Protein Intake during Weight Loss Influences the Energy Required for Weight Loss and Maintenance in Cats\textsuperscript{1–3}

Ricardo S. Vasconcellos,\textsuperscript{1} Naida C. Borges,\textsuperscript{2} Karina N. V. Gonçalves,\textsuperscript{4} Júlio C. Canola,\textsuperscript{4} Francisco J. A. de Paula,\textsuperscript{4} Euclides B. Malheiros,\textsuperscript{4} Marcio A. Brunetto,\textsuperscript{4} and Aulus C. Carciofi\textsuperscript{4*}

\textsuperscript{1}Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Departamento de Clínica e Cirurgia veterinária, 14884-900 Jaboticabal-São Paulo, Brazil; \textsuperscript{2}Universidade Federal de Goiás, Departamento de Medicina Veterinária, 74030-970 Goiânia-Goiás, Brazil; and \textsuperscript{3}Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, 14001-970 Ribeirão Preto-São Paulo, Brazil.

Abstract

The effects of 2 diets with different protein contents on weight loss and subsequent weight maintenance was assessed in obese cats. The control group (Co; \(n = 8\); body condition score (BCS) = 8.6 ± 0.2) received a diet containing 21.4 g crude protein (CP)/MJ of metabolizable energy and the high-protein group (HP; \(n = 7\); BCS = 8.6 ± 0.2) received a diet containing 28.4 g CP/MJ until the cats achieved a 20% controlled weight loss (0.92 ± 0.2%/wk). After the weight loss, the cats were all fed a diet containing 28.0 g CP/MJ at an amount sufficient to maintain a constant body weight (MAIN) for 120 d. During weight loss, there was a reduction of lean mass in Co (\(P < 0.01\)) but not in HP cats and a reduction in leptinemia in both groups (\(P < 0.01\)). Energy intake per kilogram of metabolic weight (kg\(^{-0.40}\)) to maintain the same rate of weight loss was lower (\(P < 0.04\)) in the Co (344 ± 15.9 kJ·kg\(^{-0.40}\)·d\(^{-1}\)) than in the HP group (377 ± 12.4 kJ·kg\(^{-0.40}\)·d\(^{-1}\)). During the first 40 d of MAIN, the energy requirement for weight maintenance was 398.7 ± 9.7 kJ·kg\(^{-0.40}\)·d\(^{-1}\) for both groups, corresponding to 73% of the NRC recommendation. The required energy gradually increased in both groups (\(P < 0.05\)) but at a faster rate in HP; therefore, the energy consumption during the last 40 d of the MAIN was higher (\(P < 0.001\)) for the HP cats (533.8 ± 7.4 kJ·kg\(^{-0.40}\)·d\(^{-1}\)) than for the control cats (462.3 ± 9.6 kJ·kg\(^{-0.40}\)·d\(^{-1}\)). These findings suggest that HP diets allow a higher energy intake to weight loss in cats, reducing the intensity of energy restriction. Protein intake also seemed to have long-term effects so that weight maintenance required more energy after weight loss. J. Nutr. 139: 855–860, 2009.

Introduction

Reducing the energy density of food by decreasing fat content and increasing fiber concentration is a strategy widely used for the management of obesity in pet animals. The main rationale is that these diets provide a greater satiety stimulus, because a larger volume of food is ingested (1). In addition, diets with a greater protein content have been used because they minimize the loss of lean body mass (LM)\textsuperscript{2} during the treatment of obesity (2–4). In a study on felines, Laflamme and Hannah (3) demonstrated conservation of LM during weight loss in cats fed a diet containing 45% metabolizable energy (ME) from protein. Protein-rich diets may also have satiety-stimulating or thermogenic effects (5), although this effect has been studied very little in cats (6) or dogs (7).

Increased protein content in diets for weight loss may be especially important for felines, because these animals show limited adaptation of the gluconeogenic and ureagenic enzymes involved in hepatic protein catabolism to a low amino acid intake (6). In this species, protein catabolism continues to be accelerated even in situations of low protein supply (8), which may occur in weight loss regimens as a consequence of food restriction (9), as demonstrated in rats.

Food management and diet used during weight loss may also influence energy requirements for weight management after the regimen. Dogs submitted to severe energy restriction during a weight-loss regimen became more predisposed to gaining weight after weight loss (10). The main factors implicated in this predisposition to new fat mass accumulation are reduced basal metabolism and LM as a consequence of a nutritional deficit (3,11).

