals in pet food commonly used in Korea, a total of 55 samples (27 cat feeds and 28 dog feeds) were collected. Samples were randomly collected from feed mills and markets in various locations in Korea from August to November 2022. All feed samples were ground to a particle size of less than 4 mm and stored in a freezer at -20 °C.

### 2.3. Amino acid analyzer conditions

Amino acid analyzer AAA L-8900 from Hitachi (Tokyo, Japan) was used, and the analysis column was chosen Hitachi ion exchange resin 855-4506 (Na type, 4.6 × 60 mm). The reaction column was Hitachi reaction column 852-3540 (4.6 × 60 mm). The flow rates of 0.4 mL/min (pump 1), 0.35 mL/min (pump 2), and injection volume were 20 μL. The analysis column temperature was 57 °C, and the reactor temperature was maintained at 135 °C. The gradient conditions of the mobile phase are shown on supplementary material.

### 2.4. ICP-OES conditions

Analysis was performed under the following conditions using ICP-OES (Agilent, 5100). Ion wavelength was analyzed by setting Ca 317.933 nm, P 213.618 nm, Na 589.592 nm, and K 766.491 nm respectively. RF power (kW) was set to 1.20, PlasmaFlow was set to 15.0 L/min, AuzFlow was set to 1.5 L/min, and NebFlow was set to 0.75 L/min. The stabilization time was set to 15 seconds, and the nebulizer type was set to seaspray and replicated three times for analysis.

### 2.5. Sample preparation

#### 2.5.1. Amino acids pretreatment method

0.2 g of the homogenized sample into a 50 mL centrifuge tube, add 20 mL of 6 M HCl and then hydrolyze at 110 °C for 20 hours. After cooling the sample solution to room temperature, put it in a 100 mL volumetric flask and add water to adjust the total volume to 100 mL. Take 1 mL of supernatant, concentrate, and then re-dissolve in 10 mM aqueous ammonium acetate. The extracted sample solution was filtered with a 0.2 μm (PTFE, Whatman Inc., Maidstone, UK) syringe filter, and then used as the sample solution.

#### 2.5.2. Minerals pretreatment method

All teflon vessels used for pretreatment were kept in a 10 % (v/v) $HNO_3$ solution for 24 h to remove contamination, and then rinsed with ultrapure water before use. After putting 0.5-1.0 g of the sample in a teflon vessel, adding 10 mL of 70 % nitric acid, assembling and mounting the sample container, it was decomposed using a microwave (CEM, MARS 6). The decomposition solution was transferred to a 50 mL volumetric flask, diluted with 50 mL of deionized water, filtered with Whatman No.6 (3 μm), and used as a test solution. The heating program of the microwave (CEM, United Kingdom) was set to 1800 $kg \cdot m^2/s^3$, temperature 190°C, ramp time 25 minutes, and holding time 30 minutes.

### 2.6. Method verification

For the analysis of amino acids and minerals in pet food, the feed standard analytical method notified by the National Agricultural Products Quality Management Service, which is an official feed analysis method in South Korea, was used. Prior to monitoring, the quantitative limit of each target substance was confirmed, and certified standards and samples from AAFCO's proficiency testing program were analyzed together to confirm the analyst's pretreatment proficiency and to confirm the accuracy and reproducibility of the analysis method.

## 3. Results and Discussion

### 3.1. Method verification

For the analysis of amino acids and minerals, the South Korea official method, the feed standard analytical method, was applied. For method verification, accuracy, precision, and limits of quantification were confirmed. Verification for three types of amino acids was performed using certified reference materials, and four inorganic components were performed by purchasing samples from AAFCO's proficiency testing program. The test results were evaluated by applying the accuracy and precision standards of the South Korea Ministry of Food and Drug Safety (MFDS) guidelines.[18] The quantitative limit, recovery rate, and standard deviation of each component all showed good results, and detailed results are shown in *Table* 1 and 2.

