Evaluation of arginine requirements of growing >14 week to 9 month old Labrador retrievers using differing diet formulations and the indicator amino acid oxidation technique

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Over time, the need to update amino acid requirements has become increasingly important due to genetic selection and the demand for more advanced diets. Excess or restricted amounts of amino acids can have deleterious effects on bioprocesses in the body, and to extreme cases, mortality\(^1,2\). In growing puppies, arginine deficiency has been shown to cause emesis, muscle tremors, and urinary orotic acid excretion. It has been determined that arginine requirements are affected by protein synthesis and oxidation\(^6\). Additionally, species including dogs and poultry have an antagonistic mechanism between lysine and arginine in which a surplus of one amino acid can negatively affect the absorption of the alternate amino acid\(^6\).

Minimal and recommended amino acid allowances have been established by the NRC\(^3\), but are not well backed in research. Previous research has proven that dated methods of establishing amino acid requirements, such as the use of crystalline amino acid diets, cannot always be used in practice due to high costs, and the difference of bioavailability between crystalline and commercial diets\(^4\). A newer method, the indicator amino acid oxidation technique, is less invasive and less time intensive than other methods used to determine amino acid requirements\(^5\). Little research has been completed to date on the actual dietary amino acid requirements in dogs, especially in targeted populations such as age, breed, body composition, etc. The purpose of this experiment was to explore the lysine-arginine antagonism and determine if the mechanism affects amino acid requirements in canines. This study is the arginine phase of a series aimed to define the actual dietary requirements of amino acids in growing Labrador Retrievers using the indicator amino acid oxidation technique.

INTRODUCTION
Six growing male Labrador retrievers were selected from colony at Four Rivers Kennel.
All dogs housed in controlled kennel environment.
All dogs aired outside in social groups for approximately six hours daily unless being tested.
Kenneled individually overnight.
Free access to automatic waterers, fed once daily in the morning.

All dogs were supplied with constant dietary Phe and Tyr (with excess Tyr) in the control and test diets. The constant Phe and Tyr includes the stable isotope utilized.

The control diet was fed for two days, followed by a day in which the test diet was fed, a tracer amino acid was supplied, and breath samples were collected.
On test day, a priming dose of [1-13C]Phe (Cambridge Isotope Laboratories, Inc.) based on the subject’s body weight was first supplied, followed by [1-13C]Phe doses every thirty minutes, spanning a four hour period.

A respiration mask was placed on each subject every thirty minutes (Oxymax, Columbus Instruments), 13CO2 was collected, and enrichment was determined by isotope ratio mass spectrometry (IRMS).

Results for IRMS were converted to atom percent excess (APE) and analyzed using a broken-line model of best fit (JMP Pro 14.1).
The amount of control and test food provided was based on using Metabolic BW and the NRC (2006) ME equation for puppies.
The amount of energy (4000 kcal/kg) (DM) provided daily was based on 1.2 x times NRC requirement.
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TREATMENTS

- Diets were isonitrogenous and isocaloric to ensure responses were due to differing amounts of digestible arginine included in the diet.
- Test diets were divided into Group I and Group II, which contained excess Lys with respect to Arg and lower Lys inclusion levels, accordingly. The inclusion of Lys was set at 0.1% higher than Arg in the Group II diets.
RESULTS

Results from IRMS revealed differences in amino acid requirements among the two groups (Figure 1). The estimated amino acid requirement (EAR) and population safe requirements of growing dogs in Groups I and II was determined to be 1.49 ± 0.30 and 1.38 ± 0.21 g/1000 kcal ME (mean ± 2SD), respectively.

Figure 1. $^{13}$CO$_2$ oxidation for dogs fed Group 1 diets (Excess Lys)

Figure 2. $^{13}$CO$_2$ oxidation for dogs fed Group II diets (0.1% Lys> Arg)
CONCLUSION

The amino acid requirements differed for the two groups of arginine treatments, suggesting there is reason to believe that the lysine-arginine antagonism does occur in canines. Including an excess of dietary lysine resulted in an estimated amino acid requirement and population safe requirement of growing dogs as $1.49 \pm 0.30$ g/1000 kcal ME (mean $\pm$ 2SD). Alternatively, a lys inclusion of 0.1% greater than Arg resulted in a requirement of $1.38 \pm 0.21$ g/1000 kcal ME. NRC suggest $1.33$ g/1000 kcal for Minimal Requirement (MR) and $1.65$ g/1000 kcal for Recommended Allowance (RA). The Arginine group (provided excess lysine) had a higher requirement than RA but the Arginine group fed lower levels of lysine had a very similar RA as suggested by NRC.

Czarnecki et al. (1985) conducted three studies with pointers with objective of evaluating lysine-arginine antagonism. The researchers showed 1-2% excess lysine produced no change in performance of growing pups, however 4% lysine produced classic arginine deficiencies. The research group added arginine to the high lysine diets and mitigated the arginine deficiency thus showing antagonism. Certain species such as poultry are also affected by this condition in which an excess of either lysine or arginine can affect the biological availability of the alternate amino acid. Future studies to explore the antagonist effect should be conducted.

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REFERENCES