Considering that protein intake affects LM loss during a weight-loss regimen, and an animal’s energy requirements are...
related to its LM content (1), it is possible that protein intake during weight loss may affect energy consumption both during weight loss and weight maintenance in cats. Thus, in the present study, we compared the effect of 2 diets containing different amounts of protein on body composition, energy intake, and serum leptin, insulin, and thyroid hormone concentrations during weight loss and the subsequent weight maintenance period in neutered obese cats.

Materials and Methods

This experiment was divided into 2 stages. First, obese cats were divided into 2 groups and submitted to a controlled 20% loss of body weight. Cats were considered obese when they had a body condition score (BCS) of at least 8 in a 9-point body condition system (12). The initial BCS (mean ± SEM) of the cats studied was 8.6 ± 0.20 and body weight was 5.04 ± 0.38 kg. During this phase, each group received 1 of 2 diets that differed in protein content [21.4 vs. 28.4 g crude protein (CP)/MJ ME]. After the weight loss period, all cats, regardless of the diet group, received the same diet (28 g CP/MJ ME) at amounts sufficient to maintain a constant body weight for 120 d. The Ethics Committee for Animal Well-Being of the College of Agrarian and Veterinary Sciences, Sao Paulo State University approved all experimental procedures.

Cats

This study used 8 male and 8 female adult cats, 4–8 y old, and neutered at least 24 mo before the experiment. All cats belonged to the cat colony of the Laboratory of Research in Nutrition and Nutritional Disease of Dogs and Cats, College of Agrarian and Veterinary Science, Jaboticabal campus of UNESP, Brazil. Based on their weight history and BCS, all cats had been obese for at least 12 mo prior to the study. Obesity was not induced but occurred naturally, because the cats were kept in colonies with free access to food and water and routinely participated in food palatability and digestibility assays. At the beginning of the experiment, cats had a BCS between 8 and 9 (12) and a mean body weight of 5.05 ± 0.04 kg. All BCS determinations were performed by the same investigator (R.S. Vasconcellos) throughout the study.

The health of the cats was assessed prior to the start of the study and at 4 additional times throughout the assay. The cats were submitted to clinical and hematologic examination, urinalysis, and determination of biochemical serum profiles, including glucose using the GOD-Trinder method (Glicose PAP liquiform, catalog no. 84), urea using the urease-G LDH method (Ureia UV liquiform, catalog no. 104), creatine using the Jaffé modified method (Creatinina K, catalog no. 96), alkaline phosphatase using the Bowens and McComb modified method (Fosfatasa alcalina liquiform, catalog no. 79), alanine aminotransferase using the kinetic UV-IFCC method (ALT/GPT liquiform, catalog no. 108), γ-glutamyl transferase using the Szasz modified method (Gama GT liquiform, catalog no. 108), total plasma protein using the biuret method (Proteínas totais, catalog no. 99), and albumin using the bromocresol green method (Albumina, catalog no. 19). Specific commercial kits were used for these measurements (Làbtest Diagnstica). Serum aliquots were also stored at −70°C and analyzed for insulin, leptin, total triiodothyronine (T3), total thyroxine (T4), and thyroid stimulating hormone (TSH) concentration.

Diets

Diet for weight loss. Two dry extruded diets were used for weight loss: a control (Co) and a high-protein (HP) diet (Supplemental Table 1). The diets were formulated according to the nutritional recommendations for cats from the Association of American Feed Control Officials (13) and balanced to meet maintenance requirements before being extruded and knobbled under identical processing conditions. Both formulations used the same ingredients and varied only in the inclusion rates of protein and starch sources.

Diet for weight maintenance. Once each cat lost 20% of its initial body weight, it was fed a low-energy commercial dry food for adult cats (Supplemental Table 1). All cats from both experiment groups were fed the same food during the maintenance experimental phase (MAIN).

