Lysine requirements in small, medium, and large breed adult dogs using the indicator amino acid oxidation technique[1]

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ABSTRACT: There is a lack of knowledge regarding the lysine (Lys) requirements of mature dogs and whether there are breed differences. The present study aimed to determine the Lys requirement in three breeds of mature dogs using the indicator amino acid oxidation (IAAO) technique. Thirteen adult dogs were used, four Miniature Dachshunds (5.39 ± 0.71 kg; 1.05 ± 0.02 yr old, mean ± SD), four Beagles (8.09 ± 0.40 kg; 5.03 ± 0.09 yr old, mean ± SD), and five Labrador Retrievers (29.42 ± 2.04 kg; 3.30 ± 0.69 yr old, mean ± SD). After 14 d of adaptation to a basal extruded kibble diet, dogs were fed a test diet mildly deficient in Lys (Lys concentration = 0.36%) at 17 (Miniature Dachshunds) or 13 g/kg body weight (BW; Beagles and Labradors) for 2 d. The test diet was supplemented with one of seven isonitrogenous Lys–Ala solutions, resulting in a final dietary Lys concentration of 0.36%, 0.40%, 0.44%, 0.50%, 0.54%, 0.58%, and 0.62% (as-fed basis). Dogs received dietary concentrations of Lys in random order and no dog received the same order. Following 2 d of adaptation to the experimental diets, the dogs underwent IAAO studies. During the IAAO studies, total daily feed was divided in 13 equal meals. At the sixth meal, dogs were fed a bolus of L-[1-^{13}C]-Phe (9.40 mg/kg BW); thereafter, L-[1-^{13}C]-Phe was supplied with every meal (2.4 mg/kg BW). Total production of $^{13}CO_2$ ($F^{13}CO_2$) during isotopic steady state was determined by enrichment of $^{13}CO_2$ of breath samples and total production of CO_2, measured using indirect calorimetry. A two-phase linear regression model was used to derive the mean Lys requirement, defined as the breakpoint, and the upper 95% confidence limit was calculated as the recommended allowance (RA) for Lys intake. For Miniature Dachshunds, the study was repeated with a feed intake of 14 g/kg BW, but Lys requirements could not be determined at either feed intake, suggesting a requirement below the lowest concentration and intake. Mean Lys requirements for Beagles and Labradors were 0.455% (59.16 mg/kg BW) and 0.440% (57.19 mg/kg BW), respectively, on a dry matter basis. Pooling the data for these breeds provides a mean estimate of the Lys requirement at 0.448% (58.21 mg/kg BW) with an upper 95% CL of 0.526% (68.41 mg/kg BW) on a dry matter basis. In conclusion, the Lys requirements of Beagles and Labradors are similar, while the requirement for Miniature Dachshunds is undetermined and likely lower. The estimated Lys requirement for Beagles and Labradors is higher than the National Research Council recommendation.

Key words: adult dogs, indicator amino acid oxidation, lysine, maintenance

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INTRODUCTION

Much of the work done on amino acid (AA) requirements in dogs focuses on the requirements of immature dogs and, consequently, little is known about lysine (Lys) requirements of mature dogs. Furthermore, AA requirement studies for the growing dog typically use the Beagle, which may not accurately represent the overall population of pet dogs due to differences in breed sizes, life stages, and activity levels. As a result, there is a paucity of information about the AA requirements of adult dogs across breeds.

The National Research Council (NRC, 2006) states that "no individual dose-response peer-reviewed reports could be found for the minimal requirements (MRs) of any of these amino acids -including Lys- for dogs for maintenance," and suggests an MR of 0.28% of diet on a dry matter basis. The American Association of Feed Control Officials (AAFCO; 2018) and the European Pet Food Industry Federation (FEDIAF, 2017) recommendations are subsequently based on scaling up the NRC recommendations to account for variation in digestibility of different ingredients to prevent AA deficiencies. However, formulating commercial foods in significant excess of the actual requirements of the animal presents a challenge to the sustainability of the pet food industry (Swanson et al., 2013). Therefore, empirically determining the dietary requirements of indispensable AA to avoid either deficiency or excess of individual AA and the definition of the ideal protein for adult, not growing, dogs is of importance.

