Dietary Effects of Magnesium on Histamine Metabolism and Urine Acidity in Domestic Felines

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ABSTRACT

Magnesium deficiency has been associated with increased histamine production in rats. Limitation of Mg with acidifying foods is common practice for management of urinary tract health in domestic cats. Nine healthy adult female shorthair cats were used in a 3 period random crossover experiment with fixed treatment sequences to test the effects of dietary Mg (0.06, 0.12 and 0.18% DM) on histamine in blood and urine. The dry-extruded test foods were fed in sufficient amounts to maintain ideal body weight and obtain a target urine pH of 6.3. Each experimental period was preceded by a 7d wash out period, in which the 0.06% Mg food was fed, followed by a 14d feeding period of the appropriate food. Two 24 h total urine collections were performed (d13: Acidified, d14: Un-acidified; immediately iced) and blood was collected on d14. Dry matter intake (p≥0.13) and BW (p≥0.13) were not affected by treatment. Plasma Mg concentration increased linearly with increasing dietary Mg (0.54, 0.56, 0.58 mM; p = 0.001). In contrast, plasma concentrations of threonine, histidine and tryptophan were lower in cats fed 0.12% Mg compare with 0.06 or 0.18% Mg (quadratic, p≤0.03). Urine output (p≥0.17), pH (p≥0.55), NH₃ (p≥0.21) and titratable acidity of urine (p≥0.14) were similar across treatments. Urinary histamine excretion responded quadratically (p = 0.02) to treatment (3483, 3369, 3986 ng/d), whereas urinary histamine: Creatinine (p≥0.43) and plasma histamine concentration (p≥0.55) were unaffected. Differences were not detected among treatments in total histamine, cellular + noncellular histamine, (p≥0.11) or antigen-induced (p≥0.21) histamine release in whole blood. These data indicate that dietary Mg concentration, from 0.06-0.18%, does not affect urinary acidity or circulating histamine concentrations, however, supplying Mg at 0.18% may increase urinary histamine excretion.

Keywords: Cat, Magnesium, Histamine, Urine

1. INTRODUCTION

Urine acidifying diet interventions are frequently used to manage and treat magnesium ammonium phosphate hexahydrate (struvite) crystal formation in domestic felines (Matsui, 2007). Super-saturation of magnesium, phosphate and ammonium ions in urine can lead to struvite formation, but urine alkalinity is a better predictor of struvite formation than dietary Mg (Bartges and Kirk, 2006). Nevertheless, it is common practice to control Mg content in feline maintenance diets to as little as 0.05 to 0.3% for label claims of “urinary tract health” (Matsui, 2007). Magnesium has been implicated in modulating cellular events involved in inflammation and limited Mg has been associated with increased histamine production in rats (Malpuech-Brugere, 2000; Mazur et al., 2007). The requirement for Mg in the adult domestic feline is 200 mg kg⁻¹ diet or 0.02% (NRC, 2003). However few data demonstrate the effects of dietary Mg on feline inflammatory status or relationship between dietary Mg and feline lower urinary tract disease incidence. If dietary Mg can affect the inflammatory status of the domestic feline, it may be important to more closely evaluate the advantages and disadvantages of limiting Mg in the diet to prevent struvite formation. This experiment tested the hypothesis that low levels of dietary Mg would cause alterations in histamine dynamics, resulting in elevated urinary histamine concentration.
2. MATERIALS AND METHODS

2.1. Animals

Nine domestic shorthaired spayed female cats (3.8±0.14 kg BW) between 3 to 4 years were utilized in this experiment. Cats were housed in individual cages at Spindletop Research Unit (University of Kentucky, Lexington, KY) and maintained at an ambient temperature of 25°C with a 16h light/8h dark cycle. Animals were confined to cages during feeding periods and at night. Enrichment and social time was provided between feeding periods. Felines had ad libitum access to water and were fed a complete and balanced adult maintenance diet to maintain ideal body weight (Hill’s Science Diet, Adult Maintenance Formula) prior to each experimental period and during inter-experimental periods. Animals were fed at 0730 and 1930. The experiment was performed as a random crossover design with fixed treatment sequences to test effects of dietary magnesium (0.06%, 0.12% and 0.18% Mg DM). Each experimental period consisted of a 9d “pre-feed” or “wash out” period, during which time the animals received adult maintenance diet, fed to maintain ideal body weight. Following each “wash-out” period, a one d dietary transition was performed in which cats were provided 50% adult maintenance and 50% test diet.