*Table* 1. Verification result of amino acid analytical method using certified standard materials

| No. | Compound | Concentration of CRM (%) | Recovery (%) | CV (%) | LOQ (%) |
|---|---|---|---|---|---|
| 1 | Lysine | 1.68 | 86.5 | 1.93 | 0.25 |
| 2 | Arginine | 1.78 | 90.5 | 1.91 | 0.28 |
| 3 | Taurine | 0.24 | 81.7 | 1.63 | 0.03 |

*Table* 2. Verification result of mineral analytical method using AAFCO proficiency teste sample

| No. | Compound | Concentration of AAFCO sample* (mg/kg) | Recovery (%) | CV (%) | LOQ (mg/kg) |
|---|---|---|---|---|---|
| 1 | Ca | 14.650 | 100.28 | 0.01 | 0.001 |
| 2 | P | 5.588 | 99.26 | 0.01 | 0.04 |
| 3 | Na | 5.821 | 100.72 | 0.006 | 0.05 |
| 4 | K | 7.30 | 96.85 | 0.01 | 0.01 |

*AFFCO Animal feed 202199

### 3.2. Amino acid content investigation results

Amino acids serve as an energy source in the dog's body and provide various components necessary for major metabolic functions. It is also used in the synthesis of neurotransmitters, hormones, purines, and pyrimidine nucleotides. Therefore, it is very important to adjust the concentration and ratio of amino acids consumed according to the dog's health condition. Excessive or insufficient intake of amino acids can have a negative impact on health. Therefore, information on the content of amino acids in dog food is essential to continuously improve dog nutrition.[19] A balanced diet and food composition are the main sources of these elements. In particular, in cats, taurine is classified as an essential amino acid because it cannot be biosynthesized and must be supplied through the diet.[20] The minimum required taurine content in cat food is set at 0.1 % or higher.[10] We investigated the content of the representative amino acids arginine, lysine, and taurine in 55 feed samples. In each of the 55 feed samples, the average amino acid content was found to be highest for arginine

*Table* 3. Results of investigation of 3 amino acid contents in 55 pet food samples and minimum recommended amounts of FEDIAF and AAFCO

| Type | Compound | Minimum (%) | Maximum (%) | Average (%) | FEDIAF | | AAFCO | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Early growth (<14weeks) | Adult-based on MER[a] of 110kcal/kg$^{cat0.67/dog0.75}$ | Growth & reproduction minimum | Adult maintenance minimum |
| Cat feed | Arginine | 1.10 | 2.43 | 1.79 | 1.07 | 1.00 | 1.24 | 1.04 |
| | Lysine | 0.90 | 2.58 | 1.61 | 0.85 | 0.34 | 1.20 | 0.83 |
| | Taurine | 0.11 | 0.86 | 0.28 | 0.10 | 0.10 | 0.10 | 0.10 |
| Dog feed | Arginine | 0.89 | 2.17 | 1.37 | 0.82 | 0.52 | 1.00 | 0.51 |
| | Lysine | 0.69 | 2.49 | 1.36 | 0.88 | 0.42 | 0.90 | 0.63 |
| | Taurine | 0.02 | 0.63 | 0.19 | - | - | - | - |

[a]Maintenance energy requirement.

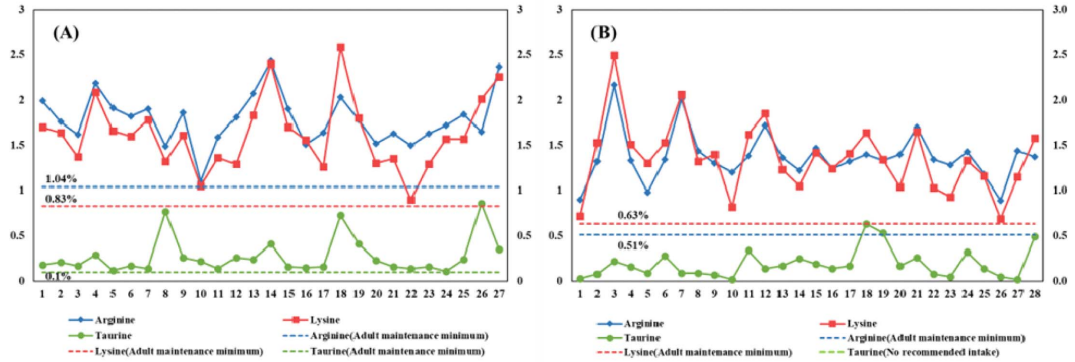


*Fig*. 1. Contents and AAFCO adult maintenance minimums of arginine, lysine and taurine in (A) cat food and (B) dog food.

and, in contrast, lowest for taurine (Arginine > lysine > taurine). However, taurine was found to be above the AAFCO and FEDIAF minimum requirements of 0.1 % in cat food. The average contents of arginine, lysine, and taurine in cat food were found to be 1.79, 1.61, and 0.28 %, respectively. In addition, the average content of arginine, lysine, and taurine in dog food was 1.37, 1.36, and 0.19%, respectively (*Table* 3).

