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Dietary Lysine: Calorie Ratios and Their Influence on Nitrogen Metabolism and Digestibility in Moderately Obese Mature Dogs

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Abstract: Problem statement: A goal during weight loss is to reduce body fat while maintaining body protein. We hypothesized that an increased dietary lysine: calorie might be beneficial during periods of caloric restriction. **Approach:** Two experiments were conducted to determine if changing the ratio of lysine: calories (lysine g/kg: Mcal ME/kg) while maintaining an ideal profile of amino acids will change nitrogen metabolism and weight loss in obese mature dogs. **Results:** The results of these experiments imply that if all essential amino acids are in adequate supply in the food, foods with an optimal balance of nutrients can reduce muscle degradation during periods of caloric restriction. **Conclusion:** Our optimum food for decreasing protein degradation was the food containing 3.0 lysine: calorie. However, the present experiments were relatively short and these results may not be sustained over longer periods of restriction.

Key words: Canine foods, weight loss, protein turnover, dietary lysine, dietary lysine: calorie, obese mature dogs, calorie ratio, Metabolizable Energy (ME)

INTRODUCTION

Nutritional formulation of foods based on essential and non-essential amino acid concentrations instead of total protein is standard for most species. Lysine is often the essential amino acid found in lowest proportion to the animal's requirement and is thus often considered the first limiting amino acid. Because of this lysine is the primary essential amino acid to which all other dietary amino acids are compared when formulating foods. A major determinant of the lysine requirement is the concentration of Metabolizable Energy (ME) in the diet. Campbell and Dunkin (1983) demonstrated that protein deposition increases linearly with the energy intake of the food.

The association between the grams of lysine in the food and the ME of the food is referred to as the lysine: calorie ratio (lysine, g/kg:ME, cal/g). Most of the previous research into the application of lysine: calorie has been conducted in growing swine. Smith *et al*. (1999) reported that increasing the lysine: calorie by varying the dietary energy density decreased back fat while increasing rate and efficiency of gain in growing pigs; suggesting a shift in lean: fat tissue accretion. Since canines are fed at maintenance for the majority of their lives, the data may not be directly transferable between species. However, we hypothesized that an increased lysine: calorie in the dry canine food might be beneficial during periods of caloric restriction. We proposed that by varying dietary lysine: calorie in mature overweight dogs, differences in nitrogen and protein metabolism might be detected. Therefore, the present studies were designed to determine how altering lysine: calorie of canine foods impact nitrogen and protein metabolism during periods of caloric restriction. This concept was investigated by two means; (1) while maintaining an ideal profile of amino acids (Baker and Czarnecki, 1991) and (2) by increasing only lysine while maintaining isonitrogenous intakes.

MATERIALS AND METHODS

Experiment 1: Six mature female crossbred hounds (24.5 + 2.8 kg BW) were used to evaluate nitrogen metabolism and protein turnover during nutrient restriction. The dogs were located in the Division of Laboratory Animal Research Facility at the University of Kentucky (Lexington, Kentucky, USA) and were cared for as specified in IACUC protocols. Dogs were housed individually in an environmentally controlled room (20-25°C with a light: dark cycle of 12:12) and were fed twice daily and had free access to water. For a majority of the experiment, the dogs were maintained in cages measuring 1×1.5 m with a slotted floor and

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access to a 1×2 m outside run with concrete flooring during the day. During periods of total urine and fecal collection the dogs were kept in their respective cages with the area restricted to 1×1 m. Each cage was cleaned twice daily and during periods of total excreta collection, feces and urine were removed three to four times daily. Dogs were provided daily social enrichment with other dogs and caretakers and were allowed to exercise for a minimum of 30 min, except during confinement for urine and fecal collections.

Feeding and treatments: The amino acid, ingredient and chemical compositions of treatments are presented in Table 1. Each food was formulated with the ideal amino acid profile as described by Baker and Czarnecki-Maulden (Baker and Czarnecki, 1991) and in accordance with the Association of American Feed Control Officials (AAFCO, 2007) nutrient guide for dogs and balanced to meet maintenance requirements. The ideal protein concept attempts to establish the ideal ratio of indispensable amino acids based upon tissue needs for maximal growth and diet utilization. Differences in the three treatments resulted from varying lysine: calorie to give ratios of 2.2, 3.0 and 3.8 (Lysine, g/kg:ME, cal/g).

