UNIVERSIDADE ESTADUAL PAULISTA - UNESP CÂMPUS DE JABOTICABAL

RELAÇÃO AMIDO:PROTÉINA E UMIDADE DO ALIMENTO, BALANÇO HÍDRICO E SUPERSSATURAÇÃO URINÁRIA PARA OXALATO DE CÁLCIO E ESTRUVITA EM GATOS

Maria Eduarda Gonçalves Tozato

Médica Veterinária

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Orientador: Prof. Dr. Aulus Cavalieri Carciofi

Dissertação de mestrado apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Medicina Veterinária (Clínica Médica Veterinária)

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: RELAÇÃO AMIDO:PROTEÍNA E UMIDADE DO ALIMENTO, BALANÇO HÍDRICO E SUPERSSATURAÇÃO URINÁRIA PARA OXALATO DE CÁLCIO E ESTRUVITA EM GATOS

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ORIENTADOR: AULUS CAVALIERI CARCIOFI

Aprovada como parte das exigências para obtenção do Título de Mestra em MEDICINA VETERINÁRIA, área: Clínica Médica Veterinária pela Comissão Examinadora:

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Jaboticabal, 04 de março de 2022

“Nós, seres humanos, estamos na natureza para auxiliar o progresso dos animais, na mesma proporção que os anjos estão para nos auxiliar. Portanto quem chuta ou maltrata um animal é alguém que não aprendeu a amar”.

(Chico Xavier)

Dedico

Às minhas avós Derci Tozato e Leonor Cherubim, que cuidaram de mim com tanto carinho, e a todas as mulheres que vieram antes de mim, que dedicaram suas vidas a seus filhos e netos, todo meu amor e gratidão.

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DADOS CURRICULARES DO AUTOR

Maria Eduarda Gonçalves Tozato nasceu em Sertãozinho-SP no ano de 1992. Em 2012 ingressou no curso de Graduação em Medicina Veterinária na Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal. Durante a graduação participou de projetos de extensão GEPA E SECITAP, realizou dois projetos de iniciação científica e foi monitora na disciplina de Nutrição de Cães e Gatos. Concluiu a graduação em fevereiro de 2017. Realizou Residência em Nutrição e Nutrição Clínica de Cães e Gatos pelo Programa de Residência Profissional em Medicina Veterinária na Universidade Estadual Paulista – UNESP Campus de Jaboticabal de 2018 a 2020. Iniciou o programa de mestrado em Medicina Veterinária na área de Clínica Médica Veterinária com ênfase em Nutrição de Cães e Gatos em março de 2020.

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"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado "**Teor de amido e umidade do alimento, balanço hídrico e supersaturação urinária para oxalato de cálcio e estruvita em gatos**", protocolo nº 3501/20, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 23 de junho de 2020.

Vigência do Projeto	01/08/2020 a 01/02/2022
Espécie / Linhagem	Felinos
Nº de animais	50
Peso / Idade	3-5 Kg / 2-6 anos
Sexo	Machos e Fêmeas
Origem	Laboratório de Nutrição de Cães e Gatos

Jaboticabal, 23 de junho de 2020.

Fabiana Pilarski

Profª Drª Fabiana Pilarski
Coordenadora – CEUA

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RESUMO:

A urolitíase representa enfermidade importante em felinos. Provenientes de espécie desértica, os gatos apresentam particularidades no balanço hídrico com produção de urina mais concentrada quando alimentados com rações secas e com alto teor de amido. Esta dissertação será apresentada em dois capítulos. No capítulo 1 é exposta a revisão de literatura com tema urolítiase e fatores dietéticos envolvidos. No capítulo 2 é descrito o estudo que avaliou o efeito de dietas com diferentes relações entre amido e proteína e teores de umidade sobre o balanço hídrico, a excreção renal de oxalato e o índice de supersaturação relativa da urina (SSR) para oxalato de cálcio e estruvita. Foram avaliados alimentos com diferentes proporções de amido (15% versus 30%) e proteína (36% versos 53%; com base na matéria seca) e dois teores de umidade (8% versus 80%), em esquema fatorial 2 x 2 totalizando 4 tratamentos experimentais. Cada tratamento foi avaliado em 9 animais, que permaneceram dez dias em adaptação às dietas e oito dias para coleta total de urina e fezes. Os resultados foram avaliados dentro de esquema fatorial 2 (relações amido:proteína) x 2 (teores de umidade do alimento) e submetidos à análise de variância considerando-se os efeitos relação amido:protéina, teor de umidade e suas interações. Quando houve interação entre relação amido:proteína*umidade, médias foram comparadas pelo teste de Tukey ($p<0,05$). A concentração de cálcio foi superior nas dietas secas e sua excreção renal maior nas dietas secas de alto amido ($p<0,05$). A concentração de fósforo foi superior nas dietas secas e com alto amido ($p<0,05$), porém sua excreção renal foi superior em gatos alimentados com as dietas úmidas ($p<0,05$). O teor de sódio na urina foi superior na dietas seca e baixa relação amido:proteína ($p<0,05$), porém sua excreção renal foi maior nas dietas úmidas ($p<0,01$). A concentração urinária de oxalato foi maior nas dietas com alto amido ($p<0,01$). A concentração urinária e excreção renal de citrato foi maior nas dietas secas ($p<0,05$). Gatos consumindo dietas com alta proteína e alta umidade tiveram maior ingestão total de água e produziram maior volume de urina ($p<0,05$). A SSR para oxalato de cálcio foi menor nos animais que ingeriram alimento úmido ($p<0,01$) e maior para dietas alto amido ($p<0,01$). A SSR para estruvita foi menor nos gatos alimentados com as dietas úmidas ($p<0,01$). Conclui-se que o consumo de alimentos com mais de 80% de umidade é eficiente para reduzir a SSR para oxalato de cálcio e estruvita e o maior consumo de proteína, reduzindo a ingestão de amido aumenta a ingestão hídrica e volume urinário, reduzindo a concentração urinária de oxalato e a SSR da urina para oxalato de cálcio.

Palavras-chave: água, citrato, urolitíase, urina.

