Processing of Dietary Casein Decreases Bioavailability of Lysine in Growing Kittens\textsuperscript{1,2}

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EXPANDED ABSTRACT

In recent years there has been renewed interest in nutrient bioavailability, and it is an important factor to consider when estimating nutritional requirements. The bioavailability of a nutrient can be adversely affected by many factors, including interaction with other nutrients and processing or storage conditions (1,2). Lysine is an essential amino acid that can be particularly sensitive to the conditions of moist-heat processing (3). These conditions favor the formation of Maillard reaction products, and lysine is commonly involved in these reactions. The epsilon amino group of lysine reacts with the carbonyl groups of reducing sugars and forms a product complex that cannot be digested or absorbed in a form usable by the animal (4). Because of its specific susceptibility to this type of processing damage, lysine shows a decreased bioavailability relative to that of other amino acids (5).

Several methods have been developed to estimate amino acid bioavailability in various proteins (6). However, bioavailability values for many nutrients are not known, even for common foodstuffs fed to economically important food animals. Information on the bioavailability of nutrients for cats and dogs is particularly lacking. Also, the most commonly accepted in vivo methods of determining bioavailability of nutrients have not been validated for many species, including the cat (7). The objectives of this study were to examine the relationship between lysine bioavailability and growth response in kittens fed heat-damaged casein and to validate the growth assay method for quantifying amino acid bioavailability in the kitten.

MATERIALS AND METHODS

All cats were born and housed in the Nutrition and Pet Care Center, University of California, Davis, and their care was in compliance with the Guide for the Use and Care of Laboratory Animals and the Animal Welfare Act. The experimental protocol was approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis.

Sixteen male 7- to 9-wk-old specific-pathogen-free domestic short-hair kittens were used. For 2 wk before the start of the study, the kittens were weaned onto a complete purified diet. When complete acceptance of the diet was achieved, as indicated by satisfactory weight gain, the kittens were sorted by age and weight and were assigned as pairs to one of eight dietary groups in an 8 \( \times \) 8 Latin-square design. Kittens were individually housed and had free access to the experimental diets and water. Each pair of kittens rotated through each dietary treatment, one at a time, for eight 10-d periods. Throughout the 80-d study, food intake and body weight were determined daily.

Commercially available casein was mixed with 50 g/kg of dextrose and an equal weight of water and autoclaved for 2 h at 121°C to simulate severe processing damage. The resulting product was dried to constant weight in a vacuum oven and ground. Eight diets were formulated for the study. Three control diets were prepared, including a basal amino acid diet (AA Control)\textsuperscript{4}. Either untreated or heated casein was added to this complete basal diet at a level of 80 g/kg diet to create the remaining control diets (C Control and HC Control, respectively). Five experimental diets were also prepared. These included two diets with all crystalline amino acids except for lysine, one containing 80 g untreated casein/kg diet and one containing 80 g heated casein/kg diet. Each dietary treatment, one at a time, for eight 10-d periods. Throughout the 80-d study, food intake and body weight were determined daily.

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\textsuperscript{4} Abbreviations used: AA Control, basal amino acid diet; ADG, average daily gain; C Control, basal diet plus 8% casein; g, gram(s); GLM, general linear models statistical procedure; HC Control, basal diet plus 8% heated casein; HCl, hydrochloride; kg, kilogram(s); –Lys/+C, basal diet minus crystalline lysine, plus 8% casein; –Lys/+HC, basal diet minus crystalline lysine, plus 8% heated casein; NRC, National Research Council; PDf, probability of difference statistical procedure; SAS, statistical computer program system; SEM, standard error of the mean; 0.4%Lys, amino acid diet containing 0.4% lysine; 0.55%Lys, amino acid diet containing 0.55% lysine; 0.7%Lys, amino acid diet containing 0.7% lysine.


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Maximal daily gain was supported by the AA Control diet, which supplied all dietary nitrogen as crystalline amino acids. Average daily weight gain and food intake were not significantly different among the three control groups. Therefore, the addition of 80 g/kg diet of untreated or heated casein to the complete basal diet (AA Control) neither enhanced nor depressed growth response or food intake. The heated casein likely added a unique element of taste or texture not present in the amino acid and untreated casein diets, and food intake was slightly increased in the HC Control group compared to that of the C and AA Control groups, although the effect was not statistically significant (Figs. 1 and 2).

A regression analysis was performed using the average daily gain data from kittens fed the diets containing increasing increments of lysine (Diets 0.4%Lys, 0.55%Lys and 0.7%Lys). There was a linear relationship between dietary lysine level and weight gain. The equation for the line was applied to the average daily gain data from kittens fed casein as the sole source of lysine (Diets −Lys/+C and −Lys/+HC) and the amount of lysine producing the growth in these kittens was extrapolated. The ratio of the amount of lysine utilized for calculation. The other three experimental diets were crystalline amino acid diets without casein, containing increasing increments of lysine (0.4, 0.55 and 0.7% lysine).