According to the AAFCO and FEDIAF guidelines, amino acid content standards in pet foods are defined according to the age of the cat or dog consuming them.[10,21,22] As a result of comparing the analysis results of 55 feed samples with each standard, it was confirmed that the amino acid content of all samples was suitable for the minimum requirements presented by AAFCO and FEDIAF (*Fig*. 1). In order to confirm the difference in the content of amino acids for each feed, the deviation of the content was checked. The inter-sample variation of arginine, lysine, and taurin in dog food was 0.27, 0.39, and 0.16 %, and the inter-sample variation of arginine, lysine, and taurin in cat food was 0.28, 0.39, and 0.20 % (*Table* 4).

### 3.3. Mineral content investigation results

Ca, P, Na, and K are known to be essential for metabolism and are commonly found in high concentrations in biological tissues.[23] P is an important component of ATP, where it reserves and releases energy, plays a structural role in bones and teeth, synergizes with calcium and helps maintain acid-base balance in the body.[24] Na, K, and Ca are particularly important for metabolism.[25] We investigated the content of four minerals (Ca, P, Na, K) in 55 feed samples. All minerals investigated were found to have higher levels in cat food than in dog food, as did amino acids. In each of the 55 feed samples, the

*Table* 4. Results of investigation of 4 mineral contents in 55 pet food samples and minimum recommended amounts of FEDIAF and AAFCO

| Type | Nutrients | Minimum (%) | Maximum (%) | Average (%) | FEDIAF | | AAFCO | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Early growth (<14weeks) | Adult-based on MER[a] of 110 kcal/ $kg^{cat0.67/dog0.75}$ | Growth & reproduction minimum | Adult maintenance minimum |
| Cat feed | Ca | 0.80 | 2.60 | 1.64 | 1.0 | 0.4 | 1.0 | 0.6 |
| | P | 0.70 | 1.60 | 1.18 | 0.84 | 0.26 | 0.8 | 0.5 |
| | Na | 0.24 | 4.48 | 0.68 | 0.16 | 0.08 | 0.2 | 0.2 |
| | K | 0.62 | 1.17 | 0.82 | 0.6 | 0.6 | 0.6 | 0.6 |
| Dog feed | Ca | 0.50 | 1.60 | 1.25 | 1.0 | 0.5 | 1.2 | 0.5 |
| | P | 0.50 | 1.30 | 0.90 | 0.9 | 0.4 | 1.0 | 0.4 |
| | Na | 0.22 | 2.85 | 0.57 | 0.22 | 0.10 | 0.3 | 0.08 |
| | K | 0.60 | 1.02 | 0.77 | 0.44 | 0.50 | 0.6 | 0.6 |

[a]Maintenance energy requirement.

average content of Ca was found to be the highest, whereas the lowest content of Na was observed (Ca > P > K > Na). However, it has been found to exceed the AAFCO minimum requirements of 0.2 and 0.3 % and the FEDIAF minimum requirements of 0.16 and 0.22 % in kitten and dog foods. The average content of the four minerals Ca, P, K, Na, and P in cat food was 1.64, 1.18, 0.68, and 0.82 %, respectively. In addition, the average contents of Ca, P, K, Na, and P in dog food were 1.25, 0.90, 0.57, and 0.77 %, respectively. According to the AAFCO and FEDIAF guidelines, mineral content standards in pet food are defined according to the age of the cat or dog consuming it.[10,22] As a result of comparing the analysis results of 55 samples with each standard, it was confirmed that the mineral content of all samples was suitable for the minimum requirements presented by AAFCO and FEDIAF. In order to confirm the difference in the content of minerals for each feed, the deviation of the content was checked. Standard deviations between samples for Ca, P, Na, and K in dog food were 0.29, 0.18, 0.53, and 0.14 %, and standard deviations between samples for cat Ca, P, Na, and K were 0.50, 0.25, 0.78, and 0.13 %, respectively (*Table* 4).