Abstract

Urolithiasis is an important disease in felines. Having evolved from a desert species, cats present particularities in the water balance with the production of more concentrated urine when fed with dry foods and with a high content of starch. This dissertation is presented in two chapters. On chapter 1 is presented a literature review on urolithiasis and the dietary factors involved in it occurence. On chapter 2 is presented a study on the effects of diets with different starch:protein ratios and moisture content on oxalate renal excretion and the relative urine supersaturation (RSS) index for calcium oxalate and struvite. Diets with different proportions of starch (15% versus 30%) and protein (36% versus 53%; on dry matter basis) and two moisture contents (8% versus 80%) were evaluated in a 2 x 2 factorial scheme, totaling 4 experimental treatments. Each treatment was evaluated in 9 cats, which remained ten days in adaptation to the diets and eight days of total collection of urine and feces. Results were evaluated in a factorial arrangement with 2 starch:protein ratios x 2 food moisture contents, and submitted to analysis of variance considering the effects starch:protein ratio, moisture content and their interactions. When interactions were verified, means were compared using the Tukey test ($p<0.05$). Calcium concentration was higher in the dry diets, and it renal excretion higher in the dry and high starch diets ($p<0.05$). Phosphorus concentration was higher in dry and high-starch diets ($p<0.05$), but it renal excretion was higher in high moisture diets ($p<0.05$). Sodium content in urine was higher in cats fed dry diets and low starch:protein ratio ($p<0.05$), but its renal excretion was higher for high moisture diets ($p<0.01$). Urinary oxalate concentration was higher in cats fed high-starch diets ($p<0.01$). The urine concentration and renal excretion of citrate were higher for dry diets ($p<0.05$). Cats consuming high-protein and high-moisture diets had higher total water intake and produced higher urine volume ($p<0.05$). The urine RSS for calcium oxalate was lower in animals fed high moisture foods ($p<0.01$) and higher for cats fed the high starch diets ($p<0.01$). The RSS for struvite was also lower in cats fed high moisture diets ($p<0.01$). It was concluded that the intake of foods with more than 80% moisture is efficient to reduce the RSS for calcium oxalate and struvite, and the higher protein consumption, reducing starch intake, increases the total water intake and urine volume, reducing the oxalate renal concentration and the urine RSS for calcium oxalate.

Keywords: water, citrate, urolithiasis, urine.

Lista abreviaturas

CEUA: Comissão de Ética no Uso de Animais

PB: Proteína bruta

MS: Matéria seca

Ca: Cálcio

P: Fósforo

Mg: Magnésio

S: Enxofre

Cl: Cloro

Na: Sódio

K: Potássio

Kcal: Quilocalorias

MEq: Miliequivalente

$Kg^{0,75}$: Peso metabólico

EM: Energia metabolizável

SSR: Supersaturação

OxCa: Oxalato de Cálcio

EB: Excesso de bases

DTUIF: Doença do trato Urinário Inferior de Felinos

MS: Matéria seca

PB: Proteína Bruta

MO: Matéria Original

EE: Extrato etéreo

ECF: Escore de condição fecal

ECC: Escore de condição corporal

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CAPÍTULO 1 – Considerações Gerais

Introdução

O Brasil possui expressivo papel no mercado Pet Food, ocupa o 2ª lugar em volume mundial de produção. Nos últimos dois anos houve crescimento de 3,6% na população de gatos, superior ao de cães que foi de 1,5% (ABINPET, 2021). A tendência é que esses números aumentem, pois devido à falta de tempo e espaço, principalmente em grandes centros, os brasileiros estão preferindo gatos como animais de companhia (Instituto Pet Brasil, 2019). Somado a isso, temos no setor de alimentos pet crescente demanda para que as dietas sejam não somente completas e balanceadas, mas promovam qualidade de vida e previnam o aparecimento de doenças, como a urolitíase nos felinos. Acredita-se que estes animais sejam mais predispostos à formação de urólitos por serem menos sensíveis à sede e desidratação, o que os levaria a ingerirem menos água de modo voluntario, concentrando a sua urina principalmente quando alimentados com dietas contendo baixa umidade (Anderson, 1982; Zoran, 2002; DiBartola, 2012; Garcia et al., 2019).

Com a popularização de dietas secas no mercado, os gatos domésticos passaram a receber quantidades consideráveis de amido e um menor teor de água via alimento (Anderson et at., 1982; Zoran, 2002; Carciofi et al., 2005). Como resultado, alguns autores especulam para certos indivíduos, que teriam predisposição à formação de urólitos, a vantagem no consumo de alimentos úmidos, que promovam maior ingestão de água, e com isso aumentam a diurese e reduzem a gravidade específica da urina (Anderson, 1982; Carciofi et al., 2005). O amido colabora na extrusão das rações, favorece na formação de viscosidade, formatação dos kibbles, influindo em sua textura e crocância e, consequentemente, na palatabilidade do produto final (Kopell et al, 2015; Baller et al, 2018). Em função disso, alimentos secos podem apresentar até 35% de amido em sua composição, especialmente em formulações com reduzida proteína e gordura. Os gatos são capazes de digerir eficientemente os carboidratos, com digestibilidade aparente de mais de 95% em alimentos extrusados (Oliveira et al., 2008). Este nutriente também não parece alterar significativamente a resposta pós-prandial de glicose e insulina, especialmente em animais não obesos, pois esta é naturalmente prolongada na espécie devido à suas particularidades na digestão de amido e metabolismo de glicose (Morris et al., 2007; Hoenig et al., 2007; Oliveira et al., 2008).

No entanto, estudos recentes de nosso grupo de pesquisa demonstraram influência do consumo de amido na excreção renal de oxalato e aumento da supersaturação relativa da urina (SSR) para oxalato de cálcio (Pedreira, 2015, Mendonça et al., 2018, Mendonça, 2020), com possível implicação na formação deste urólito. Acredita-se que a abundância de glicose oriunda da digestão do amido resulte na formação de glioxalato e síntese endógena de oxalato em gatos (Dijcker et al. 2012), com sua posterior eliminação na urina. Contudo, tais estudos utilizaram apenas formulações secas, não tendo sido avaliada a interação entre consumo de amido e a umidade do alimento. Como alimentos úmidos elevam a diurese e volume de urina (Carciofi, et al., 2005), é possível que reduzam a concentração urinária de oxalato e que mesmo com maior excreção renal do composto não ocorra elevação da SSR da urina.

Assim, é possível que a formação de oxalato resultante do consumo de amido seja relevante em dietas secas devido à limitada excreção renal de água, mas que nas dietas de elevada umidade, as implicações do consumo de amido sobre a SSR para oxalato de cálcio não sejam relevantes, devido à indução de diluição urinária. Desta forma, para se testar esta hipótese, o presente projeto de pesquisa avaliou os efeitos do consumo de alimentos com elevada ou limitada quantidade de amido, sendo este nas formulações substituído por proteína, que resultou em duas relações amido:proteína, associadas a dois teores de umidade, empregando-se para isto alimento seco extrusado e alimento úmido processado por esterilização, resultando em quatro dietas experimentais. O consumo destas dietas foi avaliado quanto aos efeitos no balanço hídrico, excreção renal de oxalato, cálcio e citrato e a SSR da urina para oxalato de cálcio e estruvita em gatos.