Before being fed to the cats, all diets were subjected to digestibility trials according to Association of American Feed Control Officials (13) guidelines. For the Co and HP diets used for weight loss, this procedure was repeated after the cats had lost 20% body weight. A 5-d test diet adaptation phase preceded 7 d of total feces and urine collection. Feces were collected twice daily, weighed, and frozen at −15°C until analysis. Urine samples were collected in plastic bottles containing 1 mL sulfuric acid solution (1 mol/L), weighed, and frozen at −15°C until analysis. Moisture, CP, acid-hydrolyzed fat, total ash, calcium, and phosphorus in food were analyzed following standard AOAC procedures (14). CP was also analyzed in feces and urine. Total dietary fiber of diets was measured according to Prosky et al. (15) and the total amount of starch of diets was measured according to the guidelines of Miller (16) and Hendrix (17). Gross energy content of diet, feces, and urine was determined with a bomb calorimeter (model 1261, Parr Instrument). All analyses were carried out in duplicate with a CV of <5%.

Experimental groups and feed management

The cats were divided into 2 groups each containing 8 cats (4 males and 4 females) matched for sex and body composition, as determined by dual-energy X-ray absorptiometry (DEXA; Table 1). The Co group received the Co diet for weight loss, whereas the HP group received the HP diet for weight loss. During the experiment, the cats were kept in individual cages (0.8 × 0.8 × 0.8 m) for 14 h (1800–0800) and then released into a collective area for exercise and socialization. To control food intake, food was offered only when the cats were inside their individual cages. Food was offered at 1800 and any remaining food was removed at 0800. Throughout the study, mean ambient temperature was 21.75 ± 0.8°C and a 12-h-dark:12-h-light cycle was provided.

Weight loss. To achieve weight loss, cats were fed 60% of their estimated maintenance energy requirements, calculated according to the NRC guidelines.

### Table 1. Body weight, BCS, body composition, and weekly weight loss rate of Co and HP cats during the weight loss and MAIN phases

<table>
<thead>
<tr>
<th></th>
<th>Obese state</th>
<th>10% weight loss</th>
<th>20% weight loss</th>
<th>End of MAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>4.91 ± 0.46a</td>
<td>4.30 ± 0.37b</td>
<td>3.86 ± 0.41c</td>
<td>3.80 ± 0.41c</td>
</tr>
<tr>
<td>HP</td>
<td>5.17 ± 0.32**</td>
<td>4.56 ± 0.32**</td>
<td>4.05 ± 0.31**</td>
<td>3.99 ± 0.27**</td>
</tr>
<tr>
<td>BCS (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.04 ± 0.01a</td>
<td>0.133 ± 0.01c</td>
<td>0.41 ± 0.1b</td>
<td>0.41 ± 0.1b</td>
</tr>
<tr>
<td>HP</td>
<td>—</td>
<td>0.129 ± 0.01c</td>
<td>0.122 ± 0.01c</td>
<td>0.122 ± 0.01c</td>
</tr>
<tr>
<td>Weekly weight loss, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.93 ± 0.21</td>
<td>0.87 ± 0.12</td>
<td>0.09 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>HP</td>
<td>0.85 ± 0.19</td>
<td>1.02 ± 0.22</td>
<td>0.10 ± 0.22</td>
<td>0.10 ± 0.22</td>
</tr>
<tr>
<td>BMC, g/cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.122 ± 0.01</td>
<td>0.109 ± 0.01b</td>
<td>0.108 ± 0.01b</td>
<td>0.109 ± 0.01b</td>
</tr>
<tr>
<td>HP</td>
<td>0.133 ± 0.01**</td>
<td>0.129 ± 0.01**</td>
<td>0.122 ± 0.01**</td>
<td>0.125 ± 0.01**</td>
</tr>
<tr>
<td>BMD, g/cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.45 ± 0.01</td>
<td>0.44 ± 0.01</td>
<td>0.44 ± 0.01</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>HP</td>
<td>0.46 ± 0.01a</td>
<td>0.46 ± 0.01**</td>
<td>0.46 ± 0.01**</td>
<td>0.46 ± 0.01**</td>
</tr>
<tr>
<td>LM, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>58.5 ± 1.6</td>
<td>65.6 ± 2.7</td>
<td>68.4 ± 1.6</td>
<td>72.2 ± 1.1</td>
</tr>
<tr>
<td>HP</td>
<td>59.3 ± 2.2</td>
<td>65.9 ± 3.2</td>
<td>73.5 ± 3.1</td>
<td>72.8 ± 2.8</td>
</tr>
<tr>
<td>FM, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>38.9 ± 1.6</td>
<td>31.8 ± 2.8</td>
<td>28.8 ± 1.6</td>
<td>24.9 ± 2.2</td>
</tr>
<tr>
<td>HP</td>
<td>38.1 ± 2.3</td>
<td>31.2 ± 3.3</td>
<td>23.4 ± 3.2</td>
<td>24.1 ± 1.9</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. n = 8 (Co) or 7 (HP). Means in a row with superscripts without a common letter differ, P < 0.05. *Different from the corresponding Co, P < 0.05.