Requirements of indispensable AA for growing animals have previously been determined using nitrogen balance and growth performance, but these methods have several limitations when applied to mature animals (Morris et al., 2004; Elango et al., 2012). Rand and Young (1999) reanalyzed nitrogen balance studies and concluded that these studies consistently underestimated the minimal Lys requirement of healthy adult humans. The indicator AA oxidation (IAAO) technique results in requirement estimates greater than those obtained with nitrogen balance studies and is a more accurate method of determining requirement estimates, especially at maintenance (Bross et al., 1998; Moehn et al., 2004). The IAAO method has been applied and validated in several species, including dogs (Shoveller et al., 2017), and since used to measure the phenylalanine (Phe; Mansilla et al., 2018), tryptophan (Trp; Templeman et al., 2019), and threonine (Thr; Mansilla et al., 2020) requirements of adult dogs. Previously, we reported no differences in Phe requirements for three breeds of dog, but Beagles had a greater Trp requirement than Labrador Retrievers and Miniature Dachshunds (Mansilla et al., 2018; Templeman et al., 2019). Beagles and Labrador Retrievers had similar Thr requirements, but a requirement could not be determined for Miniature Dachshunds (Mansilla et al., 2020). All estimates of AA requirements are greater than those currently recommended by NRC (2006) and the affiliated regulatory bodies. The objective of the present study was to determine the Lys requirements of adult dogs of three different breeds using IAAO. Considering the difference in requirement between breeds observed with Trp, we hypothesized that adult dogs of different breed sizes will differ in Lys requirements for maintenance. We also predicted that Lys requirements are higher than those presented in NRC (2006).

MATERIALS AND METHODS

Animals and Housing

The present experiment was approved by the Procter and Gamble Pet Care's Institutional Animal Care and Use Committee (IACUC). A total of 13 dogs were used, 4 adult spayed and neutered Miniature Dachshunds (5.39 ± 0.71 kg; 1.05 ± 0.02 yr old, mean ± SD), 4 adult spayed Beagles (8.09 ± 0.40 kg; 5.03 ± 0.09 yr old, mean ± SD), and 5 neutered Labradors (29.42 ± 2.04 kg; 3.30 ± 0.69 yr old, mean ± SD). Miniature Dachshunds, Beagles, and Labrador Retrievers were chosen to represent small, medium, and large dog breeds, respectively, and were all purebred dogs. All dogs resided at Procter and Gamble Pet Care (Lewisburg, OH) and were considered healthy based on a general health evaluation by a licensed veterinarian prior to the study. The study was started on Beagles and Labrador Retrievers in October, 2007, and on Miniature Dachshunds in July, 2008. During the study, dogs were pair-housed in kennels (2.4 × 2.4 m) in a temperature-controlled building (22 °C) and with a lighting schedule of 12:12 h light:dark. Dogs received daily socialization and exercise and regular veterinary care as previously reported (Shoveller et al., 2017).

Diets and Study Design

A basal diet was formulated to meet or exceed requirements for all essential AA according to NRC (2006). The analyzed nutrient content of this diet is detailed in Table 1. The extruded kibble basal diet was fed twice daily (0700 and 1300 hours) during 14 d prior to the beginning of the experiment (adaptation period) in amounts known to maintain their individual body weight (BW). After the 14-d adaptation period to the basal diet, a test diet (similar to basal diet but without added crystalline Lys; final Lys = 0.36%) was fed to the dogs at 17 g/kg of BW for Miniature Dachshunds and at 13 g/kg of BW for Beagles and Labradors for 2 d prior to each IAAO study. This feed intake was on average 95% of the feeding intake known to maintain each dog's BW. Moderate food intake restriction is required in isotope dilution studies to ensure that all dogs consume all diet to ensure equivalent food and isotope delivery among dogs of the same breed and among all dietary treatments. An important design principle of oxidation methods is that all animals are fed equivalent food with the same caloric density among all the dietary treatments as this will change overall gas exchange and macronutrient partitioning potentially contributing to the dilution of the tracer and increasing variability (Hoerr et al., 1989). Furthermore, equivalent single diet delivery among dietary treatments within breed are necessary to maintain equivalent delivery of dietary Phe and Lys among dietary treatments since Phe oxidation is sensitive to dietary supply (Mansilla et al. 2018).The test diets were supplemented with one of seven Lys hydrochloride (Skidmore Sales and Distributing, West Chester Township, OH) top-dressing solutions (0, 1.67, 3.33, 5.83, 6.35, 9.17, and 10.80 g/L) at 5.1 mL/kg BW for Miniature Dachshunds and at 3.9 mL/kg BW for Beagles and Labrador Retrievers. To maintain similar nitrogen concentrations among all top-dressing solutions,

Table 1. Ingredient composition and analyzed nutrient contents of the test diet on an as-fed basis