Revisão de Literatura

Doença do trato urinário e urolitíase

Doença do trato urinário inferior de felinos (DTUIF) é o termo utilizado para distúrbios que acometem a bexiga urinária ou uretra de gatos. Seus sinais clínicos incluem: disúria (estrangúria), hematúria, polaquiúria e periúria. Seu diagnóstico é feito a partir dos exames de urinálise, radiografia e ultrassonografia, cistorretrografia e cultura de urina (Forrester e Roudebush, 2007). Dentre as diferentes causas de DTUIF, as que são relacionadas com a presença de cristais e urólitos correspondem, a depender do estudo, entre 15% e 45% dos casos (Bartges e Kirk, 2012). Considera-se, em verdade, que a maioria dos casos de DTUIF sejam decorrentes de cistite idiopática (Silvia et al, 2013; Kruger et al, 2008).

Dos felinos atendidos entre 2005 a 2014 no Hospital Veterinário da Universidade Federal de Minas Gerais, 5% apresentavam doença do trato urinário inferior como queixa principal (Lima,2018). Segundo levantamento realizado por Camargo (2004), de um total de 774 cães e gatos atendidos no período de 1999 a 2003 pelo Serviço de Nefrologia e Urologia do Hospital Veterinário “Governador Laudo Natel” da FCAV- Unesp de Jaboticabal, 107 casos foram de urolitíase (13,82%).

A urolitíase é processo multifacetado que se inicia com a formação de microcristais na urina e termina com o urólito maduro no trato urinário (Osborne et al., 2004). Machos e fêmeas são acometidos, mas os gatos machos são geralmente mais propensos a obstruções da uretra por esta ser mais estreita e longa (Wael, 2012). O crescimento do urólito se inicia com a formação de um ninho de cristal e ou matéria orgânica, fase denominada nucleação, dependente da supersaturação da urina com cristaloides calculogênicos (Grauer, 2015). Influem na nucleação o pH, força iônica, temperatura, magnitude da excreção renal de elementos constituintes do cristaloide e/ou fatores inibidores de sua formação ou agregação (Ettinger e Feldman, 2004).

Os urólitos de maior incidência em felinos são de oxalato de cálcio e estruvita estéril (fosfato amônio magnesiano) (Forrester e Roudebush, 2007). Em geral, gatos com urólitos de estruvita tendem a ser mais jovens e com urolitos de oxalato de cálcio mais idosos (Cannon et al., 2007). Urólitos de estruvita se associam em pH urinário alcalino (>6,8) e urólitos de oxalato de cálcio em pH urinário ácido (Forrester et al., 2010; Wael, 2012). Em estudo realizado pelo Centro de Urólitos de Minnesota durante duas décadas e meia, verificou-se mudança na incidência proporcional de urólitos de oxalato de cálcio e de estruvita. Em 1981 apenas 2% das amostras analisadas eram de oxalato de cálcio e 78% de estruvita, já no ano de 2002, 55% das amostras eram de oxalato de cálcio e 33% estruvita (Osborne et al., 2009). Em um estudo mais recente realizado no México, 54,3% dos urólitos eram de oxalato de cálcio e 32,1% de estruvita (Mendonça-López, 2019). Estas proporções, no entanto, parecem ser muito variáveis, assim, ao se comparar o ano de 2005 com o ano de 2018, de um total de 3.940 urólitos de felinos analisados no laboratório da University of Davis, diminuição dos urólitos de oxalato de cálcio de 50,1% para 37,7%, e aumento dos urólitos de estruvita de 41,8% para 54,5% foi observada (Kopecny, 2021).

O fator relativamente mais bem elucidado em relação à ocorrência de estruvita é a composição mineral da dieta, pois o equilíbrio de macroelementos influencia significativamente o pH pós-prandial da urina e pode, portanto, predispor gatos a desenvolverem estes urólitos (Kienzle et al., 1991; Jeremias et al., 2013). Mudanças nos alimentos e o uso de dietas que promovam a acidificação do pH urinário, com vista à prevenção de cálculos de estruvita, podem ter favorecido ao aumento da formação de cálculos de oxalato de cálcio, considerando sua maior agregação em pH urinário ácido. Esta hipótese, no entanto, necessita de estudos confirmatórios e ainda não tem comprovação (Forrester et al., 2010). Estudo recente, inclusive, não indicou elevação da SSR para oxalato de cálcio após acidificação da urina de gatos (Bijmans et al., 2021).

Supersaturação rerlativa da urina

Pesquisas demonstram que o pH urinário, de modo isolado não permite avaliar corretamente o risco de formação de urólitos no trato urinário de cães e gatos (Robertson et al., 2002). O estudo da SSR da urina é, assim, essencial quando se pretende compreender as urolitíases (Robertson et al., 2002; Houston et al., 2011). O estado de saturação da urina é o produto da concentração de solutos presentes, seu pH, força iônica, temperatura e presença de complexos químicos pré-formados (Allen e Kruger, 2000). O aumento da concentração de substâncias calculogênicas, a exemplo do oxalato e do cálcio, com consequente aumento da SSR urinária, junto ao pH urinário fora da faixa ideal, são considerados os eventos mais importantes na formação dos cálculos (Allen e Kruger, 2000; Hostutler e Chew, 2005; Dibartola, 2012).

A técnica de avaliação da saturação urinária foi inicialmente desenvolvida para seres humanos e posteriormente adaptada para cães e gatos. O valor da SSR incorpora todos os parâmetros que influenciam a probabilidade da formação de um tipo particular de urólito, bem como as possíveis interações entre eles (Hurley et al., 2003). Para se medir a SSR urinária é necessário a obtenção das concentrações de cálcio, fósforo, potássio, magnésio, cloro, sódio, enxofre, ácido úrico, oxalato, citrato e amônia da urina, bem como seu pH. Estas informações abastecem os softwares EQUIL-93 (Department of Biochemistry and Molecular Biology, University of Florida, USA) e SUPERSAT (RSS calculator for pets, Mars, Incorporated and its Affiliates, updated version of the program developed by Robertson, 1969) que apontam o risco de formação ou não de urólitos.

A zona de subsaturação é aquela na qual não há formação de urólitos e qualquer cristal pré-existente poderá se dissolver, com excessão de urólitos de oxalato de cálcio que são indissociáveis. Estes, porém, não irão crescer. O valor de supersaturação relativa atribuído a urina subsaturada é menor que 1,0. A zona de saturação metaestável é a que apresenta valores compatíveis com a de cães e gatos saudáveis. Nesta não há formação de novos urólitos, no entanto, urólitos existentes não se dissociam e podem crescer. Os valores de SSR para a zona metaestável são de 1 a 12 para oxalato de cálcio e entre 1 e 2,5 para estruvita. Por fim, a zona de SSR supersaturada é condição de urina altamente propícia à formação, agregação e crescimento de cristais homogêneos. Os valores de SSR correspondentes à zona de supersaturação para oxalato de cálcio são os maiores de 12 e para estruvita maiores de 2,5 (Hurley et al., 2003; Jeremias, 2013).