2 According to Laflamme (12).

3 10% weight loss: from obese state to 10% weight loss; 20% weight loss: from 10 to 20% weight loss; end of MAIN: from the start to the end of the weight maintenance phase.
standard for obese cats (18), which equaled 326 MJ (kg body weight)\(^{-0.4}\). The amount of food supplied was determined by calculating the energy required for weight loss for each feline and the ME of the food determined in vivo. A body weight loss rate of 1%/wk was established for both groups; this rate was maintained by weighing the cats once per week on the same scale (Marte MB50) in the morning before the first meal. If body weight variation was >1.5% or <-0.5% relative to the previous week, the amount of food was adjusted as necessary. To determine energy consumption, the amount of supplied and refused food was recorded each day and the amount consumed was multiplied by the ME of the diet.

**MAIN.** During this stage, all cats were fed the same commercial cat food at an amount sufficient to maintain a constant body weight for 120 d. Cats were weighed once per week on the same scale in the morning before the first meal and the amount of food was adjusted if there was a body weight variation >0.2% relative to the weight at the beginning of this phase. To determine energy consumption, the amount of supplied and refused food was recorded each day and the amount consumed multiplied by the ME of the diet. Data obtained during the MAIN were divided into 3 periods: phase 1 (0–40 d), phase 2 (41–80 d), and phase 3 (81–120 d).

**Body composition measurement**

Body composition was measured at 4 times: before the beginning of the experiment (obese state), after a 10% weight loss, after a 20% weight loss, and at the end of the MAIN. Cats were deprived of food for 12 h before the examinations and then anesthetized with a combination of levimpro-mazine (Neozine 5 g/L, Aventis Pharma), tiletamine and zolazepam hydrochlorides (Zoletil 50 g/L, Virbac do Brasil Indução e Comércio) administered i.m. at respective doses of 0.5, 2.5, and 2.5 mg/kg body weight. After loss of postural reflex, cats were positioned for the exam according to the methodology described by Lauten et al. (19). Body composition was determined by DEXA (QDR 4500 Elite Windows, Student’s Guide Hologic) with 3 consecutive scans without repositioning the cats between scans. Whole-body images were analyzed using pediatric software (Pediatric whole body QDR Series uses QDR for Windows) and LM, fat body mass (FM), bone mineral content (BMC), and bone mineral density (BMD) were calculated.

**Hormone measurements**

Hormone measurements were performed at the start of the experiment, after a 20% weight loss, and at the end of the MAIN. Serum leptin concentrations were determined by RIA (Multi-species radioimmunoassay kit, Linco Research). This kit was developed to measure leptin in the plasma or serum of several species and has been validated for use in cats (20). The intra-assay CV for leptin was 5.8% and the SEM was 0.4 μg/L. Serum insulin was measured by RIA using a commercially available kit (human insulin as a standard; Diagnostic Products; P\(^{2-3}\) as tracer) that was validated for cats (21). The intra-assay CV for insulin was 6.7% and the SEM was 2.52 pmol/L. T3 was measured by RIA using a commercial kit (Coat a Count, Diagnostic Products). The intra-assay CV for T3 was 4.8% and the SEM was 10.8 ng/L. These analyses were conducted in an automatic analyzer (Diagnostic Products). T4 was measured by a solid-phase chemiluminescent competitive immunoassay (Immulite 1000 Canine total T4, Diagnostic Products) (22). TSH was measured by a solid-phase chemiluminescent competitive immunoassay (Immulite 1000 Canine TSH, Diagnostic Products) using specific automatic analyzer (IMMULITE 1000, Diagnostic Products).