Ingredient	g/kg
Corn starch	480.6
Chicken fat	130.6
Chicken meal	63.9
Yellow corn	50.6
Brewer's rice	50.6
Amino acid premix[a]	75.9
Beet pulp	30.4
Dicalcium phosphate	29.0
Chicken flavor	20.2
Potassium chloride	13.3
Sodium bicarbonate	10.1
Chicken liver flavor	5.06
Brewer's yeast	5.06
Ground flax	5.06
Choline chloride	4.47
Vitamin premix[b]	4.25
Sodium hexametaphosphate	4.05
Calcium carbonate	5.80
Mineral premix D[c]	3.44
Fish oil	2.91
Sodium chloride	1.82
Monosodium phosphate	2.33
Ethoxyquin	0.51
Nutrient content	Analyzed content
Metabolizable energy, kcal/kg (calculated)[d]	3700
Dry matter, %	92.00
Crude protein, %	10.99
Crude fiber, %	1.79
Crude fat, % min	14.25
Arg, %	0.85
Pro, %	0.38
Cys, %	0.74
His, %	0.42
Ile, %	0.68
Leu, %	0.80
Lys, %	0.36 (0.72)[e]
Met, %	0.56
Phe, %	0.61
Tau, %	0.020
Thr, %	0.77
Trp, %	0.40
Tyr, %	0.47
Val, %	0.55
Ser, %	0.32
Gly, %	0.46
Ala, %	0.46

[a]Provides per kg of final diet: 4.03 g of Arg, Cys, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, and Val each. Lys was removed for the test diets.

[b]Vitamin premix contained per kg: 6,650 K IU vitamin A, 365,000 IU vitamin D_3, 100,400 IU vitamin E, 4,100 mg thiamine, 2,500 mg niacin, 2,000 mg pyridoxine, 7,750 mg d-pantothenic acid, 115 mg folic acid, 45 mg vitamin B_{12}, 2,500 mg inositol, 13,750 mg vitamin C, and 1,200 mg β-carotene.

[c]Mineral premix contained per kg: 150 mg cobalt carbonate, 4,500 mg copper sulphate, 900 mg potassium iodine, 72,000 mg iron sulfate, 8,000 mg manganese oxide, 5,800 mg manganese sulfate, and 60,000 mg sodium selenite.

[d]Calculated metabolizable energy based on modified Atwater values.

[e]Value for Lys presented in brackets represents the basal test diet only.

alanine (Ala) was added (10.54, 8.95, 6.20, 5.69, 3.25, 1.63, and 0 g/L for solutions 1–7, respectively) to ensure total nitrogen provision did not impact AA oxidation. The final Lys concentrations in the test diet plus the top-dressing solution were 0.36%, 0.40%, 0.44%, 0.50%, 0.54%, 0.58%, 0.62% (experimental diets; as-fed basis). After the 2-d adaptation period to the experimental diet (Moehn et al., 2004), the IAAO study combined with indirect calorimetry was conducted. After each IAAO study, dogs returned to the basal diet for 4 d before feeding the test diet with a different top-dressing solution and conducting the next IAAO study. This 7-d feeding regime was repeated seven times with treatments assigned to dogs in a random order (Microsoft Excel 2010: Random function) based on a complete randomized block design, where dogs were blocked by breed and then allocated to the order of dietary treatments they were receiving. After completion of the study, all dogs were fed each of the seven experimental diets. For Miniature Dachshunds, blood samples (3 mL) were collected from the jugular vein in serum vacutainers (Becton and Dickinson) at the end of each IAAO study and represent fed state serum AA concentrations. The study procedure was repeated for Miniature Dachshunds at a lower level of feed intake (14 g/kg BW). Throughout the whole study, dogs had access to fresh water via an automatic watering system. All dogs consumed their entire daily diet offerings (basal or test diets) throughout the study.

IAAO Studies

The conduct of the IAAO study has been previously described in detail (Templeman et al., 2019) and no deviations other than dietary treatment and food provision occurred. Briefly, dogs were moved to individual respiration calorimetry chambers where the resting volume of CO_2 and O_2 produced (VCO_2; VO_2) by each dog was determined. Dogs were then fed their corresponding feed allowance divided in 13 equal meals; the first 3 meals were fed every 10 min to induce a fed state, and the other 10 meals were fed every 25 min. The total amount of feed fed during the IAAO study was based on BW measured the same day in the morning after 18 h of fasting (17 g/kg of BW for Miniature Dachshunds and 13 g/kg BW for Beagles and Labradors). Background ^{13}C enrichment was determined by the collection of CO_2 samples over three consecutive 25-min periods. The sixth meal (95 min after first feeding) contained a priming dose (9.40 mg/kg BW) of L-[1-^{13}C]-Phe (99%; Cambridge Isotope Laboratories, Inc., Tewksbury, MA). To maintain the supply of L-[1-^{13}C]-Phe, the following seven meals contained constant doses (2.40 mg/kg BW) of L-[1-^{13}C]-Phe for all dogs. Expired CO_2 was collected over the last eight 25-min periods. Additional details about the timeline for each IAAO study are presented in Mansilla et al. (2018).