Dieta e formação endógena de oxalato

Fatores relacionados ao alimento afetam o volume, pH, gravidade específica e concentração dos elementos formadores de urólito na urina (Zentek e Schulz, 2004) Desta forma, fatores alimentares podem influenciar diretamente a ocorrência ou a prevenção da urolitíase (Allen e Kruger, 2000). Assim, dispor de nutrição adequada para gatos com tendência à formação de urólitos é indispensável, a fim de se evitar recidivas e a necessidade de procedimentos invasivos para remoção dos cálculos (Markwell et al., 1998).

Com a domesticação a dieta dos gatos foi alterada. Estes, que se alimentavam de presas compostas por aproximadamente 55,8% de proteína bruta, 23,6% de gordura, 8,8% de extrativos não nitrogenados e 11,8% de matéria mineral na matéria seca, com cerca de 67% de umidade (Dierenfeld et al., 2002), passaram a ingerir menos proteína, maior quantidade de amido e menos água via alimentos comerciais secos (Verbrugghe et al., 2012). Alimentos comerciais secos extrusados para felinos contêm de 25% a 60% de carboidratos em sua composição (de Oliveira et al., 2008), sendo a maior parte destes amido, usualmente com menos de 8% de umidade.

O amido é usado em petfood por seu baixo custo quando comparado à proteína e gordura, por apresentar boa digestibilidade pelos felinos e por ser vantajoso para que o processo de extrusão ocorra adequadamente (Spears e Fahey, 2004; Baller et al., 2018). Por ser um polímero de glicose, quando digerido e absorvido no trato gastrintestinal, ocorre disponibilização de glicose na corrente sanguínea. A glicose, por sua vez, é um dos açucares precursores de oxalato na via do glioxilato (Behnam et al. 2006). A via metabólica do glioxalato é a principal responsável pela síntese endógena de oxalato em gatos. Esta síntese ocorre predominantemente no fígado e é dependente do glioxilato presente nos hepatócitos (Dijcker et al. 2011). Quando o glioxalato está disponível, pode sofrer redução a glicolato pela enzima glioxilato redutase/hidroxipiruvato redutase (GR/HPR), ou, então, ser transaminado à glicina pela ação da enzima alanina:glioxilato aminotransferase (AGT1).

Essa via dependente da enzima AGT1 está presente somente na mitocôndria dos felinos. Isso, possivelmente, pode ser explicado pelo hábito alimentar dos carnívoros: em função do baixo consumo de carboidratos e glicolato, durante o processo evolutivo provavelmente estes dispensaram a existência de sistema de detoxificação do glioxilato nos peroxissomos, tal como está presente nos herbívoros (Danpure et al., 1990; Dijcker et al., 2011). Quando não transaminado a glicina, o glioxalato será, então, oxidado a oxalato pela enzima L-lactato desidrogenase (L-LDH), presente no citoplasma do hepatócito de felinos (Behnam et al., 2006; Dijcker et al., 2011), ou sofrerá ação da glicolato desidrogenase (GD) nos peroxissomos, sendo também convertido a oxalato (Ribaya-Mercado e Gershoff, 1984; NG et al., 1995; Dijcker et al.,2011). Estas vias metabólicas encontram-se ilustradas na Figura 1.

O oxalato é produto final do metabolismo que não possui função específica, sendo em sua totalidade excretado através da filtração glomerular (Holmes et al. 2005; Knight et al., 2007). Em elevadas concentrações na urina, o oxalato estará mais disponível para se ligar ao cálcio e formar o urólito indissolúvel de oxalato de cálcio. Estudos recentes demonstraram relação entre o consumo de dietas extrusadas secas ricas em amido e a produção e excreção renal de oxalato, representando possível fator de risco à formação de urólitos de oxalato de cálcio nestes animais (Dijcker et al., 2011).

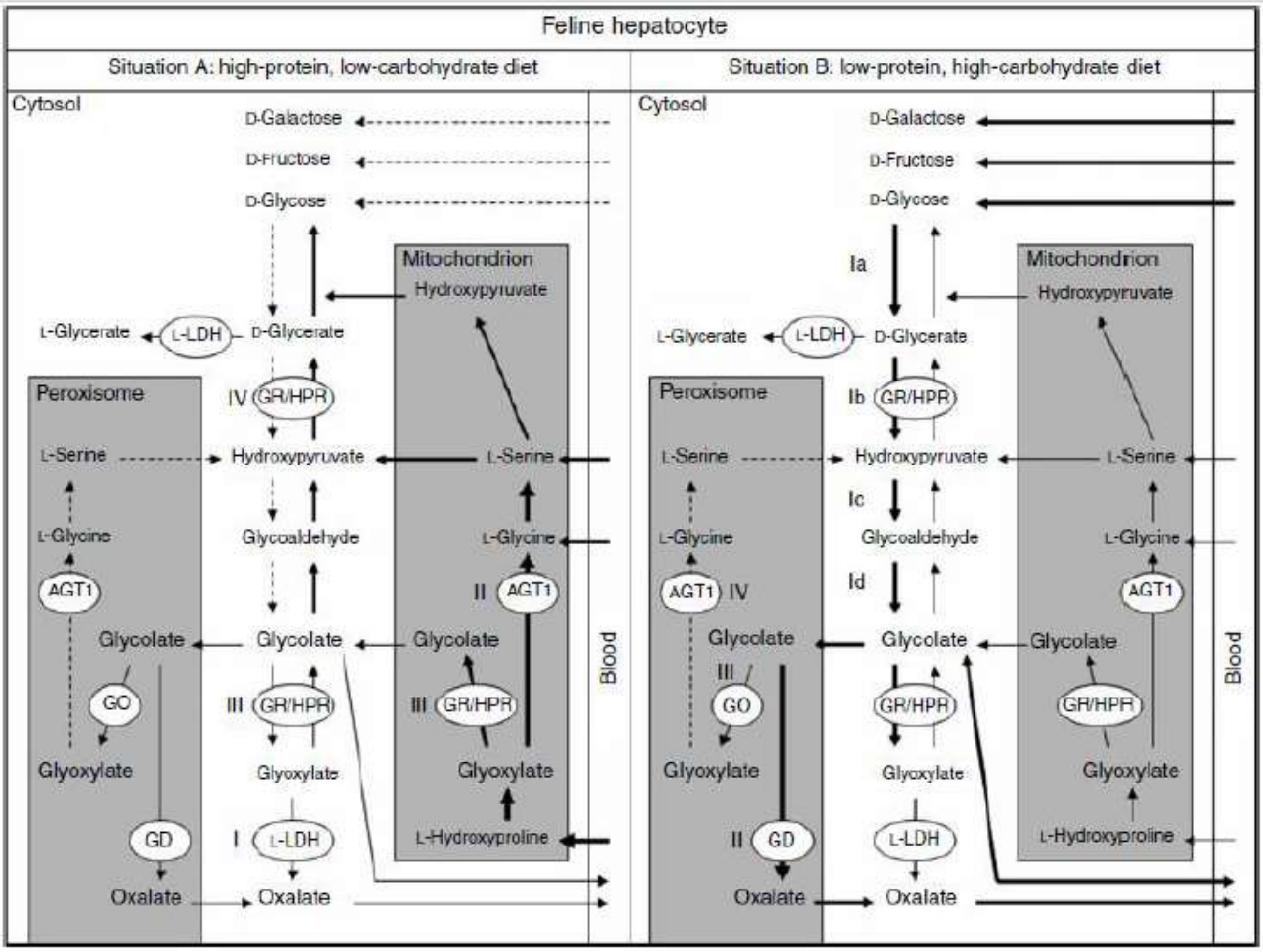

Figura 1. Produção endógena de oxalato a partir de hidroxiprolina (situação A) e açúcares (situação B), pela via do glioxilato no hepatócito felino. Fonte: Dijcker et al., 2011.