**Nitrogen balance**

Nitrogen balance (NB) was determined at the beginning (after a 7-d diet adaptation period) and at the end of the weight loss program. The cats were kept for 120 h in stainless steel metabolic cages (0.8 × 0.8 × 0.8 m) equipped with an apparatus for the separate collection of urine and feces. The amount of food supplied corresponded to the requirement for body weight loss. Food consumption and total fecal and urine excretion were recorded. Feces were collected twice daily, weighed, and frozen (−15°C) until analysis. Urine samples were collected in plastic bottles containing 1 mL of sulfuric acid solution (1 mol/L), weighed, and frozen (−15°C) until analysis. Nitrogen was measured in food, feces, and urine by the Kjeldhal method according to AOAC (14) procedures. The NB was calculated as the difference between ingested nitrogen (N\(_{\text{intra}}\)) and nitrogen excreted into the feces (N\(_{\text{fc}}\)) and urine (N\(_{\text{ur}}\)) according to the following formula:

\[
\text{NB (mg · kg body weight}^{-1} \cdot \text{d}^{-1}) = N_{\text{intra}} - (N_{\text{fc}} + N_{\text{ur}}),
\]

**Statistical analysis**

The study followed a randomized block design (body composition and sex) in a split-plot treatment arrangement with the 2 diets (Co and HP) as the plots and the periods (obese state, 10 and 20% weight loss, and maintenance) as the subplots. Each cat represented an experimental unit. Data were statistically analyzed using the general linear model function of the SAS software (23). The effects and interactions between cats, diets, and periods were determined and when differences were detected with an ANOVA F-test (P < 0.05), multiple comparisons of means were made using Tukey’s test (P < 0.05). All variables were first tested for normality by the Shapiro-Wilk method and were found to have normal distribution. Pearson correlations were also performed to evaluate the relationship between body composition, nutrient intake, and the results of hormonal measurements (P < 0.05). Data are reported as mean ± SEM.

**Results**

The diets were well consumed by the cats. One male cat had to be removed from the HP group due to the development of urinary obstruction during the first week of the experiment. All other cats maintained a good health condition with no changes in clinical examination, blood count, or biochemical analyses (data not shown). The calculated nutrient intake for the cats of both groups met the minimum recommendation of the NRC (18), except for ME during the weight loss phase. For both the Co and HP diets, cats in the obese state and after a 20% weight loss had similar results for food digestibility and ME, with no effect of weight reduction on diet digestibility. Due to this, data presented (Supplemental Table 1) refer to the mean ME results of both trials for Co and HP diets.

**Body weight and composition**

**Weight loss.** Weekly weight loss was similar for both experimental groups leading to an overall reduction of BCS, with no difference between groups (Table 1). The LM of Co cats was reduced by 232 ± 50.2 g during weight loss, which was a loss relative to the beginning of the study (P < 0.01; data not shown). Although cats that received the HP diet had a reduction in LM of 97 ± 83.9 g, there was no change in LM from to the beginning of the study (data not shown). BMC was reduced in both groups (P < 0.05), but BMD was not. FM was also reduced in both groups (P < 0.01) but at a greater magnitude in the HP group, resulting in differences in body composition between Co and HP cats at the end of the weight loss phase (P = 0.006), with a higher LM (%) for the HP group (Table 1).

**MAIN.** The HP and Co groups did not change in body composition, weight, or BCS during the MAIN. However, LM tended to increase (P = 0.14) and FM tended to decrease (P = 0.14) in the Co group so that by the end of the MAIN, there was no longer any difference in body composition between the groups (Table 1).

**Nutrient intake and NB**

**Weight loss phase.** To obtain a 0.9–1%/wk body weight loss, Co cats received 343.6 ± 11.3 kJ ME·kg\(^{-0.4}\) d\(^{-1}\) and HP cats received 377 ± 10.1 kJ ME·kg\(^{-0.4}\) d\(^{-1}\) (P < 0.04; data not shown). This difference was even more prominent at the beginning...
of the weight loss phase when the Co group required a greater food restriction (P < 0.0001) (Table 2).

Protein intake varied between groups (P < 0.0001) but not over time during the weight loss phase. At the beginning of the study, NB differed between the groups (P < 0.03) and was negative for the Co group (−13.6 ± 3.91 mg kg body weight−1 d−1) and positive for the HP group (71.4 ± 6.15 mg kg body weight−1 d−1). When the cats reached a 20% weight loss, the NB was positive in Co (33.6 ± 3.53 mg kg body weight−1 d−1) and HP group (70.9 ± 7.55 mg kg body weight−1 d−1), with no difference between the 2 groups.