Because of concerns that excessive amounts of Ca and P may have a harmful effect during the growing season of large breed dogs, AAFCO sets the maximum permitted recommended standards (based on the dry matter). Additionally, the AAFCO Pet Food Commission sets a maximum calcium limit of 1.8 % DM for all dog foods and a limit for phosphorus of 1.6 % DM to ensure that all Life Stage products are properly formulated. In addition, FEDIAF sets and manages

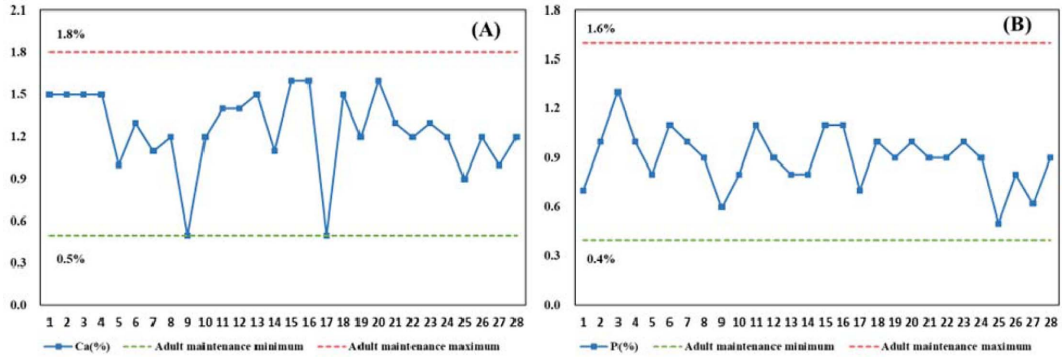


*Fig*. 2. Ca (A) and P (B) content in dog food and AAFCO recommended minimum and maximum intake for adult maintenance.

the maximum intake of calcium and phosphorus for adult dogs at 2.5 % and 1.6 %. In this regard, it was confirmed that the contents of Ca and P among all the investigated feeds were properly included in the recommended standards of AAFCO and FEDIAF (*Fig*. 2).

## 4. Conclusions

In this study, the contents of three amino acids (arginine, lysine, and taurine) and four minerals (Ca, P, K, and Na) were investigated in 55 dog and cat foods. All amino acids and minerals investigated were found to have higher levels in cat food than in dog food. Among the three amino acids, arginine was found to have the highest content, and the average content in cat and dog food was 1.79 and 1.37 %, respectively. In contrast, the lowest content of taurine was observed, but was found to be above the minimum requirement of 0.1 % for AAFCO and FEDIAF in cat food. And it was confirmed that the amino acid content of all 55 samples investigated was suitable for the minimum requirements presented by AAFCO and FEDIAF. As a result of investigating the four minerals, Ca was observed the most, and the average content was confirmed to be 1.64 and 1.25 % in cat and dog food, respectively. On the other hand, Na was found to be the lowest, but was found to be above the AAFCO minimum requirements of 0.2 and 0.3 mg/kg and FEDIAF minimum requirements of 0.16 and 0.22 % for young cat and dog foods. In addition, the mineral content of all 55 samples investigated was confirmed to be suitable for the minimum requirements presented by AAFCO and FEDIAF. In addition, the content of calcium and phosphorus was confirmed to be below the maximum recommended intake standard (based on dry matter).

It was confirmed that amino acids (arginine, lysine, and taurine) and minerals (Ca, P, K, and Na) in all distribution feeds investigated in this study were properly included in the standards recommended by AAFCO or FEDIAF. From these results, it can be seen that the feeds used in the study contained the recommended amounts of amino acids and minerals suitable for the health of pets. Additionally, as confirmed through the results of this study, the content of amino acids and minerals differed between products. The appropriate content levels of amino acids and minerals may vary depending on the pet's weight, health condition, etc., so it seems necessary to pay attention to the content of these ingredients when feeding. Investigation of these nutrients can be used as basic data for the health and well-being of pets in the future, and deficiencies in certain nutrients such as taurine can cause diseases such as cardiomyopathy, so continuous management is necessary. In addition, it is expected that continuous monitoring of more diverse nutrients will be needed in the future, and this information can be used as basic data for establishing a future pet food quality management system.

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