The first day after the diet transition was considered d 1 of a 14-d feeding period, during which time the cats received assigned test diet (Table 1). Refusals were collected at 0730 daily, weighed, recorded and discarded. Representative feed samples were obtained from each 9.1 kg bag opened during the experiment for dry matter analysis. After d 14 of each feeding period, animals underwent a one day diet transition back to adult maintenance diet and were maintained thusly for 7 d as a “wash out.”

2.2. Total Urine Collection

On d 12, 13 and 14, cats were confined to their individual cages for urine collection. Urine was collected via Smart Cat Box Collection System (Providence House Mfg. Inc., Seal Rock, OR). This system utilized a grated tray containing polyethylene beads (Smart Cat Box, Providence House Mfg. Inc., Seal Rock, OR) (cat litter substitute) which drained into a lower tray equipped with a funneled floor and drain. These boxes are fashioned to function with a urine reservoir; however, the needs of this collection required that the urine samples be immediately preserved over ice, post elimination. In order to achieve this, the central hole which would normally drain to a urine reservoir was permanently attached to a 33 mm laboratory funnel.

Funnel was fitted to 46 cm BARD urine collection tubing (C.R. BARD, Inc., Covington, GA). Tubing exited the cage into a Styrofoam cooler, through a hole drilled into the side, approximately 5-10 cm from the bottom of the cooler. The tubing was attached to a 265 mL BARD Urine Collection Bag (C.R. BARD, Inc., Covington, GA), inside the cooler, preserved on ice. Samples were collected such that starting on d12; cats were given 24 h to urine void. If after 24 h, no void was produced, the first urinary elimination was collected and considered to be the first 24 h urine void. After the first sample was collected, urine collection bags were acidified with 5 mL of 6N HCl and were preserved over ice. Acidified urine samples were collected in the same manner as those, not acidified, with 24 h provided and stored at -20°C until assayed.

Acidity was determined from aliquots of urine sample and titrated via the addition of 0.1M NaOH until a sustained pH of 7.4 was attained. The amount of NaOH required to titrate the sample was recorded and reported as a proportion of the aliquot size (mL NaOH/mL urine). Acidified urine samples were aliquoted into 12×75 mm polyethylene tubes or 1.5 mL micro-centrifuge tubes and stored at -20°C until assayed.

| Ingredient (%) | 0.06 | 0.12 | 0.18 |
|----------------|------|------|------|
| Brewers rice   | 47.77| 47.67| 47.57|
| Corn gluten meal| 26.10| 26.10| 26.10|
| Choice white grease | 11.00 | 11.00 | 11.00 |
| Pork by-product meal | 3.67 | 3.67 | 3.67 |
| Fish meal      | 2.30 | 2.30 | 2.30 |
| Lamb meal      | 2.24 | 2.24 | 2.24 |
| Fish oil       | 0.80 | 0.80 | 0.80 |
| Palatability enhancer | 1.50 | 1.50 | 1.50 |
| L-Threonine    | 0.40 | 0.40 | 0.40 |
| Taurine        | 0.25 | 0.25 | 0.25 |
| DL-Methionine  | 0.15 | 0.15 | 0.15 |
| L-Lysine-HCl   | 0.13 | 0.13 | 0.13 |
| L-Tryptophan   | 0.05 | 0.05 | 0.05 |
| Potassium chloride | 1.00 | 1.00 | 1.00 |
| Calcium sulfate | 0.70 | 0.70 | 0.70 |
| Choline chloride | 0.53 | 0.53 | 0.53 |
| Salt, Iodized  | 0.35 | 0.35 | 0.35 |
| Dicalcium phosphate | 0.30 | 0.30 | 0.30 |
| Potassium sulfate | 0.18 | 0.18 | 0.18 |
| Magnesium oxide | --- | 0.10 | 0.20 |
| Vitamin E      | 0.31 | 0.31 | 0.31 |
| Vitamin premix | 0.10 | 0.10 | 0.10 |
| Mineral premix | 0.10 | 0.10 | 0.10 |
| Palatability enhancer | 1.50 | 1.50 | 1.50 |
| Preservative   | 0.07 | 0.07 | 0.07 |
2.3. Blood Sampling and Handling