**MAIN.** The energy intake required to maintain a constant body weight increased during the MAIN period in both groups (P < 0.05). In the first phase of MAIN (0–40 d), the Co and HP groups had the same ME intake, but in the second (41–80 d) and third (81–120 d) phases, the HP group required more food to maintain a constant body weight (P < 0.0001). The supply of ME increased by ∼31.4 ± 1.6% relative to the beginning of the MAIN phase in the HP group and by 18.2 ± 1.4% in the Co group (P < 0.01; data not shown). Even considering ME consumption per kg LM, the Co (256 ± 8.4 kJ ME/kg LM) and HP (286 ± 10.4 kJ ME/kg LM) groups differed (P < 0.0001) at the end of the MAIN phase (data not shown).

**Hormone concentrations**
The leptin concentration decreased during the weight loss phase and at the end of the MAIN phase in both groups (P < 0.05) regardless of protein consumption (Table 3). There was a positive correlation between FM (%) and serum leptin concentration (r = 0.666; P < 0.01). The insulin concentration did not change during the weight loss phase but increased in both groups at the end of the MAIN phase (P < 0.05), with no effect of protein intake. T3, T4, and TSH concentrations did not change during the study or differ between treatments, remaining in the normal range for felines (data not shown).

**Discussion**
In the present study, a diet with 28.4 g CP/MJ permitted maintenance of LM during weight loss in cats compared with a diet containing 21.4 g CP/MJ. Laflamme and Hannah (3) demonstrated a similar effect with a diet containing ∼31.8 g CP/MJ. The replacement of carbohydrates with protein in foods designed for weight loss has proven to be beneficial by facilitating the maintenance of LM in obese humans (4,5,24) and dogs (25). In addition, a higher protein concentration seems to increase satiety, body thermogenesis, loss of body fat (9), and insulin sensitivity in obese cats (26).

Beyond the protein content of the diet, feed management and the rate of weight loss are also important factors influencing alterations to body composition that occur during weight loss (25). In a previous study, when a weight loss rate of 1.6% body weight/wk was achieved (60% higher than that in the present study), cats fed a HP diet (30.4 g CP/MJ) had a high proportion of LM loss (24% of the lost body weight; −430 g per cat) (6).

The greatest LM loss occurred in the Co group during the final phase of weight loss (10–20% weight loss). In this phase, cats already had less body fat. During this period, the Co group lost 1.65 g fat/g LM lost, as opposed to 19.4 g fat/g LM lost in the HP group (data calculated from DEXA results in grams; data not shown). This change in metabolic preference for energy substrates has been previously described in rats by Dunn et al. (27), who detected increased protein oxidation with decreased body fat. The greater availability of dietary amino acids in the HP group probably ameliorated this trend, resulting in a smaller loss of lean mass in the cats receiving this diet (8,28,29).

The consumption of 4.2 g digestible protein/kg0.67 (699 mg N/kg−0.67) was not enough to maintain a positive NB in the Co group at the beginning of the study even though this value was higher than that recommended for cat maintenance by the NRC (18) and reported by Green et al. (28) for neutral NB in cats. The difference between the results of these investigators and the present study is probably due to the concomitant restriction of protein and energy supply. At the end of the weight loss period, Co cats had a positive NB with the same protein intake. It is possible that adaptive responses occurred in the cats due to the reduction of LM, leading to a new equilibrium between nitrogen intake and excretion. However, more studies are needed to confirm and explain these findings.

The energy intake required for weight loss in the present study, regardless of the experimental group, corresponded to 67% of NRC (18) recommendations for the maintenance of obese adult cats (considering the current body weight of the cats). These values were close to those reported by Laflamme and Hanna (3) (~71% of NRC) and by Villaverde et al. (30) (~69% of NRC) for the same rate of weight loss. Our result of the effect of protein intake on the energy required for weight loss has not been related in felines by other authors (3,6), although this effect was demonstrated in humans (31,32).