Sampling: Each experimental period was 28 days in length with a 7 day adjustment period to the new foods in order to avoid gastric upset. Prior to the start of the experiment, dogs were fed on an ad libitum basis a high calorie, high protein food (Canine Active Diet, Hill's Science Diet, Topeka, Kansas 28% fat/30% CP) for up to 28 days to ensure they had achieved adequate body condition to maintain an increased body weight throughout the study. A body weight of approximately 120% of ideal was targeted based on each dog's records for the previous year. Following this preconditioning phase, the dogs were switched to their respective treatment foods and fed to maintain body weight for the first 14 days of each experimental period. Once this hyperalimentation phase was completed, the dogs were restricted to 75% of their calculated maintenance energy (145 kcal ME*kg ideal BW 0.67) for the final 14 days. All food was weighed and divided into two portions. Each dog was fed two times a day, half of the daily ration at each feeding and allowed 12 h to consume each meal; all food not consumed was weighed and recorded. Throughout the experiment, food samples were taken daily and composited for nutrient content analysis.

The final six days of the experiment (days 23-28) dogs were confined for urine and fecal collection. Fecal and urine output was collected continuously for the next 6 days and samples were placed into labeled containers. Urine samples were removed every 12 h via catch pans into vessels containing 5 mL 6 N H3PO4 and a light spray of water was used to wash down the catch pan to insure complete urine collection. Urine output was measured and subsequently divided into two equal parts. Half of the output was kept for 15N urea analysis and total urea excretion, while the other half was composited for the period and analyzed for total nitrogen output. Feces was removed 3-4 times per day, placed in labeled plastic bags, composited for the period and stored frozen for further analysis.

At 0700 on day 25 of the experiment the dogs were all given an oral dose of 92 15N-glycine (Cambridge Isotope Laboratory, Andover, MD, USA) at 5 mg glycine/kg BW to monitor nitrogen metabolism. Subsequent urine collections were analyzed for excreted 15N present in urinary urea.

Blood samples were taken on days 22 and 28, 3 h after the morning feeding. Venous blood was drawn from a foreleg and 5 cc were placed in a vacutainer containing heparin and placed on ice. The vacutainer tubes were then centrifuged at 5,000×g for 15 min and the plasma was transferred into storage vials and frozen until further analysis

Experiment 2: Four treatments consisting of dry extruded kibble with lysine: calorie of 2.2, 3.0, 3.8 and 4.6 (g lysine/Mcal ME) were formulated and prepared as described above (Table 2) with the major difference being all foods were formulated to be isonitrogenous as lysine was the only amino acid increased. Feeding procedures were as described above.

Dogs: Eight female crossbred hounds (24.5 + 2.8 kg BW) were randomly divided into two groups of four dogs each. Each group was assigned randomly to a 4×4 Latin square. All dogs were cared for, housed and socialized as described above in accordance with IACUC protocols.

Sampling: Total fecal and urine output were collected for the final 6 days (days 23-28) of the period, with a 15N107 glycine (Cambridge Isotope Laboratory. Andover, MD, USA) dose at 7.5 mg kg^{-1} BW administered orally via a gelatin capsule before feeding on day 25 at 0700. Urine and feces were collected and processed as described above. Blood samples were taken as described for Experiment 1.

Table 1: Ingredient composition and nutrient contents of foods formulated to maintain an ideal amino acid profile while varying the lysine: calorie in Experiment 1[a]

Formulated Lysine, g/kg:ME, cal/g			
Ingredients, g/kg dry matter	2.2	3	3.8
Rice, Brewer's	436.9	398.1	416.1
Corn starch	230.0	263.0	230.9
Low-ash poultry meal	139.4	135.0	134.8
Choice white grease	75.5	75.5	75.5
Corn gluten meal	45.0	45.0	45.0
Soybean oil	20.0	20.0	20.0
Cellulose	20.0	20.0	20.0
Potassium Chloride	10.0	10.0	10.0
Pal enhancer	10.0	10.0	10.0
Dicalcium Phosphate	7.1	7.1	7.1
Iodized salt	2.5	2.5	2.5
Choline chloride	1.7	1.7	1.7
Calcium carbonate	1.1	1.1	1.1
Vitamin premix	1.0	1.0	1.0
Mineral premix	0.5	0.5	0.5
Magnesium Oxide	0.3	0.3	0.3
L-Lysine	-	4.3	7.7
DL-Methionine	-	1.6	3.1
L-Threonine	-	1.6	3.1
L-Tryptophan	-	0.9	1.3
L-Valine	-	0.6	3.0
L-Isoleucine	-	0.3	1.6
L-Histidine	-	-	1.1
L-Tyrosine	-	-	2.7
Nutrient Composition, g/kg Dry matter			
Dry Matter	929.5	934.5	932.0
Crude Protein	180.0	186.5	199.5
Crude Fat	136.0	131.8	137.6
Ash	39.9	39.9	39.9
Crude Fiber	17.2	16.1	17.2
Alanine	11.7	12.3	11.9
Arginine	9.8	11.1	10.3
Aspartate	14.3	15.0	14.5
Cystine	2.4	2.4	2.4
Glutamate	27.0	29.2	27.8
Glycine	12.4	12.8	12.7
Histidine	3.4	4.1	4.3
Isoleucine	5.3	7.2	6.9
Leucine	15.1	16.6	15.2
Lysine	9.0	12.1	15.0
Methionine	3.6	4.8	5.9
Phenylalanine	7.5	8.2	7.7
Proline	11.9	11.0	11.8
Serine	8.3	8.2	8.4
Threonine	6.8	8.2	9.8
Tyrosine	4.7	4.8	6.8
Valine	7.1	9.2	10.2

