

### Introduction

In order to ensure adequate growth and maintenance of body tissues, amino acids must be accurately supplied to canines. Feed a deficiency of amino acids can have deleterious effects such as weight loss, disease, or in extreme cases, death. Of the amino acids, methionine is an essential amino acid in that it must be consumed in order to meet the body requirements. The requirement of cysteine, another sulfur containing amino acid, can be met through either metabolism of methionine to cysteine or through dietary cysteine. Thus, the two amino acids are often referred to as the “total sulfur amino acids.”

Amino acid requirements of the essential amino acids have been determined in the past; however, it is important to continually predict requirements as genetics change over time. Requirements can be determined through a variety of methods, with each method having its own advantages and disadvantages. A more novel technique, the indicator amino acid oxidation technique, involves the use of a stable isotope in order to determine the amino acid requirement. The method is based on the idea that when one indispensable amino acid is deficient, then all other IDAA, including the indicator amino acid (in this case, the stable isotope), will be oxidized. Advantages of this method are its ease and quick response time. Ensuring rapid responses is important when determining amino acid requirements in developing mammals such as puppies.



### Objective

The objective of this experiment is to use the indicator amino acid oxidation technique to determine the sulfur amino acids requirements for growing Labrador Retrievers.

### Experimental Design

A total of twelve dogs were subjected to twelve diets with varying levels of methionine and cysteine, ranging from deficient to excess. Diets were formulated to 1.6x National Research Council values for all indispensable amino acids. Dogs were fed a control diet for two days, followed by one day of testing. During the experimental testing period, the test diet was fed, a tracer amino acid was supplied, and breath samples were collected.

To ensure a steady state was achieved before samples were obtained, a priming dose of L-[1-<sup>13</sup>C]Phe (Cambridge Isotope Laboratories, Inc.) was supplied to each animal based on their body weight. Following the priming dose, a dose of [1-<sup>13</sup>C]Phe was supplied every thirty minutes, spanning a four hour period. The process of obtaining samples consisted of placing a respiration mask on each subject every thirty minutes (Oxymax, Columbus Instruments). <sup>13</sup>CO<sub>2</sub> was collected and isotopic enrichment of the breath sample was determined by isotope ratio mass spectrometry (IRMS). Data gained through the IRMS measurements were converted to atom percent excess and analyzed using a broken line model (JMP<sup>®</sup> Pro 15).

### Results

Amino acid requirements of sulfur amino acid acids for growing Labrador Retrievers are shown in Table 1. The estimated Met and TSAA mean and population requirements of growing dogs was determined to be 0.78 ± 0.16 and 1.53 ± 0.21 g/1000 kcal ME (mean ± 2SD), respectively. Tables 2 and 3 contain published data from the NRC and AAFCO.

**Table 1. Four Rivers amino acid requirements for methionine and total sulfur amino acids in > 14 wk growing Labrador Retrievers**

g/1000 kcal ME	Met	TSAA
Minimum Requirement	0.78	1.53
Recommended Allowance	0.94	1.74

**Table 2. NRC Amino Acid Recommendations for >14 wk growing pups**

g/1000 kcal ME	Met	TSAA
Minimum Requirement	0.53	1.05
Recommended Allowance	0.65	1.33

**Table 3. AAFCO Amino Acid Recommendations for dogs**

	Met	TSAA
Growth and Reproduction (g/1000 kcal ME)	0.88	1.75

### Conclusions

The inclusion of amino acids in dog food can affect the dog’s health and metabolic functions. For example, a deficiency in total sulfur amino acids can lead to disease or ability to overcome immune related stress. As the pet food industry strives to ensure healthy animals from puppies to adults, it becomes essential to understand the amino acid requirements of each age group in order to create more accurate diet formulations.



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