A systematic review of 5 studies on humans indicated an increased satiety stimulus and rate of weight loss with HP diets, with this effect being stronger on a short-term basis (5). The short-term effect of protein on weight loss was also demonstrated in the present study, because Co cats required greater energy restriction at the beginning of weight loss (0–10% weight loss).
loss) to maintain the same weight loss rate as the HP cats. The difference in energy restriction decreased during the second phase of weight loss (10-20% weight loss), when the energy supply became similar for both groups (Table 2). Although the exact mechanism by which protein enables weight loss has not been fully elucidated (5), the postprandial thermogenic effect of protein and its effect on protein turnover are thought to be involved in the process (33).

At the start of the MAIN period, mean energy intake for weight stabilization was ~25% lower than NRC (18) recommendations for obese cats, suggesting that using the NRC (18) recommendations is not appropriate for cats after weight loss. A reduction in the energy requirement after weight loss has been observed in humans (34) and in cats (30). Factors that may induce a reduction of the energy requirement after weight loss include: changes in basal metabolism and body composition, as evidenced in humans (34) and rats (35); alterations in the feedback regulation of fat stores (36); or a reduction in cellular energy consumption (37).

There is no consensus, however, about whether or not these changes in energy requirement are irreversible, because some studies have reported the persistence of reduced energy requirements in rats (35,38) and cats (30), whereas others have reported that this effect was reversible in rats (39,40). In the present study, a gradual increase in caloric intake to stabilize body weight occurred during the MAIN period in both groups, suggesting that at least some increase in energy requirement exists in cats after weight loss. It is not possible, however, to determine whether the energy consumption of the cats stabilized during the 120-d MAIN phase or if a longer period of time may be necessary for the cats to reach their maintenance energy intake.

At end of the MAIN period, the HP group reached the maintenance energy requirement recommended by the NRC (18) for obese cats, although this requirement remained 18% lower in the Co group. These results suggest that the energy requirements for weight maintenance after energy restriction depend on protein intake during weight loss. This could be an important observation, because it is possible that greater energy intake would facilitate long-term maintenance of body weight in cats; this hypothesis should be evaluated further. This assumption is reinforced by a study in humans that demonstrated a lower incidence of weight gain after weight loss in participants who ate more protein during weight loss (4).

Hoenig et al. (26) reported that nutrient oxidation increases in diets with a HP content for lean cats. Thus, it is possible that the consumption of 6.2–8.3 g of digestible protein/kg$^{\cdot0.67}$ during the MAIN phase, when the cats had 28 to 24% FM, favored the elevation of nutrient oxidation and energy consumption. To confirm this hypothesis, however, it would be necessary to compare cats in the MAIN phase receiving diets with different protein:energy ratios and this was beyond the scope of the current experiment.

The absence of a detectable interaction between diet and hormone concentrations suggests that changes in leptin and insulin were simply due to variation in body composition and energy consumption. In contrast to the data obtained in this study on felines, Agus et al. (32) observed in humans that consumption of diets with a higher protein concentration during energy restriction promoted a greater reduction of plasma leptin concentration that was attributed to the concomitant reduced secretion of insulin, one of the main secretagogues of leptin.

Leptin concentration is directly proportional to percent of body fat in cats (20), changing as a function of animal adiposity (41). The elevation of the plasma leptin concentration in obese cats has been associated with the development of insulin resistance and of type II diabetes mellitus (26), indicating the importance of weight loss programs for felines.

Insulin concentrations were within reference values for the species in all cases (42). Elevation of insulin at the end of the MAIN phase seemed to reflect the increased food intake and substrate oxidation during this phase. Although this study did not detect any change in the concentration of thyroid hormones, other investigators have detected changes in thyroid hormone concentrations during weight loss in cats (43). In dogs, reduced T3 and T4 concentrations seemed to be related to the extent of energy restriction (44). Thus, in the present study, it is possible that the energy restriction was insufficient to produce major changes in these hormones. In view of the connection between thyroid hormones and metabolic rate (34), the study of free rather than total T3 and T4 may perhaps result in a better relationship between these metabolites and energy intake and weight loss in cats.

The present study confirmed that increased protein intake favors the maintenance of body lean mass during weight loss in obese cats. The results also suggest that protein may reduce the energy restriction needed for weight loss. Protein intake also seems to act on a long-term basis, resulting in greater energy requirements during the subsequent phase of weight maintenance. These aspects are important for successful weight loss and maintenance in cats and deserve further study.

Acknowledgment
We thank Rodrigo Sousa Bazolli from Mogiana Alimentos S.A. for the technical support for the experiment.

Literature Cited