[a]Formulated to supply at least (g/kg of food): 0.6 Mg, 1.8 Na, 7.0 K, 7.6 Cl, (mg/319 kg of food) 211 Fe, 163 Zn, 13 320 Cu, 13 Mn, 0.4 Se, 1.5 I (IU/g of food) 18.2 Vitamin A, 1.0 Vitamin D, 0.18 Vitamin E (mg/kg of food) 0.3 Biotin, 1484 Choline, 1.9 Folic acid, 62 Niacin, 18 Pantothenic acid, 8.6 Pyridoxine, 8.0 Riboflavin, 41 Thiamin and 0.13 Vitamin B12

Table 2: Ingredient composition and nutrient contents of foods formulated to be isonitrogenous 324 while varying the lysine: calorie in Experiment 2[a]

Formulated Lysine, g/kg:ME, cal/g				
Ingredients, g/kg dry matter	2.2	3	3.8	4.6
Rice, Brewer's	436.9	438.8	440.4	436.9
Corn starch	230.0	230.0	230.0	230.0
Low-ash poultry meal	139.4	132.3	126.9	139.4
Choice white grease	75.5	75.5	75.5	75.5
Corn gluten meal	45.0	45.0	45.0	45.0
Soybean oil	20.0	20.0	20.0	20.0
Cellulose	20.0	20.0	20.0	20.0
Potassium Chloride	10.0	10.0	10.0	10.0
Pal enhancer	10.0	10.0	10.0	10.0
Dicalcium Phosphate	7.1	7.1	7.1	7.1
Iodized salt	2.5	2.5	2.5	2.5
Choline chloride	1.7	1.7	1.7	1.7
Calcium carbonate	1.1	1.1	1.1	1.1
Vitamin premix	1.0	1.0	1.0	1.0
Mineral premix	0.5	0.5	0.5	0.5
Magnesium Oxide	0.3	0.3	0.3	0.3
L-Lysine	-	4.2.0	8.0	-
Nutrient Composition, g/kg Dry matter				
Crude Protein	180.0	180.0	180.0	180.0
Crude Fat	139.0	138.0	137.0	136.0
Crude Fiber	17.0	17.0	17.0	17.0
Potassium	7.7	7.6	7.6	7.5
Calcium	6.1	6.0	5.8	5.7
Phosphorous	5.2	5.1	5.0	4.9
Magnesium	0.7	0.7	0.7	0.7
Arginine	11.0	10.7	10.4	10.1
Histidine	3.8	3.7	3.7	3.6
Isoleucine	7.0	6.8	6.7	6.5
Leucine	15.7	15.4	15.1	14.8
Lysine	8.6	12.0	15.0	18.3
Methionine+Cystine	6.4	6.3	6.1	6.0
Phenylalanine+Tyrosine	13.0	12.7	12.5	12.2
Threonine	6.8	6.6	6.5	6.3
Tryptophan	2.0	1.9	1.9	1.8
Valine	8.7	8.5	8.3	8.1

[a]Formulated to supply at least (g/kg of food): 18.2 Vitamin A, 1.0 Vitamin D, 0.18 Vitamin E (mg/kg of food): 0.3 Biotin, 1484 Choline, 1.9 Folic 327 acid, 62 Niacin, 18 Pantothenic acid, 8.6 Pyridoxine, 8.0 Riboflavin, 41 Thiamin and 0.13 Vitamin B12 and at least (g/kg of food): 0.6 Mg, 1.8 Na, 7.0 K, 7.6 Cl, (mg/kg of food): 211 Fe, 163 Zn, 13 Cu, 13 Mn, 0.4 Se, 1.5 I

Laboratory analyses: Feed was ground using a conventional (Hamilton-Beach ®, Mexico) 14-speed blender, composited by dog and period and stored at room temperature. Dry matters for food intake and fecal output were determined in fecal and whole food samples by loss in sample weight before and after drying in a 55°C forced air oven. Ground food and fecal samples were dried overnight in a 100°C vacuum oven. Feed samples were analyzed for crude fat (method 920.39), crude fiber (method 962.09), ash (method 942.05), calcium (method 968.08), potassium (method 968.08), magnesium (method 968.08), phosphorus (method 965.17) and amino acids (method 994.12) using standard methods (AOAC, 2005).