On d14 of experimental period, cats were sedated with Torbugesic (0.01mg kg\(^{-1}\) BW, Fort Dodge, Pfizer Animal Health, Wyeth) and Dormitor (40 µg kg\(^{-1}\) BW Pfizer Animal Health, Exton, PA). Blood samples (8 mL) were drawn from the jugular vein, placed into heparinized (23.5 IU per mL heparin sulfate) tubes, a 1 mL aliquot was placed into heparinized whole blood. Total histamine (intracellular + extracellular) was measured in heparinized whole blood and represented the total concentration (mg/dL) of histamine in a representative sample. Total histamine release was determined by incubating whole blood in releasing buffer in the presence of anti-IgE. Spontaneous histamine release was measured by incubating whole blood in releasing buffer without the addition of whole blood. Basophilic granulocytes are the primary cells in blood that contain and release histamine (Galoppin et al., 1989). Thus, in vitro total release represents the induced release of histamine from basophils and spontaneous release is the fraction that is released or leaked in the absence of antigen.

2.4. Chemical Analysis of Diets

Dietary analyses (Table 2) for moisture (930.15), ash (942.05), crude protein (954.02), and magnesium (962.09) content according to the AOAC, 2005. Crude protein content of the diets was determined using a Leco CN2000 nitrogen analyzer (Leco Corp., St. Joseph, MI).

2.5. Plasma Magnesium

One milliliter plasma samples were transported on ice and immediately analyzed (within 2-4 h) for Mg concentration with Nova 8 Electrolyte/Chemistry Analyzer (NOVA Biomedical Corp, Waltham, MA).

2.6. Histamine ELISA

Histamine ELISA kits (Ref #: BA 10-1000; Rocky Mountain Diagnostics, Colorado Springs, CO) were utilized for the determination of histamine concentration of plasma, urine and whole blood. Urine samples were diluted 1:9 with provided diluent. Plasma samples were de-proteinized with 1% Trichloacetic acid solution; 0.150 mL plasma and 0.150 mL 1% TCA combined in micro-centrifuge tube and maintained at room temperature for 1-2 h for histamine release ELISA, while remaining 7 mL were stored on ice until centrifuged at 7,000×g for 15 min and plasma harvested and stored for analysis (-20°C).

2.7. Urine Ammonia

Previously acidified urine samples were thawed and vortexed thoroughly. Samples were diluted 500:1 with distilled water in 10 mL polyethylene test tubes and vortexed for 30 sec. Ammonia concentration was determined enzymatically using a Konelab Analyzer (Thermo Electron Corp., Vantaa, Finland).

2.8. Urine Creatinine

Acidified urine samples were used in an enzymatic method to determine concentration of creatinine in which creatinine was converted to creatine under the activity of creatinase (Myers, 2006). Creatine was then acted upon by creatinase to form sarcosine and urea. Sarcosine oxidase converted sarcosine to glycine and hydrogen peroxide and the hydrogen peroxide reacted with a chromophore in the presence of peroxidase to produce a colored product that was measured at 546 nm (secondary wavelength = 700 nm; Roche/Hitachi Modular Analytical System, Roche, Basel, Switzerland).

2.9. Determination of Total Amino Acids in Plasma

Plasma amino acids were determined by isotope dilution with Gas Chromatography-Mass Spectrometry (GC-MS) as previously described (Calder et al., 1999; El-Kadi et al., 2006). To a known weight (0.5 g) of fresh plasma was added an equal known weight of a solution containing 0.2 mg hydrolyzed [U-\(^{13}\)C] algae protein powder (99 atoms %; Martek Biosciences, Waltham, MD), 100 nmol [indole-\(^{2}\)H\(_{3}\)] tryptophan, 200 nmol [\(^{15}\)N] glutamine, 25 nmol [methyl-\(^{3}\)H\(_{3}\)] methionine and 3 µmol [15N2] urea and the samples stored frozen (-80°C).