Dissertação de mestrado conduzida em nosso laboratório comparou dietas extrusadas secas com diferentes teores de amido e proteína. Gatos consumindo alto amido apresentaram aumento na concentração urinária e excreção renal de oxalato ($p<0,01$), induzindo maior supersaturação da urina para oxalato de cálcio (0,62 na dieta alto amido *vs* 0,22 na dieta baixo amido; p<0,05). A eliminação renal de oxalato (mg/kg/d), por outro lado foi semelhante entre grupos (Pedreira, 2015). Em estudos posteriores de nosso laboratório, a indução de formação e excreção renal de oxalato foi confirmada, tanto em função de consumo elevado de amido como do aminoácido hidroxiprolina (Mendonça et al., 2018; Mendonça, 2020).

Balanço hídrico, produção e características da urina

Além dos fatores citados anteriormente, o consumo de alimento extrusado seco, muito popular entre os proprietários atualmente, parece resultar na eliminação renal de menor quantidade de urina (Lekcharoensuk et al., 2001; Carciofi et al., 2005). Na natureza boa parte da água ingerida pelos felinos é proveniente do alimento, maior parte da composição química de suas presas (Dierenfeld et al., 2002). Gatos tem menor resposta à sede e desidratação que cães e outros animais onívoros (NRC, 2006), o que parece resultar em não compensação do consumo de água quando alimentados com alimentos secos (Anderson et at., 1982; Zoran, 2002). Como resultado, a urina produzida pelos gatos alimentados com rações secas pode apresentar aumento nas concentrações de substâncias formadoras de urólito, embora isto seja apenas uma hipótese ainda não suficientemente estudada (Allen e Kruger, 2000; Hostutler et al., 2005).

Um mecanismo proposto para se reduzir a SSR da urina é o aumento da excreção renal de água, com consequente maior volume de urina e possível diluição dos compostos litôgenicos presentes (Hashimoto et al., 1995; Hawthorne and Markwell, 2004). Maior excreção renal de água depende de aumento na ingestão hídrica dos gatos. Alternativas avaliadas neste sentido incluiram alimentos com elevada umidade, usualmente maior do que 75% como as dietas úmidas e estratégias alimentares com maior consumo de nutrientes que aumentam a excreção de água pelos rins, destacando-se estudos com proteína e sódio (Mendonça, 2020).

Dentre os estudos com felinos, maior consumo de demonstrou efeito positivo em elevar a turnover hídrico e produção de urina proteína (Hashimoto et al., 1995; Hawthorne e Markwell, 2004; Garcia et al., 2020), embora ainda não esteja claro o efeito real destas medidas sobre a SSR da urina e a formação de urolitos de oxalato de cálcio e estruvita. Elevado consumo de proteína resulta em maior produção de ureia (Lekcharoensuk et al., 2001), principal responsável pelo aumento da carga de soluto nos rins (Dibartola, 2012). Em excesso, a ureia vai precisar de água para ser excretada, pois, no filtrado glomerular vai induzir aumentos na tonicidade, osmolalidade e retenção de água no túbulo renal, ocasiona aumento do volume de urina dos gatos (Dibartola, 2012; Pablack et al., 2014). A depender do estudo, aumentos no volume de urina de 74% a 218% foram reportados na literatura, com dietas variando de 49,84% até 64% de proteína bruta, (Hawthorne e Markwell, 2004; Zentek e Schulz, 2004; Pedreira, 2015; Garcia et al., 2019; Mendonça, 2020). Estes aumentos de volume urinário não são, de modo geral, acompanhados por redução da densidade específica da urina. Assim, apesar do maior volume a gravidade específica permanece elevada quando da maior diurese induzida por elevado consumo de proteína bruta, usualmente com valores maiores do que 1,050 (Hashimoto et al., 1995; Hawthorne e Markwell, 2004; Pedreira, 2015; Garcia et al., 2019; Mendonça, 2020). Isto pode ser explicado pelo modo de ação e indução de diurese nas dietas ricas em proteína, que atuam por elevação da carga de solutos e consequente osmolalidade.

Pak (1991) e Dijcker et al. (2011) demonstraram que o aumento da proteína alimentar pode acidificar o pH da urina dos gatos, devido a carga ácida advinda da metabolização dos aminoácidos sulfurados (metionina, cistina e taurina), bem como gerar aumentos na concentração de cálcio urinário e redução do citrato na urina, um dos inibidores da formação de oxalato de cálcio. Em outros estudos gatos ingerindo dietas com elevada proteína tiveram aumento do consumo hídrico e volume de urina, com redução do pH urinário, sem, no entanto, afetar a excreção de Ca urinário (Hashimoto et al., 1996; Funaba et al., 1996; Pablack et al., 2014). Estudos mais recentes demonstraram, por outro lado, que o balanço de macroelementos da dieta é o real determinante do pH da urina e que é possível se formular dietas com elevada proteína que induzam produção de urina neutra ou mesmo alcalina (Pedreira, 2015, Mendonça, 2018, 2020). Assim, este é ainda ponto controverso que necessita de mais estudos. De qualquer forma, estes resultados abrem oportunidade não somente de se explorar possível diminuição da formação e excreção renal de oxalato ao se substituir amido por proteína na dieta, como se obter maior excreção renal de água e turnover hídrico em gatos alimentados com elevada proteína, somando assim dois possíveis benefícios à redução da SSR da urina para oxalato de cálcio, hipótese que merece estudos científicos.

Em relação ao teor de água do alimento, dois estudos, já mais antigos, não verificaram diferenças no balanço hídrico de gatos alimentados com dietas diferindo no teor de umidade (Thrall e Miller, 1976; Burger, 1980). Já Markwell et al. (1998) demonstraram que com umidades entre 10 e 45% nos alimentos, a produção de urina não se alterou, mas mediante consumo de alimento com 75% de umidade o volume urinário aumentou. Estes resultados foram confirmados em estudo posteriordo nosso grupo de pesquisa, que verificou que umedecer alimento seco para se atingir umidade final próxima a 50% não altera o balanço hídrico de gatos, mas a produção de urina aumenta e sua densidade aparente diminui quando gatos são alimentados com alimento úmido enlatado, com 80% de água (Carciofi, 2005).