Samples that were stored frozen were allowed to reach room temperature before analysis. Fecal, ground food and urine samples were placed in ceramic boats and combusted for total nitrogen content using the LECO CN2000 (St. Joseph, MI, USA) nitrogen analyzer. After thawing, individual urine samples, urine composites and plasma samples were analyzed for urea content using the Technicon Auto-Analyzer (Bran + Luebbe, Buffalo Grove, IL, USA) and a colorimetric procedure (Marsh *et al*., 1965).

After determination of the urea content of the samples, the urine was centrifuged for 10 min at 1,000×g at 4°C. An aliquot of urine containing 100 μmol of urea was mixed with 4.0 mL of water, the pH was adjusted to 2.5 and it was poured onto a 1.8 mL AG 50W-X8, 100-200 mesh, H+ form (Bio-Rad Laboratories, Richmond, CA, USA) cation exchange column. The first 5 mL of filtrate was discarded and then 20 mL of deionized H2O was added to the column and the eluent collected. The samples were then dried overnight in a 60°C oven and reconstituted with 2 mL 0.1 M pH 7.0 phosphate buffer. Following reconstitution, 100 μL of the sample was placed in a 25 mL Erlenmeyer flask with 3 mL of 0.1 M pH 7.0 phosphate buffer. Two 0.6 cm filter paper disks were suspended from the stopper and 5 μL of 2.5 M KHSO4 was added to the disks to trap ammonia. Urease was then added and the flask was immediately stoppered. The flasks were incubated for 20 min at 25°C in a Dubnoff shaking incubator. Two hundred microliters of 3 N NaOH was injected through the stopper into the flask and the flasks were shaken for an additional hour and then allowed to stand for 24 h. The stoppers were then removed and placed in a desiccators containing an open container of concentrated H2SO4 and allowed to dry for an additional 24 h. When the filter papers were dry, they were placed in an opened Sn cup and the cup was folded around the filter papers in preparation for analysis. Samples were then sent to the University of California, Davis Stable Isotope Facility and placed in a PDZ Europa Anca Sample Preparation Unit to be analyzed for 15N using a PDZ Europa 20-20 Isotope Ratio Mass Spectrometer.

Blood glucose was analyzed using glucose hexokinase adapted for use on a Konelab 20XTi Analyzer (ThermoElectron Corp., Waltham, MA, USA). Plasma creatinine was determined colorimetrically using alkaline picric acid as described previously (Toro and Ackermann, 1975).

Calculations: Nitrogen turnover was calculated using 15N-glycine as a marker for protein turnover as described 143 previously (Waterlow *et al*., 1978). Briefly:

- Protein turnover (g N/d) = (rate of urea excretion, g N/d) / (fractional recovery of 15N from 15N-glycine in urinary urea)
- Protein degradation (g N/d) = Protein turnover-(absorbed N)
- Protein synthesis (g N/d) = Protein turnover-N excreted in urine

Statistics: Data from both 148 experiments were analyzed as replicated Latin Squares using the General Linear Models procedure of SAS (2003). The experimental unit was dog, the model included square, dog (square), period (age) and treatment and the error was residual error mean square. Data from one dog in Experiment 1 was omitted for the determination of protein metabolism due to outlying values.

For blood data collected on day 22 and 28, the Proc Mixed Procedure of SAS (2003) was used and day and treatment by day were included in the model. The model included the fixed effect of treatment and random effects of dog, day and period. Means were separated using polynomial contrasts for the effects of the lysine: calorie. Only five dogs were used for the blood chemistry analysis in Experiment 1 due to hemolysis contaminating the plasma samples of one dog. Differences were considered significant when $p<0.05$ with a tendency for $p<0.10$.

RESULTS

Experiment 1: Because restricted intakes were adjusted to ideal body weight, there were no differences in dry matter intake (Table 3). As expected the greatest amount of weight change occurred during the first week of restriction but there was no effect of treatment. During the second week of restriction dogs receiving the 3.0 lysine: calorie treatment lost more weight (quadratic; $p = 0.03$) and over the 2-week restriction there was a tendency (linear; $p = 0.09$) for increasing weight loss as the lysine: calorie increased.

Because ideal amino acid profiles were maintained across foods, N intake (Table 4) increased linearly with increasing lysine: calorie ($p<0.0001$); however, N excreted in the feces and urine did not differ across treatments. Apparent nitrogen absorption increased as lysine: calorie increased, with the greatest difference occurring between 2.2 and 3.0 (quadratic; $p = 0.01$). Nitrogen retention followed a similar numerical pattern, however, only a slight linear increase ($p = 0.13$) was observed. The fraction of dietary N absorbed remained the same between foods as did the fraction of N retained. Urea excretion was similar across treatments. Protein synthesis, degradation and turnover were all lowest for the 3.0 lysine: calorie treatment and highest for the 3.8 lysine: calorie (quadratic; $p<0.05$).