Sample Collection and Analysis

Nitrogen content in the basal diet was analyzed with a LECO analyzer (LECO Corporation, MI). Amino acid content in the test diet was analyzed using AOAC method 999.12 (AOAC International, 2000). Calorimetry data were collected automatically using Qubit calorimetry software (Customized Gas Exchange System and Software for Animal Respirometry; Qubit Systems Inc.). Measured VCO_2 during fasting and fed states were averaged over the collection periods to obtain a mean fasting and fed VCO_2 for each dog. Background and enriched samples of CO_2 were collected by trapping subsamples of expired CO_2 in 1-M NaOH. The NaOH solution was subsampled and stored at room temperature until further analysis. The enrichment of ^{13}C in breath CO_2 captured in NaOH solution was measured by continuous-flow isotope ratio mass spectrometry (20/20 isotope analyzer; PDZ Europa Ltd., Cheshire, UK). Enrichment of CO_2 samples were expressed above background samples (atom percent excess; APE).

Calculations

The rate of $^{13}CO_2$ released per kilogram of BW per hour ($F^{13}CO_2$, mmol.kg^{-1}.h^{-1}) was calculated using the following equation:

$$F^{13}CO_2 = (FCO_2)\,(ECO_2)\,(44.6)\,(60)\,/\,[(BW)\,(1.0)\,(100)]\,,$$

in which FCO_2 is the average production of CO_2 during the isotope steady state phase (mL/min); ECO_2 is the average $^{13}CO_2$ enrichment in expired breath at isotopic steady state (APE, %); and BW is the weight of the dog (kg). The constants 44.6 (mmol/mL) and 60 (min/h) convert the FCO_2 to micromoles per hour; the factor 100 changes APE to a fraction; and the 1.0 is the retention factor of CO_2 in the body due to bicarbonate fixation as reported previously (Shoveller et al., 2017). Resting and fed energy expenditure (REE and FEE) was calculated based on VO_2 and VCO_2 using the modified Weir equation (Weir, 1949):

$$\text{REE (kcal/day)} = [3.94\,(VO_2) + 1.11\,(VCO_2)] \times 1440$$

Energy expenditure (kcal/d) was expressed in relation to metabolic BW ($BW^{0.75}$) for all dog breed sizes.

Body Composition Determination

Lean body mass (LBM) was determined during the 7-wk study using an X-Ray Bone Densitometer (QDR4500, Hologic Inc., Bedford, MA). After 18 h of fast, dogs were sedated using dexmedetomidine (0.02 mg/kg; Dexdomitor, Pfizer) and carprofen (2 mg/kg; Rimadyl, Pfizer) administered intramuscularly. Propofol (5 mg/kg; Propoflo, Abbott) was administered intravenously for anesthesia induction. Anesthetized dogs were positioned in sternal recumbency with the cranial aspect of ante brachium placed on the table and phalanges pointing caudally. Body mass composition (i.e., mineral, fat, lean, and water content) was determined in the left and right arms and legs, trunk, and head (data not shown). Following the scan, atipamezole (0.2 mg/kg; Antisedan, Pfizer) was administered intramuscularly. Whole body composition was determined by the sum of all regions measured on individual dogs.

Statistical Analysis

The effect of Lys concentration in the test diet on $F^{13}CO_2$ was analyzed using PROC MIXED of SAS (v. 9.4; SAS Institute Inc., Cary, NC) with diet as a fixed effect and dog as a random effect. The estimate of the mean Lys requirement and the upper 95% confidence limit (CL) for individual dog breeds and pooling data from Beagles and Labradors Retrievers were derived by breakpoint analysis of the $F^{13}CO_2$/kg BW using a two-phase linear regression model as previously reported (Shoveller et al., 2017). Differences in AA concentration in blood were determined comparing each dietary Lys concentration against the lowest Lys diet (0.36%) using the Dunnett's test. The mean Lys requirements were also calculated with Lys concentration in serum data using the two-phase linear regression model. Within different breed sizes, BW, and the calorimetry data were analyzed using PROC MIXED of SAS (v. 9.4; SAS Institute Inc., Cary, NC) with diet as a fixed effect and dog as a random effect. The same data was also pooled per dog and was compared using breed as a fixed effect and dog as a random variable. Results were considered statistically significant at $P \le 0.05$ and a trend when $P \le 0.10$.

RESULTS

All dogs remained healthy throughout the study and consumed all feed during the IAAO studies. Body weight, energy expenditure (EE), and respiratory quotient (RQ) did not differ across dietary treatments for all individual breeds, except for BW in Beagles, which was highest at the highest dietary Lys concentration (Table 2). Body weight, LBM, and calorimetry data were pooled for comparison between breeds. Body weight and LBM were different ($P < 0.05$) between all breeds as expected (Table 3). Lean body mass was not significantly different when expressed as a percentage of BW ($P > 0.10$) between Beagles and Labradors but was lower for Miniature Dachshunds ($P < 0.05$). Fasting RQ did not differ among breeds ($P > 0.10$) but fed RQ was higher for Miniature Dachshunds compared to the other breeds ($P < 0.05$). Rate of O_2 and CO_2 differed among breeds with the highest rate for Labradors and the lowest for Miniature Dachshunds as might be expected due to size differences ($P < 0.05$). Resting EE and FEE per unit of metabolic BW were lower for Miniature Dachshunds compared to Beagles or Labradors ($P < 0.05$).