Buckey (2011), utilizando dieta padronizada com inclusões crescentes de água (6,3%; 25,4%; 53,2; 73,3%), avaliou o papel da umidade da ração no balanço hídrico e SSR urinária para oxalato de cálcio e estruvita. Gatos alimentados com a dieta com 73,3% de umidade tiveram maior ingestão diária total de líquidos e produziram urina com densidade significativamente menor (1,036 vs 1,054), o que resultou em menor valor de SRR para Oxalato de cálcio (1,14 vs 2,29) em relação a dieta com 6,3% de umidade ($p < 0,001$).

Hipóteses e Objetivos

Diante do exposto, são hipóteses do presente estudo que o consumo de alimentos úmidos, com mais de 80% de água, resultará em maior ingestão e excreção de água, com redução da SSR da urina para oxalato de cálcio e estruvita em comparação ao consumo de alimento seco. Menor ingestão de amido, resultante do consumo de alimento com maior teor proteico resultará em menor síntese endógena e excreção renal de oxalato, maior volume de urina e menor SSR para oxalato de cálcio nos gatos.

Considerando as hipóteses estabelecidas, este estudo teve como objetivo avaliar o balanço hídrico, características da urina, excreção renal de oxalato, cálcio e citrato e determinar a SSR da urina para oxalato de cálcio e estruvita de gatos alimentados com rações secas ou úmidas, com duas relações amido:proteína.

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Starch to protein ratio and moisture content of the food influence water balance and urine supersaturation for calcium oxalate and struvite in cats[1].

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Starch to protein ratio and moisture content of the food influence water balance and urine supersaturation for calcium oxalate and struvite in cats.

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Abstract

Two starch to protein ratios (high starch [HS]: 25% starch and 36% protein; high protein [HP]: 15% starch and 53% protein on dry matter basis), and two moisture contents (5%, dry kibbles; 80%, wet food) were compared in a 2 x 2 factorial arrangement totaling 4 diets. Each diet was evaluated in 9 cats, with 8 days of total collection of urine and feces. Results were submitted to analysis of variance considering the effects starch to protein ratio, moisture content and their interactions ($P<0.05$). Urine density was lower and their volume higher in cats fed wet foods ($P<0.01$). Ca intake was higher for dry and HS foods, but it urine concentration higher for dry and HP diets ($P<0.05$). Oxalate urine concentration was 60% higher for cats fed the HS formulations ($P<0.05$). The relative supersaturation (RSS) of urine for calcium oxalate was higher for dry foods and HS formulations ($P<0.01$), while for struvite RSS was lower for wet foods, and among the dry diets lower for HS than for HP formulation ($P<0.01$). Foods with more than 80% moisture reduced urine density and RSS for calcium oxalate and struvite, and high protein to starch ratio reduces urine RSS for calcium oxalate.

Keywords: citrate, kibble diets, stones, urolithiasis, wet food

Introduction

Urolithiasis is a complex biomineralization process that starts with microcrystals formation and ends with a mature urolith development in the urinary tract[1,2]. Despite some changes over the years on the relative prevalence, calcium oxalate and magnesian ammonium phosphate (struvite) are the main uroliths which affect cats [3,4,5,6]. The incidence of the conditions is relevant, with estimates ranging from 13% to 18% of the cat population affected with feline lower urinary tract disease[3,4,5,6] .

Many controversies exist about their etiology and the role of diet in the development or prevention of urolith formation[7,8,9], but adequate nutrition for urolith-forming cats is considered essential to avoid recurrences and invasive procedures to remove stones[4]. Dietary intake and their metabolic formation will influence the renal excretion of calculogenic substances, what together with water balance and the consequent urine volume will finally determine the concentration on urine of the substances that may promote or prevent mineralization. Urine pH is another important factor, strictly controlled by diet macro mineral composition[10], that determines the force of aggregation and crystals biomineralization in the urine[11]. However, the use of urine pH only is not adequate to predict urolith formation, which is better accessed by the calculation of the relative supersaturation of the urine (RSS)[12,13,14].

Dry kibble diets, very popular among cat owners induces lower water intake via food as it has usually less than 10% moisture. Although some controversial results were published[15,16], most studies described that cats do no compensate for this low moisture on food presenting low total water intake, and consequently, reduced urine volume, and increased urine density in comparison to the intake of wet foods or dry foods supplemented with more the 70% water [17,18]. However, the impact on the urine RSS for calcium oxalate and struvite of this induced dilution on urine promoted by wet food consumption is still not clear

Dry kibble diets are also relatively high in starch and low in protein content in comparison to preys, the ancestral diet of felines[20]. Starch is used to favor extrusion and kibble formation[21,22], and to be a good metabolizable energy source, with high digestibility for cats[21]. Starch also do not induce noticeable changes on post-prandial glucose and insulin responses, especially in non-obese animals[23,24]. However, high starch and moderate protein kibble diets intake may result in low urine production, as protein intake is known to induce water renal excretion and urine output in cats[25,26]. High protein intake induces elevated production of urea, which in the glomerular filtrate increases tonicity, water retention and urine volume. As urea increases urine osmolality, in this situation although a larger amount of urine is produced, its osmolality and density remain high[14]. Additionally, some experiments reported increased calcium renal excretion in cats fed high protein foods[27,] and due these aspects it is not known yet the impact of the increased urine volume induced by protein intake on the urine RSS of cats.

On the other side, increased starch intake results in higher metabolic availability of glucose, a precursor de oxalate through the glyoxylate pathway[28]. Due this, high starch diets has been incriminated to increase endogenous oxalate synthesis in feline hepatocytes[29]. This hypothesis is reinforced by a study that demonstrated lower urine volume, higher oxalate urine concentration and higher RSS for calcium oxalate in cats fed high starch in comparison to high protein dry diets[30]. This effect of starch intake, however, was only evaluated on dry kibble diets and no study about the effect of starch was localized for wet foods.

Taking this in consideration, are hypothesis of the present study that the intake of wet foods with more the 80% moisture will induce higher total water intake and urine volume, reducing the urine density and RSS for calcium oxalate and struvite in comparison to the intake of dry foods. Lower starch intake by cats, resulting from the intake of high protein formulations will result in lower endogenous synthesis and renal excretion of oxalate, higher urine volume and lower RSS for calcium oxalate. To evaluate this, the preset study evaluated the water balance, urine characteristics, renal excretion of oxalate, calcium, and citrate, and measure the urine RSS for calcium oxalate and struvite of cats fed dry or wet foods, with two starch to protein ratios.