Plasma urea and creatinine concentrations from samples collected on days 22 and 28 exhibited no day x treatment interaction therefore treatment means are presented (Fig. 1). Plasma urea concentration was lowest for the 3.0 lysine: calorie treatment (quadratic; $p = 0.04$) and plasma creatinine concentration tended (quadratic; $p = 0.06$) to be lowest for the 3.0 lysine: calorie treatment. There was no change in plasma glucose concentrations with treatment (data not shown).

Table 3: Dry matter intake and body weight change in dogs fed varying lysine: calorie during caloric restriction in Experiment 1

	Treatment Lysine:calorie			Contrasts		
	2.2	3	3.8	SEMa	Linear	Quadratic
Intake, g dry matter/day	190.00	192.00	192.00	0.901	0.18	0.22
Weight change d15-21, kg	-0.80	-0.80	-0.95	0.215	0.32	0.55
Weight change d22-28, kg	-0.15	-0.34	-0.19	0.108	0.60	0.03
Total Change, kg	-0.95	-1.14	-1.14	0.144	0.09	0.28

[a] Standard error of mean n = 6

Table 4: Nitrogen metabolism during nutrient restriction in dogs fed varying l 334 ysine:calorie in Experiment 1

	Treatment Lysine: calorie			Contrasts		
Item, g N/day	2.2	3.0	3.8	SEMa	Linear	Quadratic
Intake	5.310	5.680	5.900	0.043	<0.0001	0.21
Fecal excretion	0.520	0.530	0.590	0.040	0.2300	0.64
Urine excretion	4.790	4.750	4.900	0.159	0.6200	0.65
Absorbed	4.790	5.140	5.300	0.023	<0.0001	0.01
Retained	0.001	0.390	0.400	0.160	0.1300	0.37
Absorbed/Intake	0.902	0.906	0.899	0.683	0.7900	0.53
Retained/Intake	-0.001	0.070	0.067	0.030	0.1600	0.35
Urea Excretion	4.490	4.670	4.760	0.260	0.5100	0.89
Protein Synthesis	12.210	11.650	14.840	0.465	0.0200	0.03
Protein Degradation	11.860	11.380	14.830	0.392	0.0060	0.02
Protein Turnover	16.140	15.630	18.780	0.400	0.0100	0.02

[a] Standard error of mean n = 5

Table 5: Dry matter intake and body weight in dogs fed varying lysine: calorie at isonitrogenous intakes in Experiment 2

	Treatment Lysine: calorie				Contrasts			
Item	2.2	3.0	3.8	4.6	SEM[a]	Linear	Quad	Cubic
Intake, g DM/day	148.00	148.00	147.00	148.00	0.800	0.317	0.684	0.720
Weight change d15-21, kg	-0.35	-0.31	-0.38	-0.27	0.111	0.739	0.719	0.571
Weight change d22-28, kg	0.13	-0.04	0.25	0.14	0.111	0.564	0.779	0.105
Total Change, kg	-0.21	-0.35	-0.14	-0.13	0.166	0.543	0.669	0.452

[a] Standard error of mean n = 8

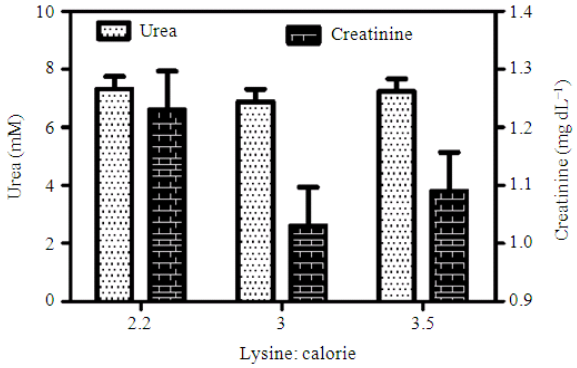

Fig. 1: Plasma urea and creatinine concentrations collected from dogs on days 22 and 28 in Experiment 1. Day x treatment (p>0.10) for urea and creatinine. Urea quadratic (p = 0.04), creatinine linear (p = 0.08), quadratic (p = 0.06)

Experiment 2: Because the foods were restricted and adjusted for the dog's ideal body weight, dry matter intake was not affected by lysine: calorie (Table 5). Dogs on each treatment lost body weight during the first week of restriction. In contrast, body weight change was negligible or was positive across treatments during the second week. Nevertheless, varying the lysine: calorie did not affect weight loss in either week; exception being a tendency for cubic response (p = 0.10) in the second week.

Because the foods were formulated to be isonitrogenous, there was no change in N intake (Table 6). Nitrogen excreted in the feces and urine were also not affected by lysine: calorie. The N absorbed and N retained, whether expressed as g/day or as a fraction of intake were not affected by lysine: calorie. The rate of urea excretion was not affected by treatment; however, protein turnover, synthesis and degradation all decreased (linear; p<0.05) as lysine: calorie increased.