All dogs consumed their meals immediately after feeding during the IAAO study. Isotopic steady state was reached by all dogs, confirmed by the last four analyzed breath samples (data not shown). Using the two-phase linear regression relating $F^{13}CO_2$ to the levels of Lys in the test diets, the breakpoint for Lys could not be determined for Miniature Dachshunds at an intake of 17 g/kg BW (Fig. 1A). The study was repeated with Miniature Dachshunds at an intake of 14 g/kg BW and again no breakpoint could be determined. For Beagles, based on the $F^{13}CO_2$ ($mmol.kg^{-1}.h^{-1}$), the mean Lys requirement was estimated to be 0.423% (55.02 mg/kg BW) of diet (as-fed basis) with an upper 95% CL of 0.507% (65.89 mg/kg BW; Fig. 1B). For Labradors, the Lys requirement was estimated to be 0.409% (53.18 mg/kg BW) with an upper 95% CL of 0.477% (61.96 mg/kg BW) on an as-fed basis (Fig. 1C). The Lys requirement for Beagles and Labradors was not different ($P > 0.10$). Because the estimate of the Lys requirement did not differ between Beagles and Labradors and they received the same feed intake, we pooled the data. Pooling

Table 2. BW, fasting and fed energy expenditure and RQ for Miniature Dachshunds, Beagles, and Labrador Retrievers fed diets containing graded levels of Lys

Miniature Dachshunds									
	Dietary Lys[a], %							Pooled ANOVA	
	0.36	0.4	0.44	0.5	0.54	0.58	0.62		
	n = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	SE[b]	*P*-value
BW, kg	4.81	4.78	4.82	4.79	4.74	4.78	4.76	0.18	0.739
REE, kcal/kg $BW^{0.75}$	32.6	46.4	40.1	26.1	30.7	36.9	27.7	9.2	0.077
FEE, kcal/kg $BW^{0.75}$	49.3	70.7	72.3	53.4	54.3	63.0	49.5	10.6	0.191
Fast RQ	0.775	0.774	0.765	0.762	0.797	0.763	0.780	0.019	0.843
Fed RQ	0.843	0.854	0.848	0.844	0.846	0.847	0.840	0.007	0.716
Beagles									
	Dietary Lys, %							Pooled ANOVA	
	0.36	0.4	0.44	0.5	0.54	0.58	0.62		
	n = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	SE	*P*-value
BW, kg	7.89	8.47	8.47	8.49	7.67	7.79	9.29	0.29	0.008
REE, kcal/kg $BW^{0.75}$	51.1	55.2	46.2	64.0	64.1	68.6	60.9	6.0	0.140
FEE, kcal/kg $BW^{0.75}$	55.7	67.8	53.5	72.6	76.9	76.2	81.5	8.4	0.178
Fast RQ	0.727	0.747	0.754	0.763	0.762	0.745	0.774	0.014	0.102
Fed RQ	0.830	0.847	0.829	0.834	0.839	0.827	0.833	0.008	0.675
Labrador Retrievers									
	Dietary Lys, %							Pooled ANOVA	
	0.36	0.4	0.44	0.5	0.54	0.58	0.62		
	n = 5	*n* = 5	*n* = 5	*n* = 5	*n* = 5	*n* = 5	*n* = 5	SE	*P*-value
BW, kg	29.5	29.6	29.9	29.3	30.0	29.4	29.5	0.99	0.470
REE, kcal/kg $BW^{0.75}$	62.9	63.9	64.7	61.8	66.8	58.5	66.8	6.0	0.927
FEE, kcal/kg $BW^{0.75}$	74.0	78.1	76.0	76.3	81.4	70.0	80.0	8.0	0.933
Fast RQ	0.750	0.784	0.768	0.755	0.773	0.747	0.757	0.013	0.187
Fed RQ	0.831	0.832	0.844	0.832	0.842	0.813	0.818	0.011	0.228

[a]Lys content in the diet is in as-fed basis

[b]standard error of the mean based on *n* = 4 for each level of Lys in the diet for Miniature Dachshunds and Beagles, *n* = 5 for Labrador Retrievers.