Essential amino acids (except lysine) were provided in all diets at twice the NRC requirement. The recommended dietary level of lysine HCl is 10 g/kg diet (8). All diets contained the following essential amino acids (g/kg diet; Ajinomoto USA, Teaneck, NJ): arginine HCl, 23.4; methionine, 7.5; histidine HCl, 7; isoleucine, 10; leucine, 24; cysteine, 7.5; phenylalanine, 8; tyrosine, 9; threonine, 14; tryptophan, 3; valine, 12; and the following dispensable amino acids (g/kg diet; Ajinomoto USA): alanine, 26.1; glycine, 26.1; glutamine, 26.1; glutamate, 11.2; aspartic acid, 14.9; proline, 22.4. In addition, lysine HCl was added to appropriate diets as follows (g/kg diet; Ajinomoto USA): alanine, 26.1; glycine, 26.1; glutamine, 26.1; glutamate, 11.2; aspartic acid, 14.9; proline, 22.4. (g/kg diet; Ajinomoto USA): alanine, 26.1; glycine, 26.1; glutamine, 26.1; glutamate, 11.2; aspartic acid, 14.9; proline, 22.4.

All diets also contained chicken fat (200 g/kg diet; Foster Farms, Livingston, CA), hydrogennated beef tallow (50 g/kg diet; A.C. Humko Corp, Memphis, TN), cornstarch (100 g/kg diet, Melojeal; National Food Starch and Chemical, Bridgewater, NJ), taurine (1.5 g/kg diet; Taisho Pharmaceutical, Torrance, CA), choline chloride (3 g/kg diet; International Mineral and Chemical Corp., Terre Haute, IN), vitamin mixture [10 g/kg diet (9)] and mineral mixture [50 g/kg diet (9)]. In addition, diets contained sodium acetate (Fisher Scientific, Santa Clara, CA) at equimolar concentrations to that of the hydrochlorides (of arginine, histidine and lysine), to replace an equal weight of dextrose, and dextrose (286–295 g/kg diet; A.E. Staley, Decatur, IL).

Statistical analyses were performed using SAS for Windows, GLM procedure (Version 8, SAS Institute, Cary, NC). Weight gain and food intake were the main effects. When significance was revealed (P < 0.05), the PDIF procedure of GLM was used to determine mean differences.

RESULTS AND DISCUSSION

For all kittens, average daily gains were not statistically significantly different during periods two through six; however, gains were decreased significantly for periods one, seven and eight (P < 0.05). The decreased gain during period one may have been the result of the kittens adapting to the protocol. Although the kittens had been consuming a similar diet and had been housed individually for 2 wk before the start of the study, they may have experienced some adjustment to the experimental diets or to the husbandry schedule during period one. Daily weight gain peaked during period six, followed by a significant decrease during periods seven and eight. The decreased gain observed in the last two periods was likely a result of the period of rapid linear growth beginning to taper off. The kittens ranged in age from 18 to 20 wk and, although their growth was still linear, it seems apparent that by period seven it had slowed (Table 1).

TABLE 1
Average daily gain (ADG) for all kittens for each ten day period1

<table>
<thead>
<tr>
<th>Period</th>
<th>ADG (g)</th>
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<tbody>
<tr>
<td>1</td>
<td>13.68 &lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>19.14 &lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
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<td>3</td>
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<td>7</td>
<td>18.37 &lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>13.07 &lt;sup&gt;e&lt;/sup&gt;</td>
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</tbody>
</table>

1 Values are least-square means pooled SEM = 1.77, n = 16. Values with differing superscripts are significantly different at P ≤ 0.05.

FIGURE 1 Daily weight gain of male kittens fed three purified control diets with complete amino acids only (AA Control), with amino acids and untreated casein (C Control) and with amino acids and heated casein (HC Control), and kittens fed five purified experimental diets with untreated casein and no added lysine (−Lys/+C), with heated casein and no added lysine (−Lys/+HC), and three diets with increasing levels of added lysine and no added casein (0.4%Lys, 0.55%Lys and 0.7%Lys). Values are least-squares means ± SEM, n = 16. Values with different letters were statistically different, P ≤ 0.05.

FIGURE 2 Daily food intake of male kittens fed three purified control diets with complete amino acids only (AA Control), with amino acids and untreated casein (C Control) and with amino acids and heated casein (HC Control), and kittens fed five purified experimental diets with untreated casein and no added lysine (−Lys/+C), with heated casein and no added lysine (−Lys/+HC), and three diets with increasing levels of added lysine and no added casein (0.4%Lys, 0.55%Lys and 0.7%Lys). Values are least-squares means ± SEM, n = 16. Values with different letters were statistically different, P ≤ 0.05.
growth to the amount of total lysine in the casein (6.28 g/kg diet) is the estimated fraction of bioavailable lysine in that protein for growing kittens.

For the untreated casein (Diet $\text{Lys/+/C}$),

$$\frac{6.04 \text{ g/kg diet}}{6.28 \text{ g/kg diet}} \times 100 = 96.2\% \text{ bioavailable lysine}$$

and for heated casein (Diet $\text{Lys/+HC}$),

$$\frac{3.55 \text{ g/kg diet}}{6.28 \text{ g/kg diet}} \times 100 = 56.5\% \text{ bioavailable lysine}$$

It should be noted that when the data are plotted using total lysine intake and total gain, rather than dietary concentration and average daily gain, there is little difference between the resulting estimates of lysine bioavailability and those reported here.

The results of this study indicate that the lower growth rate of kittens fed heated casein reflects a decreased bioavailability of lysine in heated casein resulting from heat-processing damage. We conclude that the growth assay method is a satisfactory method for estimating amino acid bioavailability in the kitten. Using this method to estimate bioavailability of other amino acids may help us to better understand the effects of various processing procedures on overall nutrient bioavailability of commercial feline diets.

**LITERATURE CITED**