Thawed samples were de-proteinized by addition (1mL) of sulfosalicylic acid (15% w:v), the supernatant desalted by cation (AG-50, H\(^{+}\) form) exchange and amino acids and urea eluted with 2 mol/L/NH\(_{4}\)OH.
followed by water. Eluate was lyophilized to dryness and amino acids converted to their t-butyldimethylsilyl derivative. Under electron impact mode, the following ions (m/z) were monitored: Urea 231, 233; alanine 260, 263; glycine 246, 248; valine 288, 293; isoleucine 302, 308; praline 286, 291; methionine 292, 295; serine 390, 393; threonine 404, 408; phenylalanine 234, 242; aspartate 302, 304; glutamate 432, 437; lysine 300, 306; histidine 440, 446; glutamine 168, 169; tyrosine 302, 304; and tryptophan 244, 249. For leucine isotope enrichment and concentration, ions at 302, 303 ([l-13C] leucine), 305 ([2H3] leucine) and 308 ([13C6] leucine, internal standard) were monitored. Calibration curves were generated from gravimetric mixtures of labeled and unlabeled amino acid.

2.10. For Leucine
Correction was also made for spillover of [l-13C] leucine (m/z 303) into [2H3] leucine (m/z 305) and spillover of [2H3] leucine into [13C6] leucine (m/z 308). All enrichments were expressed as Atoms Percent Excess (APE) relative to background natural abundance (El-Kadi et al., 2006).

2.11. Statistical Analysis
Data were analyzed as a random crossover design with fixed treatment sequences using the MIXED procedure of SAS (version 8.0, SAS Institute). Actual magnesium concentration for each diet varied slightly from the formulation target. Thus, backward stepwise regression was performed using actual intake of magnesium, where second order model (Quadratic) was tested and if not significant, the analysis was repeated with a first order (Linear) model. All data are presented as Least Squares Means ± SEM. Effects were considered significant at p≤0.05 and a tendency for significance at p≤0.10.

3. RESULTS
Dry matter intake (p≥0.13) and BW (p≥0.16) were not affected by treatment (Table 3). Plasma Mg increased linearly with increasing dietary Mg (p = 0.001). Urine output (p≥0.17), pH (p≥0.55), NH3 (p≥0.21) and titratable acidity of urine (p≥0.14) were similar across treatments. Urinary histamine concentration was lower for the mid-level Mg diet compared with either the low-or high-level diet (Quadratic; p = 0.05). Urinary histamine excretion rate responded quadratically (p = 0.02) to increasing dietary Mg treatment with the high Mg diet having a greater excretion rate than the low and mid-level Mg diets. However, urinary histamine: Creatinine was similar across treatments (p≥0.43). Plasma histamine concentration (p≥0.55) was also unaffected by dietary Mg. Differences were not detected among treatments in total histamine, cellular + non-cellular histamine, (p≥0.11) or antigen-induced (p≥0.21) histamine release in whole blood (Table 4). Plasma lysine concentration tended to increase (linear; p = 0.09) with increasing dietary levels of Mg (Table 5). In contrast, threonine (p = 0.01), histidine (p = 0.02) and tryptophan (p = 0.03) demonstrated a quadratic effect with dietary treatment; and leucine (p = 0.09), methionine (p = 0.07), phenylalanine (p = 0.09) and aspartate (p = 0.08) exhibited quadratic trends with dietary treatment (Table 4). In the case of each amino acid, the quadratic response was attributable to lower plasma concentrations for the 0.12% Mg diet compared with either the 0.06 or 0.18% Mg diet.

| Table 3. Effects of dietary magnesium on biochemical analytes of urine and plasma |
|---------------------------------------------------------------|-------------------|-------------------|
| % Dietary Magnesium | P Value | SEM | Lin | Quad |
| 0.06 | 0.12 | 0.18 | SEM | Lin | Quad |
| Body weight (kg) | 3.85 | 3.81 | 3.79 | 0.03 | 0.160 | 0.28 |
| Dry matter intake (g/24 h) | 39.30 | 39.60 | 39.70 | 2.04 | 0.130 | 0.70 |
| Urine Output (g/24h) | 24.90 | 28.80 | 29.00 | 2.99 | 0.170 | 0.48 |
| pH | 6.15 | 6.22 | 6.17 | 0.05 | 0.550 | 0.95 |
| NH3 (mmol/L) | 354.30 | 313.70 | 333.50 | 19.32 | 0.440 | 0.21 |
| Titratable acidity a | 0.18 | 0.17 | 0.16 | 0.01 | 0.140 | 0.78 |
| Creatinine (mg/dL) | 337.10 | 282.20 | 318.00 | 22.95 | 0.740 | 0.20 |
| Histamine (ng/mL) | 140.70 | 117.60 | 138.60 | 13.86 | 0.790 | 0.05 |
| Histamine: Creatinine b | 4.34 | 4.35 | 4.38 | 0.96 | 0.720 | 0.43 |
| Histamine (ng/24 h) | 3483.00 | 3369.00 | 3986.00 | 511.45 | 0.210 | 0.02 |
| Plasma Histamine (ng/mL) | 1.30 | 1.00 | 1.10 | 0.38 | 0.550 | 0.76 |
| Magnesium(mmol/L) | 0.54 | 0.56 | 0.58 | 0.01 | 0.001 | 0.48 |