Table 3. BW, LBM, and indirect calorimetry data (+/- SEM[a]) of the dogs used

	Miniature Dachshunds	Beagles	Labrador Retrievers	Pooled ANOVA
	n = 4	*n* = 4	*n* = 5	*P*-value
BW, kg	4.97 ± 0.17[c]	8.33 ± 0.52[b]	29.61 ± 0.82[a]	<0.001
LBM, kg	3.48 ± 0.27[c]	7.21 ± 0.13[b]	24.48 ± 2.02[a]	<0.001
LBM, % BW	70.1 ± 1.3[b]	87.0 ± 3.3[a]	82.4 ± 5.2[a]	<0.001
REE, Kcal/$BW^{0.75}$	43.0 ± 2.68[b]	58.6 ± 3.16[a]	64.0 ± 3.51[a]	<0.001
FEE, Kcal/$BW^{0.75}$	61.79 ± 3.68[b]	69.14 ± 4.10[ab]	77.42 ± 4.20[a]	0.023
Fasting RQ	0.774 ± 0.006	0.754 ± 0.008	0.762 ± 0.010	0.122
Fed RQ	0.847 ± 0.003[a]	0.833 ± 0.005[b]	0.830 ± 0.007[b]	0.014
Fed O_2, L/min	1.85 ± 0.19[c]	2.78 ± 0.28[b]	8.27 ± 0.37[a]	<0.001
Fed VCO_2, L/min	1.57 ± 0.16[c]	2.32 ± 0.23[b]	6.89 ± 0.30[a]	<0.001

[a]Standard error of the mean, *n* = 4 for Miniature Dachshunds and Beagles, *n* = 5 for Labrador Retrievers.

[a,b,c]Values in a row with different superscript are different ($P < 0.05$).

these data, the estimated requirement for Lys was 0.416% (54.13 mg/kg BW) with an upper 95% CL of 0.489% (63.62 mg/kg BW) on an as-fed basis (Fig. 1D). Requirements are presented on a dry matter basis for comparison with NRC, AAFCO, and FEDIAF recommendations in Table 4.

For Miniature Dachshunds, serum Lys concentration at any dietary Lys level did not differ when compared to that at the lowest level of dietary Lys (Table 5). A breakpoint could not be determined using the serum AA data for Miniature Dachshunds and suggests that plasma Lys may be

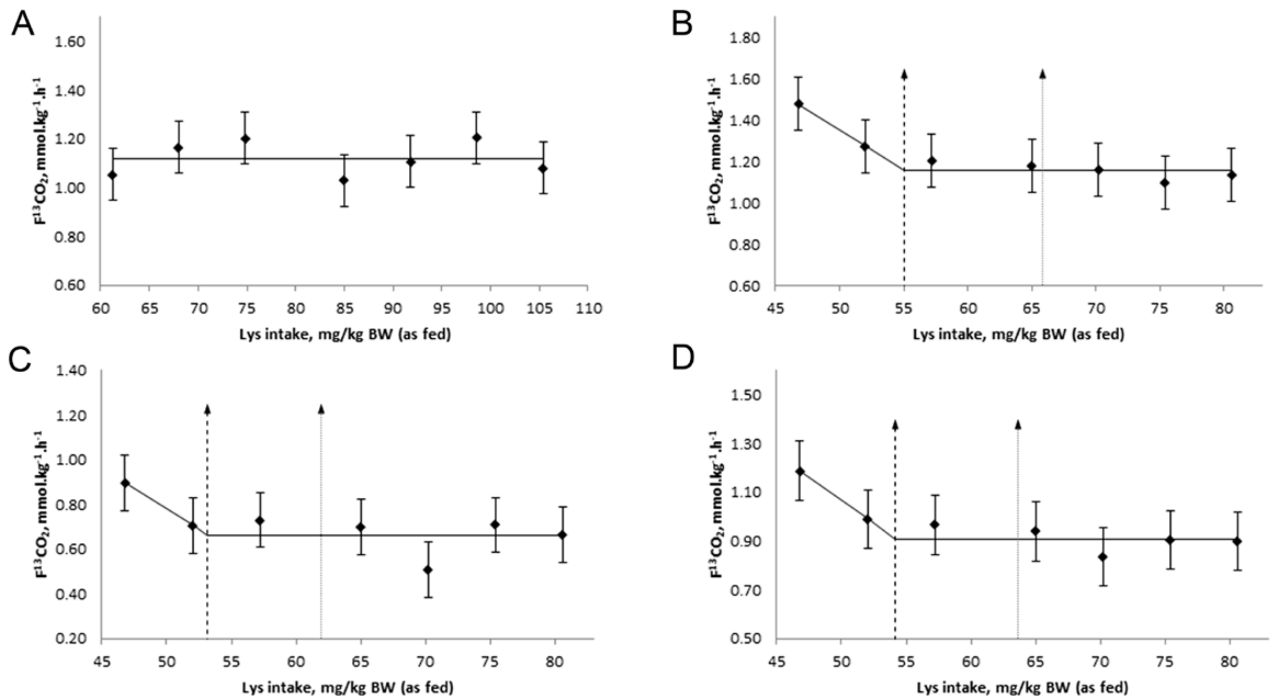

Figure 1. Production of $^{13}CO_2$ from the oxidation of orally administered L-[1-^{13}C]-Phe in adult dogs of different breeds fed diets with increasing levels of Lys. Miniature Dachshunds (A), Beagles (B), Labrador Retrievers (C), Beagles and Labrador Retrievers (D). Dashed lines represent mean Lys requirement; dotted lines represent upper 95% confidence limit.