For blood samples collected on the first and last day of collections (Day 22 and 28) there was no day x treatment interaction therefore treatment means are presented (Fig. 2). Blood urea nitrogen concentration increased (p<0.01) linearly with increasing lysine: calorie; however concentrations decreased at the 3.0 lysine: calorie (cubic; p<0.05).

Table 6: Nitrogen metabolism during nutrient restriction in dogs fed varying lysine: calorie at isonitrogenous intakes in Experiment 2

	Treatment Lysine: calorie				Contrasts			
Item, g N/day	2.2	3.0	3.8	4.6	SEM[a]	Linear	Quad	Cubic
Intake	4.430	4.430	4.430	4.390	0.020	0.109	0.239	0.529
Fecal excretion	0.750	0.730	0.620	0.710	0.060	0.363	0.390	0.270
Urine excretion	4.990	4.530	4.810	4.980	0.400	0.892	0.446	0.644
Absorbed	3.680	3.690	3.810	3.680	0.590	0.662	0.234	0.202
Retained	-1.310	-0.840	-1.000	-1.300	0.440	0.947	0.389	0.803
Absorbed/Intake	0.832	0.833	0.860	0.840	0.013	0.418	0.429	0.235
Retained/Intake	-0.291	-0.208	-0.224	-0.308	0.099	0.884	0.414	0.945
Urea Excretion	2.890	2.940	2.920	3.020	0.189	0.660	0.920	0.820
Protein Synthesis	20.830	19.200	14.050	13.390	2.524	0.030	0.850	0.490
Protein Degradation	22.380	19.780	14.740	14.280	2.576	0.030	0.690	0.560
Protein Turnover	26.840	24.240	19.030	18.680	2.621	0.030	0.680	0.540

[a] Standard error of mean n = 8

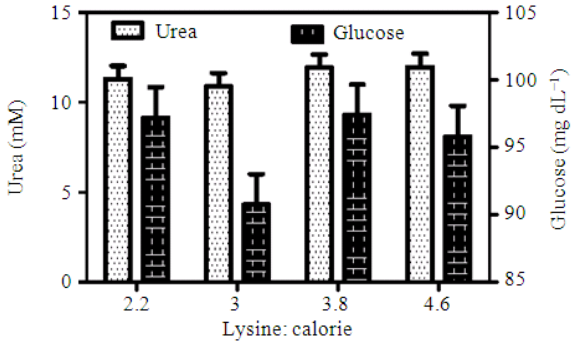

Fig. 2: Plasma urea and glucose concentrations collected from dogs on days 22 and 28. Day x treatment ($p>0.10$) for both. Urea linear ($p = 0.01$) and cubic ($p<0.05$), glucose cubic ($p<0.05$)

Plasma glucose concentrations also responded cubically ($p<0.05$) to increasing lysine: calorie as concentrations decreased at the 3.0 lysine: calorie then increased at 3.8 and decreased at 4.6. Other blood components such as creatine phosphate, total protein, triglyceride, cholesterol and albumin were unaffected by treatment (data not shown).

DISCUSSION

Experiment 1: The purpose of this study was to determine the effects of differing dietary lysine: calorie on whole body N metabolism and weight loss in mature, moderately obese dogs. Studies in rats, horses and humans have found that amino acid supplementation and shifting the percentage of calories from dietary protein can have benefits such as reduced loss or maintenance of lean muscle mass and increased fat mass loss during a weight-reduction (Das *et al*., 2004; Diez *et al*., 2002; Graham-Thiers and Kronfeld, 2005; Volpi *et al*., 1998; Wakshlag *et al*., 2003). In older canines, maintenance of muscle mass becomes increasingly important as muscle wasting becomes more prevalent with age. Kealy (1999) noted that an inadequate supply of protein increases loss of lean muscle mass. Because obesity is also common in older animals, maintaining lean muscle mass while losing fat mass becomes a primary focus. The greater weight loss during the initial 7 days of food restriction likely results from changes in gastrointestinal fill occurring with the reduced food intake (Table 3). The greater weight loss associated with the 3.0 lysine: calorie treatment is difficult to explain; however, both of the increased lysine: calorie foods tended to promote greater weight loss.

Dietary protein concentration for the 2.2 lysine: calorie food, i.e.,18%, was chosen to represent an amount that is adequate for a mature dog fed at maintenance (AAFCO, 2007). This is lower than most commercially available foods, but is two-fold higher than the minimum recommendations for maintenance (NRC, 2006).