a Probability of larger F-statistic; b; n = 9; c mL 0.1 N NaOH/mL urine; d; Ratio of [analyte]/[creatinine]; e Value ×10^−9
Magnesium deficiency has been implicated in modulating cellular events involved in inflammation (Mazur et al., 2007) and limited Mg has been associated with increased histamine production in rats (Malpuech-Brugere, 2000). Magnesium deficient rats exhibited peripheral vasodilatation of the ears associated with an elevation in blood histamine levels resulting from elevated mast cell degranulation (Nishio et al., 2003). Sprague-Dawley rats fed 0.05% Mg diet increased blood histamine 4 to 5 fold (60 to 80 µg/100mL Vs ~10 µg/100 mL ) compared with controls; furthermore, mast cell number and extent of degranulation were increased with a Mg deplete diet (Kraeuter and Schwartz, 1980). Magnesium deficiency causes leukocytosis in rats with infiltration of tissues such as heart, lung, skeletal muscle, intestine and thymus, but accumulation of eosinophil occurs in certain tissues: Urinary tract and gastrointestinal tract, suggesting heightened sensitivity (Mazur et al., 2007) and these cells are not only increased in number, they are primed for action. Magnesium depleted rats demonstrate elevated levels of pro-inflammatory cytokines (e.g., IL-6 and high sensitivity C reactive protein) and demonstrated increased plasma substance P (Mazur et al., 2007; Song et al., 2007).

The mechanistic link between Mg deficiency and inflammation likely includes calcium signaling, neurotransmitter release of substance P, membrane oxidation and activation of Nuclear Factor kappa B (NFkB) (Mazur et al., 2007). In vitro work has shown that there is an increased response of Mg depleted cells to calcium signaling (Malpuech-Brugere et al., 1998), but Mg acts a natural antagonist of calcium and it is likely that decreased extracellular Mg induces an increase in intracellular calcium (Mazur et al., 2007). Furthermore, Mg deficiency alters the stress response by affecting neuromediator activity or production of acetylcholine, catecholamine and substance P, implicating the deficiency strongly in neurogenic inflammation (Mazur et al., 2007). Lastly, NFkB is an important family of transcription factors which, when activated, translocate to the nucleus and stimulate transcription of genes that control immune and inflammatory responses (Altura et al., 2003). It has been shown that low extracellular Mg induces lipid peroxidation and activation of NFkB in cultured canine cerebral vascular smooth muscles cells and supports a potential role for NFkB in Mg deficient inflammation (Altura et al., 2003).

The oxidative stress placed on the cellular environment due to inflammation is vast and affects tissue, erythrocyte and lipoprotein peroxidation, oxidative modification of proteins, antioxidant status reduction and elevated plasma Nitric Oxide (NO) (Mazur et al., 2007). An example of this inflammatory and oxidative stress is seen in the development of cardiac lesions in experimentally Mg deprived rodents subjected to ischemic-reperfusion stress (Tejero-Taldo et al., 2004). It

### Table 4. Effect of dietary magnesium on histamine release in heparinized whole blood

| % Dietary Magnesium | P Value * | SEM b | Lin | Quad |
|---------------------|----------|-------|-----|------|
| 0.06                | 10.1     | 12.7  | 14.6 | 2.84 |
| 0.12                |          |       |      | 0.14 |
| 0.18                |          |       |      | 0.86 |

* Probability of larger F-statistic; b: n = 9; ng/mL; Total Release: Histamine released in response to antigen treatment; Total: Cellular + noncellular histamine concentration