Table 4. Recommended dietary Lys inclusions by AAFCO, FEDIAF, NRC, and the present study

			NRC[c]		Miniature Dachshunds		Beagles		Labrador Retrievers		Beagles and Labrador Retrievers	
	AAFCO[a]	FEDIAF[b]	MR	RA	MR	CL	MR	CL	MR	CL	MR	CL
g/100 g DM[d]	0.63	0.46	0.28	0.35	–	–	0.455	0.545	0.440	0.512	0.448	0.526
g/Mcal ME	–	1.22	0.70	0.88	–	–	1.23	1.47	1.19	1.39	1.21	1.42
mg/kg BW	–	–	–	–	–	–	59.16	70.86	57.19	66.62	58.21	68.41

[a]Association of American Feed Control Officials Manual, 2018.

[b]European Pet Food Industry Federation Nutritional guidelines for complete and complementary pet food for cats and dogs, 2017.

[c]Nutrient requirements of dog and cats (NRC, 2006).

[d]Values for g/100 g dry matter (DM) are determined assuming a dietary energy density of 4,000 kcal ME/kg.

a poor predicator of whether the dog is below or above its requirement.

DISCUSSION

Lysine is an essential AA for dogs and cannot be synthesized endogenously but there is a lack of information about the Lys requirements of adult dogs (NRC, 2006). In growing animals, nitrogen balance and growth performance are commonly the outcomes assessed when investigating AA requirements, but these responses are insensitive in adult animals due to a slower rate of protein turnover and result in an underestimation of requirements for mature animals at maintenance (Morris and Rogers, 1994; Pencharz and Ball, 2003; Morris et al., 2004). The IAAO method is more sensitive to changes in the AA pool than the nitrogen balance method and does not require the lengthy adaptation period of up to 7 d that is necessary for nitrogen balance studies (Pencharz and Ball, 2003; Elango et al., 2008). Protein deposition is prioritized in nitrogen balance and growth studies, but the IAAO method takes into account the use of the AA for both protein synthesis and oxidative pathways, more suitable for adults (Pencharz and Ball, 2003; Elango et al., 2012).

In the current study, the slope of the breakpoint model for the $F^{13}CO_2$ in Miniature Dachshunds data was not significant, and Lys requirements could not be determined for this breed at either of the two feed intakes (17 or 14 g/kg BW) investigated.

Table 5. Serum Lys concentration in adult Miniature Dachshunds fed diets containing increasing levels of Lys*

	Miniature Dachshunds							
	Dietary Lys, %							
	0.36	0.4	0.44	0.5	0.54	0.58	0.62	SEM[a]
	$n = 4$	$n = 4$	$n = 4$	$n = 4$	$n = 4$	$n = 4$	$n = 4$	
Lys, µM	249	194	286	288	321	246	286	65.0

[a]Standard error of the mean, $n = 4$ at each level of dietary Lys for Miniature Dachshunds.

*Significantly different ($P < 0.05$) when compared to the lowest level of dietary Lys (Lys = 0.36%) using the Dunnett's test.

Moreover, no breakpoint could be determined for this breed when using the serum Lys concentration data collected at the 14 g/kg BW level of feed intake. This suggests that the Lys requirement for Miniature Dachshunds is below 0.36% based on the IAAO data. The pooled estimated 95% upper CL of 0.526% based on $F^{13}CO_2$ (68.41 mg/kg BW; dry matter basis) for Beagles and Labradors is higher than the NRC (2006) MR of 0.28% and the FEDIAF (2017) recommendation of 0.42% but lower than the AAFCO (2018) recommendation of 0.63% (Table 4). The calculated upper 95% CL is an estimation of the recommended allowance (RA) for Lys.