As lysine: calorie increased in Experiment 1 total intake of N increased because the foods were formulated to maintain ideal amino acid profiles (Baker and Czarnecki, 1991) which necessitated increasing total protein as lysine increased. By maintaining similar amino acid profiles across the foods, any secondary effects or deficiencies as a result of limiting amino acids could be eliminated. However, increasing nitrogen intake with increasing lysine: calorie may have reduced the ability to observe small differences in total nitrogen metabolism. However, the additional N supplied by increasing the lysine: calorie was absorbed (Table 4) with no change in urinary or fecal excretion. These results are similar to a study by Williams *et al*. (2001) using dogs, who found that increasing protein concentrations in an almost isocaloric food linearly increased nitrogen intake and total N absorption (g/d). The net result of the greater absorption observed in

Experiment 1 and no change in Experiment 2 was a trend for increased N retention for the 3.0 and 3.8 lysine: calorie treatments. This would suggest that the greater weight loss exhibited by dogs on the 3.0 and 3.8 lysine: calorie treatments was perhaps composed primarily of body fat reserves rather than lean muscle mass.

Because most excess nitrogen is excreted in the urine as urea, the rate of urea excretion closely reflects true N metabolism and was similar across treatments. Any excess protein and amino group the body does not use for protein synthesis, or is not transaminated, or does not recycle to the gastrointestinal tract is excreted as urea. Because daily urea excretion was similar across treatments, the increased dietary protein intake was likely being utilized for protein accretion. Specifically, amino acid supplementation in foods 3.0 and 3.8 supplied amino acids that resulted in a tendency for an increase in N retention and thus the use of the amino acids for protein accretion. This is also supported by the quadratic response seen in plasma urea and creatinine concentrations (Fig. 1) as plasma urea and creatinine concentrations were lower for the 3.0 lysine: calorie treatment. Creatinine excretion is generally positively associated with muscle mass and reductions in plasma concentration may relate to a decreased turnover of muscle protein. Haverberg *et al*. (1975) showed that creatinine excretion paralleled changes in body weight in restricted rats and that excretion increased during energy and protein restriction.

The changes (linear and quadratic; $p<0.05$) in protein turnover, synthesis and degradation indicated that the 3.0 lysine: calorie treatment resulted in the lowest protein rates of turnover, synthesis and degradation. As the lysine: calorie increased from 2.2 to 3.0 protein turnover, synthesis and degradation decreased perhaps indicating a conservation of muscle mass. With the further increase to 3.8 there was an increase in protein synthesis, degradation and turnover associated with the increased protein intake above that with the basal 2.2 lysine: calorie treatment. Williams *et al*. (2001) fed three different amounts of protein using a basal, isocaloric food and the mid-ranged food (which had a lysine: calorie of about 2.8) showed the greatest decrease in protein degradation by providing an apparently ideal amount of essential amino acids. Our results suggest that the 3.0 lysine: calorie may be the optimum lysine: calorie to feed dogs with a greater propensity for muscle wasting as it may slow the degenerative process of sarcopenia by maintaining muscle mass by decreasing muscle turnover. Similar results have been found in humans when the interactions of protein intake and muscle degradation and aging were examined. Volpi *et al*. (1998) found that infusing essential amino acids in elderly individuals stimulated protein synthesis.

Urea excretion (g/d) did not change between treatments, despite the reduction in protein degradation and turnover for the 3.0 lysine: calorie treatment. This suggests that with the improvement in lysine: calorie amino acids were utilized for protein synthesis rather than deaminated for excretion and is supported by the tendency for increased N retention (linear; $p = 0.13$). Williams *et al*. (2001) determined that increasing dietary protein from 16 to 32% increased protein synthesis and degradation in dogs. They also used 15N-glycine for calculating protein metabolism, albeit with a different dosing scheme. Their foods produced lysine: calorie of 2.0, 3.0 and 2.5 for the 16, 24 and 32% crude protein foods, respectively. As lysine: calorie increased from 2.0 to 3.0 there was a sizable increase in protein synthesis and degradation in geriatric dogs but not in young adult dogs. The young adult dogs decreased protein synthesis and degradation on the 3.0 lysine: calorie treatment, as was seen in the present experiment. However, it must be remembered in the Williams *et al*. (2001) experiment dietary crude protein was also changing substantially along with lysine: calorie. The dogs in the study by Williams *et al*. (2001) were fed to meet maintenance energy requirements in comparison to the dogs in the present study which were calorically restricted. These results are in disagreement with Salter *et al*. (1990) who reported increases in protein synthesis and degradation for protein deficient pigs supplemented with essential amino acids and because protein synthesis was greater than protein degradation, a net increase in protein accretion was observed. In the current study, there were changes in protein degradation with changing proportions of dietary protein similar to those found in the experiment performed by Salter *et al*. (1990), however, protein synthesis also decreased resulting in no change in protein accretion.