### Table 5. Plasma concentration (nmol/g) of amino acids with dietary magnesium treatment

| Amino acid | % Dietary Magnesium | P- Value * | SEM b | Lin | Quad |
|------------|---------------------|------------|-------|-----|------|
| Valine     | 98                  | 97         | 103   | 9.17| 0.28| 0.20 |
| Leucine    | 84                  | 82         | 90    | 8.94| 0.23| 0.09 |
| Isoleucine | 47                  | 46         | 48    | 3.76| 0.37| 0.23 |
| Methionine | 52                  | 47         | 54    | 4.47| 0.81| 0.07 |
| Threonine  | 88                  | 83         | 90    | 8.25| 0.37| 0.01 |
| Phenylalanine | 70          | 62         | 70    | 7.35| 0.89| 0.09 |
| Lysine     | 75                  | 77         | 83    | 7.19| 0.09| 0.33 |
| Histidine  | 91                  | 83         | 89    | 10.07| 0.92| 0.02 |
| Tryptophan | 47                  | 43         | 49    | 5.42| 0.53| 0.03 |
| Alanine    | 397                 | 360        | 391   | 51.30| 0.95| 0.35 |
| Glycine    | 308                 | 285        | 313   | 27.26| 0.35| 0.15 |
| Proline    | 135                 | 124        | 131   | 16.64| 0.79| 0.38 |
| Serine     | 151                 | 135        | 134   | 18.32| 0.44| 0.11 |
| Aspartate  | 14                  | 13         | 14    | 1.00| 0.71| 0.08 |
| Glutamate  | 38                  | 31         | 31    | 5.11| 0.30| 0.25 |
| Glutamine  | 323                 | 281        | 292   | 27.74| 0.39| 0.11 |
| Arginine   | 113                 | 116        | 130   | 32.57| 0.35| 0.53 |
| Tyrosine   | 45                  | 41         | 44    | 4.09| 0.61| 0.12 |

* Probability of larger F-statistic; n = 9, except for 0.06% Mg (n = 5) and 0.18% Mg (n = 8) diets

4. DISCUSSION

Magnesium has been implicated in modulating cellular events involved in inflammation (Mazur et al., 2007) and limited Mg has been associated with increased histamine production in rats (Malpuech-Brugere, 2000). Magnesium deficient rats exhibited peripheral vasodilatation of the ears associated with an elevation in blood histamine levels resulting from elevated mast cell degranulation (Nishio et al., 1987). Sprague-Dawley rats fed 0.05% Mg diet increased blood histamine 4 to 5 fold (60 to 80 µg/100mL Vs ~10 µg/100 mL ) compared with controls; furthermore, mast cell number and extent of degranulation were increased with a Mg deplete diet (Kraeuter and Schwartz, 1980). Magnesium deficiency causes leukocytosis in rats with infiltration of tissues such as heart, lung, skeletal muscle, intestine and thymus, but accumulation of eosinophil occurs in certain tissues: Urinary tract and gastrointestinal tract, suggesting heightened sensitivity (Mazur et al., 2007) and these cells are not only increased in number, they are primed for action. Magnesium depleted rats demonstrate elevated levels of pro-inflammatory cytokines (e.g., IL-6 and high sensitivity C reactive protein) and demonstrated increased plasma substance P (Mazur et al., 2007; Song et al., 2007).
was found that treatment with antioxidants (Vitamin E and porbucol) significantly protected against lesion development. Non-protected rat cardiac muscle generated excess NO when exposed to ischemic-reperfusion stress, which contributed to lesion formation (Tejero-Taldo et al., 2004). Substance P released from c-fibers is implicated in neurogenic inflammation and when NMDA receptors (participatory in substance P release from c fibers) were blocked, perivascular inflammatory infiltrates were reduced and cardiac lesion formation was reduced (Tejero-Taldo et al., 2004).