To our knowledge, this is the first dose-response study evaluating the Lys requirements of adult dogs at maintenance. The discrepancy between the results of this study and the NRC (2006) may be in part because current NRC (2006) recommendations are based on long-term studies during which no signs of AA deficiencies were reported in dogs fed low-protein diets (Ward, 1976; Sanderson et al. 2001). Also, crystalline Lys has an estimated bioavailability of 100%. Thus, estimating Lys requirements with crystalline AA will result in a lower estimate of the requirement when compared to estimates generated from feeding diets formulated with natural protein sources with a lower bioavailability of AA (Hirakawa and Baker, 1986). This may in part explain the discrepancy between our estimated RA and the AAFCO (2018) recommendation, as this recommendation is the result of safety factors applied to the NRC values to ensure nutritional adequacy of commercial foods using a wide variety of ingredients. Up to 56% of Lys in extruded maintenance dog diets may be bound and unavailable to the dog, suggesting that total Lys content is an unreliable predictor of the sufficiency of a food to meet dog Lys requirements (Williams et al., 2006). Accurate estimates of requirements are, therefore, essential and need to be considered when formulating diets with ingredients typically used in commercial pet foods. Further, the pet food industry may benefit from adopting the practice of formulating diets on the basis of individual AA, rather than total protein, limiting the need to formulate diets in large excess of requirements and improving the sustainability of pet foods by optimizing nutrient delivery (Fiacco et al., 2018).

Growing dogs have higher AA requirements in contrast to adults due to high protein deposition rate in addition to physiological maintenance. The adequate intake (AI) of Lys for immature Beagles was determined to be 0.577% and 0.692% (as-fed basis) of the diet for female and male dogs, respectively (Milner, 1981). Adequate intake is the estimated requirement of a given nutrient to sustain a particular stage of life when no MR has been determined (NRC, 2006). As mentioned, bioavailability also needs to be considered; Pointer puppies may have a lower Lys requirement of 0.70% of the diet when determined using diets with crystalline AA compared to diets with intact proteins where the requirement was estimated at 0.8% (Hirakawa and Baker, 1986). The present study used a diet with a combination of crystalline AA and intact proteins, resulting in an estimate between those obtained from the sole use of intact proteins or crystalline AA. These results, including the determination of an MR for Lys for adult dogs, are necessary to improve the formulation of diets for the adult dog that are appropriately differentiated from diets intended for dogs in the growth phase.

Resting EE, FEE, and RQ did not differ within each breed at all dietary Lys levels, so the data were pooled and compared between breeds. Both Labradors and Beagles had a significantly higher LBM as a percentage of BW than Miniature Dachshunds. This difference is of interest because, in humans, lean tissue has demonstrated a regionally higher rate of AA metabolism than adipose tissue (Patterson et al., 2002). The lower LBM percentage for Miniature Dachshunds may attribute to the difference in AA requirements and inability to detect a requirement in this breed in this study. Miniature Dachshunds also had a higher food intake compared to the other breeds in this study, which may have affected the prediction of the requirement as food intake does affect gas exchange and may have altered macronutrient partitioning (Hoerr et al., 1989). The fasting RQ values should represent fat oxidation for all breeds due to the 18-h fast prior to each IAAO study, and these values were not different between breeds. However, the fed RQ was higher in Miniature Dachshunds compared to the other breeds, suggesting that this breed had a higher proportional postprandial carbohydrate oxidation. This could perhaps be due to the fasting period not lasting long enough to deplete carbohydrate reserves or a greater emphasis on carbohydrate as an energy source in Miniature Dachshunds. Mansilla et al. (2018) and Templeman et al. (2019) observed that, for Miniature Dachshunds, the fasting RQ was higher and the increment between the fasting and fed RQ was smaller than that of Beagles and Labradors, suggesting a difference in macronutrient utilization for this breed. No such difference was seen in the present study. However, the differences in REE, FEE, and fed RQ for Miniature Dachshunds offer support to the hypothesis that small breed dogs will have differences in macronutrient metabolism, and perhaps AA requirements, compared to larger breeds.

The current paper is part of a series of studies aimed at determining the AA requirements for mature dogs of different breeds. The results of this study suggest that the NRC (2006) maintenance requirements for Lys are underestimated and that the dietary Lys level of commercial foods should not be less than 0.53% (68.41 mg/kg BW; 95% CL) to meet Lys requirements for maintenance for adult dogs. Our estimated requirements are lower than those estimated for growing dogs in the literature, as would be expected for adult dogs, but higher than the current recommendations for adult dogs at maintenance. As only spayed and neutered dogs were included in this study, further investigation is required to determine whether AA requirements differ between intact or altered adult dogs. An estimated requirement for Lys for Miniature Dachshunds could not be determined in this study, suggesting that small breed dogs may have different Lys requirements than larger breeds, a hypothesis supported by differences in body composition between Miniature Dachshunds and the larger breeds. Further studies investigating the differences in macronutrient utilization and other AA requirements among different breeds of adult dogs are warranted.

Conflict of interest statement. The work was funded by the Procter and Gamble Co. A.K.S. and L.F. were employees of the Procter and Gamble Co.; A.K.S. was an employee of Mars Petcare; L.F. is now employed by Mars Petcare, and A.K.S. is now faculty at the University of Guelph. K.A.K.S. and W.D.M. have no conflicts of interest.

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