Plasma urea and creatinine concentrations both changed quadratically ($P = 259$ 0.04 and $P = 0.06$, respectively) both showing a nadir at the 3.0 lysine: calorie. This would also support an improvement in N efficiency occurring at the 3.0 lysine: calorie.

Experiment 2: Experiment 1 used foods increasing in lysine: calorie balanced to maintain similar amino acid profiles. This approach resulted in increasing dietary protein concentration as lysine increased. This experiment used foods increasing in lysine: calorie, but with a constant crude protein and ME. This was accomplished by increasing only lysine.

As seen in Experiment 1 weight loss was greater during the first week of restriction. This was not unexpected because the foods were isocaloric (3986.5 ± 4.5 ME kcal/kg) and the average dry matter intake across all four foods was constant (148 ± 0.63 g/day).

Feeding an isonitrogenous food and maintaining a constant nitrogen intake (4.42 ± 0.04 g/day) resulted in no changes in N excretion, absorption or retention. Nitrogen retention was negative (-1.11 ± 0.27 g/day) across all foods indicative of the imposed nutrient restriction. It was hypothesized that the optimum lysine: calorie would give the N retention closest to zero, which occurred for the 3.0 food at -0.84 g/day. However, this was not different (quadratic $P > 0.38$) from the other treatments. This data suggests that with foods that are isonitrogenous and isocaloric, adding dietary lysine to achieve a 3.0 lysine: calorie would not improve N efficiency. This observation is further supported by the rate of urea excretion as it remained constant (0.374 ± 0.02 g N/day) across all treatments. A constant rate of urea excretion across the treatments implies that even though the lysine: calorie increased across foods, amino acid utilization was similar. Urinary urea excretion has been shown to decrease when limiting amino acids are supplied (Brown and Cline, 1974). In the present study it was hypothesized that adding additional lysine would increase use of other amino acids for nitrogen and protein metabolism, resulting in an increased efficiency of nitrogen use. The static patterns across all four foods in nitrogen metabolism and rate of urea excreted suggest that efficiency was not improved.

Increasing lysine supplementation resulted in linear ($p<0.05$) decreases in protein turnover, synthesis and degradation. Leclercq (1998) reported that supplementing lysine to deficient broilers decreased protein synthesis and degradation while increasing net protein accretion and this response differed for different muscles. The N metabolism data described above would suggest that dogs in the present experiment were not lysine deficient. Dogs in the present experiment also differ in that they were not growing, in fact, they were losing weight. This might explain why supplying lysine decreased protein turnover without apparently affecting N metabolism. Similar methodologies have been used in pigs studying lysine supplementation (Salter *et al*., 1990). These authors fed pigs a lysine deficient food and supplemented crystalline lysine to make pigs adequate or excess in dietary lysine. They found that protein synthesis and degradation increased linearly as lysine increased; however, the increase in the lysine excess pigs was comparatively small. These results may contrast with the present study but differences may be the result of a mature animal model fed at restricted intakes.

Previous studies that have used the 15N-glycine method for calculating whole-body protein turnover in dogs (Williams *et al*., 2001) and humans (Pannemans *et al*., 1995) have examined the effects of increasing dietary protein. Pannemans *et al*. (1995) reported that increasing the dietary protein intake in adult humans increased the rates of whole-body protein degradation, while Williams *et al*. (2001) reported that increasing the dietary protein levels linearly increased the protein degradation of mature dogs. In the present study increasing only lysine decreased protein turnover, synthesis and degradation.

In previous studies (Williams *et al*., 2001; Salter *et al*., 1990), increasing concentrations of supplemental amino acids have been shown to increase protein synthesis and degradation but none of these studies have evaluated these during weight loss. From these results it would appear that supplying a limiting amino acid during weight loss may reduce tissue turnover. In contrast Experiment 1 indicated that when all essential amino acids were supplied there was an optimum lysine: calorie for minimizing protein turnover. Plasma urea concentrations also suggested that the 3.0 lysine: calorie was optimal. Similarly, plasma glucose concentration was also lower for the 3.0 lysine: calorie whereas other treatments were similar. The significance of this is difficult to ascribe as all values were in the normal range. It could coincide with reduced gluconeogenesis from amino acids and lower blood urea.

CONCLUSION

The present experiments suggest benefits to optimizing the lysine: calorie in canine foods. Reductions in protein turnover during weight loss with improved lysine nutrition may reduce muscle losses without affecting weight loss. The results of this experiment imply that if all essential amino acids are in adequate supply in the food, foods with a an optimal lysine: calorie can reduce muscle degradation during periods of caloric restriction. Our optimum food for decreasing protein degradation was the food containing 3.0 lysine: calorie. The results from this experiment may improve regimes for companion animals affected by high rates of protein degradation, especially for older animals that have a propensity for muscle loss. However, the present experiments were relatively short and these results may not be sustained over longer periods of restriction.

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