Results

Item	Dry foods		Wet Foods	
	High starch[1]	High protein[2]	High starch[3]	High protein[4]
Moisture (%)	4.78	4.74	81.61	81.61
	%, DM basis			
Starch	28.75	15.33	25.32	6.69
Crude Protein	36.89	53.67	36.91	53.31
Essential amino acid				
Arginine	2.14	2.58	1.88	2.76
Histidine	0.81	1.08	0.95	1.25
Isoleucine	1.51	2.21	1.55	2.30
Leucine	2.53	3.79	2.96	4.43
Lysine	2.03	2.16	2.34	3.36
Methionine	0.76	0.93	0.68	0.93
Methionine + Cysteine	1.24	1.84	1.41	2.05
Phenylalanine	1.52	2.50	1.80	2.74
Phenylalanine + Tyrosine	2.54	3.88	2.84	4.23
Threonine	1.26	1.71	1.54	2.29
Leucine	2.53	3.79	2.96	4.43
Serine	1.13	2.05	1.37	2.17
Valine	1.74	2.49	1.98	2.97
Tryptophan	0.34	0.52	0.45	0.61
Taurine	0.48	0.40	0.45	0.42
Dispensable amino acids				
Glycine	2.75	3.16	2.09	2.86
Hydroxyproline	0.67	0.60	0.21	0.26
Aspartic acid	2.76	3.21	2.98	4.39
Glutamic acid	5.38	13.00	5.30	8.43
Proline	2.39	4.89	2.15	3.39
Tyrosine	1.02	1.38	1,78	1.49
Cystine	0.48	0.91	0.73	1.12
Alanine	2.05	2.35	1.85	2.68
Acid-hydrolyzed fat	17.01	17.47	24.06	23.92
Crude fiber	2.38	2.95	1.81	1.04
Ash	9.08	6.01	9.11	7.97
Calcium	1.57	0.60	1.15	1.16
Phosphorus	1.22	0.58	0.98	1.17
Magnesium	0.09	0.06	0.07	0.09
Sodium	0.91	0.79	1.13	1.41
Chlorine	1.71	1.60	1.22	1.51
Potassium	0.75	0.77	0.56	0.64
Sulfur	0.63	0.78	0.68	0.93

Table 1. Analyzed chemical composition of experimental diets for cats with different starch to protein ratios and moisture content. Values on dry matter basis

[1] Ingredients: Broken rice, Poultry by-product meal, Poultry fat, Wheat gluten, , Wheat bran, Swine-isolated protein, Sugarcane fiber, Palatant enhancer, Beet pulp, Common salt, Potassium chloride, Vitamin-mineral Premix, Choline chloride, Calcium carbonate, Taurine, Salmon oil, Mold Inhibitor, Antioxidant, DL-Methionine, Urine acidifier

[2] Ingredients: Wheat gluten, Poultry by-product meal, Broken rice, Poultry fat, Swine-isolated protein, Sugarcane fiber, Palatant enhancer, Beet pulp, Common salt, Potassium chloride, Wheat bran, Vitamin-mineral Premix, Choline chloride, Calcium carbonate, Taurine, Salmon oil, Mold Inhibitor, Antioxidant, DL-Methionine, Urine acidifier, Phosphoric acid

[3] Ingredients: Water, Chicken meat, Swine kidney, Cassava starch, Swine liver, Swine Plasma, Wheat gluten, Sugarcane fiber, Beet pulp, Xanthan gum, Common salt, Potassium chloride, Vitamin-mineral Premix, Choline chloride, Calcium carbonate, Taurine, Salmon oil, Mold Inhibitor, Antioxidant, DL-Methionine, Liquid Palatant, Urine acidifier

[4] Ingredients: Water, Chicken meat, Swine kidney, Swine liver, Swine Plasma, Wheat gluten, Sugarcane fiber, Beet pulp, Xanthan gum, Common salt, Potassium chloride, Vitamin-mineral Premix, Choline chloride, Calcium carbonate, Taurine, Salmon oil, Mold Inhibitor, Antioxidant, DL-Methionine, Liquid Palatant, Urine acidifier

Item	Starch to protein ratio		Mean	SEM[1]	P value	
	High starch	High protein			S:P[2]	Moisture
Body weight (kg)						
Wet food	3.59	3.60	3.59	0.26		
Dry food	4.24	4.47	4.36	0.27		
Mean	3.92	4.07		0.19	0.563	<.001
Body Condition Score						
Wet food	5.22	5.11	5.16	0.11		
Dry food	5.44	5.44	5.44	0.32		
Mean	5.33			0.05	0.860	0.281
Dry Matter intake (g/kg/day)						
Wet food	10.0	9.6	9.80	0.60		
Dry food	11.5	11.1	11.30	0.60		
Mean	10.8	10.3		0.42	0.324	0.001
Crude Protein intake (g/kg/day)						
Wet food	3.70	5.24	4.47	0,17		
Dry food	4.26	5.76	5.01	0,17		
Mean	3.98	5.50		0,12	<0.001	0.001
Starch intake (g/kg/day)*						
Wet food	2.46[b]	0.56[d]	1.52	1.74		
Dry food	3.20[a]	1.62[c]	2.41	1.57		
Mean	2.83	1.09		1.90	<.001	<.001

Table 2. Body weight, body condition score, and dry matter, crude protein and starch intake of cats fed experimental diets with different starch to protein ratios and moisture contents.

[1] SEM - standard error of the mean (n = 9 cats per food)

[2] S:P - effect of the starch to protein ratio of the diet

* Starch to protein ratio * moisture content interaction (P<0.05)

[a, b, c] Inside a variable, means without a common superscript letter differs (P<0.05)

Item	Starch to protein ratio		Mean	SEM[1]	P value	
	High starch	High protein			S:P[2]	Moisture
Total tract apparent digestibility (%)						
Dry Matter						
Wet food	87.51	82.63	85.07	1.13		
Dry food	83.20	87.30	85.25	1.09		
Mean	85.35	84.98		0.80	0.626	0.772
Crude Protein*						
Wet food	93.70[a]	91.13[b]	92.40	0.74		
Dry food	90.06[b]	90.55[b]	90.31	0.74		
Mean	91.86	90.84		0.53	0.060	<0.001
Acid-hydrolyzed fat*						
Wet food	91.25[ab]	87.40[b]	89.33	1.60		
Dry food	92.29[a]	93.84[a]	93.06	1.50		
Mean	91.77	90.62		1.08	0.302	0.002
Starch						
Wet food	99.92	99.86	99.89	0.03		
Dry food	99.89	99.90	99.90	0.02		
Mean	99.90	99.88		0.02	0.301	0.772
Crude Energy*						
Wet food	87.82[a]	83.48[b]	85.65	1.22		
Dry food	86.75[ab]	88.48[a]	87.62	1.18		
Mean	87.29	85.98		0.85	0.135	0.027
Fecal characteristics						
g/kg/day, As-is						
Wet food	6.90[ab]	7.93[b]	7.41	0.59		
Dry food	6.87[ab]	5.65[a]	6.24	0.58		
Mean	6.88	6.77		0.41	0.803	0.008
g/kg/day, DM basis						
Wet food	2.79	3.16	2.98	0.28		
Dry food	3.12	2.54	2.83	0.58		
Mean	2.96	2.85		0.10	0.619	0.482
Score						
Wet food	3.87	3.63	3.74			
Dry food	3.85	3.92	3.89			
Mean	3.86	3.77			0.226	0.060

Table 3. Coefficients of nutrient digestibility and fecal characteristics of cats fed experimental diets with different starch to protein ratios and moisture contents.