Urinary histamine appears to be primarily produced in the cortical glomeruli of the kidney from circulating plasma histidine via histidine decarboxylase (Abboud et al., 1982). This has been confirmed in cultured cortical glomerular cells incubated with L-Histidine and has been attributed as the major site of intra-renal histamine synthesis and accumulation (Abboud et al., 1982). Very small concentrations of circulating plasma histamine stimulate the accumulation of cAMP in the glomeruli and increase filtration rate, but much lower concentrations exist in plasma (Abboud et al., 1982). In the current experiment, a quadratic effect was seen on 24 h urinary histamine excretion demonstrating that with increased dietary Mg, domestic felines generate elevated histamine levels in urine. However, when histamine concentration was normalized to creatinine concentration no treatment differences were detected. Creatinine is a metabolite of creatine metabolism in skeletal muscle; it is freely filtered across the glomerulus and is not reabsorbed, secreted, or metabolized by any cell type in the nephron and can be used for normalization of renal filtered or excreted analytes (Koeppen and Stanton, 2008). The inconsistent results between 24 h histamine excretion rate and histamine: Creatinine in the current experiment are not obvious. Given that histamine and creatinine exhibited a similar pattern for both urinary concentration and excretion rate it may suggest that excretion rate was complicated by either incomplete urine recovery or frequency of urination. Not all cats voided during the 24 h urine collection period and thus the first subsequent voiding was used as the 24 h output. Although felines usually urinate multiple times daily, it is not uncommon for them to withhold urine for multiple days during balance studies (Burger and Smith, 1987; Matandos and Franz, 1980). Previous efforts using 14C-labelled inulin, a non-metabolized and rapidly excreted compound, indicate that multiple days of urine collection are necessary for accurate quantitative measures of urinary metabolite excretion (Hendriks et al., 1999). The observed data does not agree with the experimental hypothesis that decreased levels of dietary Mg would be associated with elevated levels of histamine in urine as would be consistent with rat models. However, in murine models, dietary treatments below Mg requirement were provided (Mazur et al., 2007; Song et al., 2007); the current study did not utilize a diet below Mg requirement. However, normal physiological plasma Mg levels are between 0.8 and 1.2 mmol L\(^{-1}\) in felines, but may reach as low as 0.4 mmol L\(^{-1}\) (Altura and Altura, 1984; Kaneko et al., 1997; Rude and Singer, 1981); our data fall below the normal plasma Mg values expected in felines, but elevations in urine histamine were still not seen. It is not surprising that histamine levels are not altered in plasma, as histamine can be rapidly catabolized and cleared from plasma (Schayer et al., 1954). Magnesium exists within all intracellular compartments and is primarily (80-90%) bound to ATP (Rude, 1998). The principle role of Mg is related to enzyme activity; there are over 300 enzyme systems, which are dependent on association with Mg and of great interest, all enzymes requiring ATP, require Mg for substrate formation (Rude, 1998). Furthermore, intracellular free Mg acts as an allosteric activator of enzyme action (e.g., adenylate cyclase, phospholipase C and Na/K-ATPase) (Connolly et al., 1985; Dorup and Clausen, 1993; Garfinkel and Garfinkel, 1985; Maguire, 1984).

Our diets were formulated well above minimum amino acid requirements and our plasma amino acid concentrations were comparable to those found in felines fed to 100% minimum amino acid requirement (Bioruge et al., 1994). Plasma lysine concentrated tended to increase linearly with dietary Mg. However, plasma concentrations of threonine, histidine and tryptophan demonstrated a quadratic effect with dietary treatment and leucine, methionine, phenylalanine and aspartate exhibited quadratic trends with dietary treatment. The quadratic responses of these amino acids to dietary treatment can be characterized as a decrease plasma concentration for the intermediate (0.12%) dietary Mg treatment. The dietary composition of the intermediate treatment does not reflect any depression in crude protein or amino acid content compared to the other treatments. Individual observations do not indicate any outlying cats in the dietary treatment means, which might skew the data. It is unclear based on the data collected in this experiment what underlying mechanism may be contributing to these effects.

5. CONCLUSION

In adult spayed female domestic cats, 0.06, 0.12, 0.18% dietary Mg did not affect urine output or acidity. Urinary histamine excretion rate was elevated in cats fed
0.18% Mg, however, when urinary histamine concentration was normalized to creatinine, no differences were observed between dietary treatments. In contrast to previous observations in rodents fed Mg deficient diets, dietary Mg supplied at or above requirement did not alter circulating concentrations of histamine. Moreover, dietary Mg did not influence total or antigen-induced histamine release in whole blood. Based on the above findings, our hypothesis that low levels of dietary Mg would elevate plasma and urine histamine concentration in felines was rejected. Accordingly, it seems unlikely that alterations of dietary Mg, within the aforementioned ranges, would alter the histamine inflammatory status of the domestic feline significantly contributing to idiopathic cystitis.

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