[1] SEM - standard error of the mean (n = 9 cats per food)

[2] S:P - effect of the starch to protein ratio of the diet

* Starch to protein ratio * moisture content interaction (P<0.05)

[a, b] Inside a variable, means without a common superscript letter differs (P<0.05)

Item	Starch to protein ratio		Mean	SEM[1]	P value	
	High starch	High protein			S:P[2]	Moisture
Urine characteristics						
Density (g/l)*						
Wet food	1,020[c]	1,019[c]	1,020	0,002		
Dry food	1,046[a]	1,056[b]	1,051	0,001		
Mean	1,033	1,038		0,002	0.001	<.001
pH						
Wet food	6.10	6.07	6.08	0.07		
Dry food	6.12	6.25	6.19	0.07		
Mean	6.11	6.16		0.05	0.358	0.055
Water balance (ml/kg/day)						
Water from food*						
Wet food	43.44[b]	54.70[a]	49.07	1.47		
Dry food	0.58[c]	0.53[c]	0.56	1.43	<.001	<.001
Mean	22.01	27.61		1.03		
Drinking water						
Wet food	0.17	0.19	0.10	0.91		
Dry food	20.90	20.69	20.80	0.91		
Mean	10.37	10.44		0.65	0.913	<.001
Metabolic water*						
Wet food	5.10[a]	4.30[b]	4.70	0.21		
Dry food	5.00[a]	4.90[a]	4.95	0.21		
Mean	5.05	4.60		0.15	0.012	0.111
Total water intake*						
Wet food	49.43[b]	59.10[a]	54.26	2.12		
Dry food	28.44[c]	27.55[c]	28.00	2.05		
Mean	38.94	43.32		1.47	0.010	<.001
Urine water excretion*						
Wet food	28.15[b]	39.90[a]	34.03	1.53		
Dry food	15.02[c]	16.06[c]	15.54	1.43		
Mean	21.59	27.98		1.04	<.001	<.001
Fecal water excretion						
Wet food	2.07	2.58	2.32	0.32		
Dry food	2.30	1.75	2.03	0.32		
Mean	2.19	2.17		0.22	0.935	0.190
Insensible losses						
Wet food	16.46	16.18	16.32	0.28		
Dry food	10.69	9.32	10.00	1.37		
Mean	13.57	12.75		0.82	0.403	<.001

Table 4. Urine characteristics and water balance of cats fed experimental diets with different starch to protein ratios and moisture contents.

[1] SEM - standard error of the mean (n = 9 cats per food)

[2] S:P - effect of the starch to protein ratio of the diet

* Starch to protein ratio * moisture content interaction ($P<0.05$)

[a, b, c] Inside a variable, means without a common superscript letter differs ($P<0.05$)

Item	Starch to protein ratio		Mean	SEM[1]	P value	
	High starch	High protein			S:P[2]	Moisture
Ca intake (mg/kg/d)						
Wet food	113.9	107.3	110.6	5.08		
Dry food	176.6	63.5	120.1	5.24		
Mean	145.3	85.4		3.65	<.001	0.014
Ca urine concentration (mg/dL)						
Wet food	1.43	1.49	1.46	0.52		
Dry food	4.03	5.64	4.83	0.49		
Mean	2.73	3.56		0.36	0.049	<.001
Ca renal excretion (mg/kg/d)						
Wet food	0.46	0.59	0.52	0.10		
Dry food	0.65	0.82	0.73	0.10		
Mean	0.55	0.70		0.07	0.037	0.005
P intake (mg/kg/d)						
Wet food	94.6	108.0	101.19	14.12		
Dry food	141.7	60.4	101.07	12.66		
Mean	118.0	84.2		9.48	<.001	0.975
P urine concentration (mg/dL)*						
Wet food	164.8[b]	169.0[b]	166.9	1.61		
Dry food	260.3[a]	183.5[b]	221.9	1.86		
Mean	212.5	176.3		1.15	0.003	<.001
P renal excretion (mg/kg/d)						
Wet food	50.5	65.1	57.8	3.20		
Dry food	39.0	29.3	34.2	3.01		
Mean	44.8	47.2		2.20	0.848	<.001
Mg intake (mg/Kg/d)						
Wet food	7.15	8.34	7.74	0.47		
Dry food	11.10	7.17	9.13	0.47		
Mean	9.12	7.76		0.33	0.001	0.001
Mg urine concentration (mg/dL)						
Wet food	3.07	3.62	3.35	0.43		
Dry food	9.21	11.86	10.53	0.46		
Mean	6.14	7.75		0.31	0.001	<.0001
Mg renal excretion (mg/kg/d)						
Wet food	1.05	1.46	1.26	0.17		
Dry food	1.41	1.87	1.64	0.17		
Mean	1.23	1.66		0.12	0.001	0.004
Na intake (mg/Kg/d)						
Wet food	142.1	108.4	125.3	5.65		
Dry food	105.6	87.9	96.7	5.83		
Mean	123.9	98.1		4.06	<.001	<.001
Na urine concentration (mg/dL)						
Wet food	299.5	286.0	292.7	20.86		
Dry food	543.9	439.8	491.9	23.32		
Mean	421.7	362.9		16.64	0.002	<.001
Na renal excretion (mg/kg/d)						
Wet food	93.2	115.5	104.4	1.81		
Dry food	66.2	75.8	71.0	10.81		
Mean	79.7	95.6		7.65	0.045	0.001
Cl intake (mg/kg/d)*						
Wet food	120.4[d]	143.7[c]	132.1	6.42		
Dry food	196.9[a]	171.7[b]	184.3	6.81		
Mean	158.7	157.7		0.97	0.836	<.001
Cl urine concentration (mg/dL)						
Wet food	580.77	449.96	515.37	99.45		
Dry food	1272.16	1197.04	1234.60	99.45		
Mean	926.47	823.50		102.97	0.153	<.001
Cl renal excretion (mg/kg/d)						
Wet food	151.0	190.9	170.9	12.85		
Dry food	193.6	196.2	194.9	12.85		
Mean	172.3	193.5		9.08	0.047	0.046

K intake (mg/kg/d)						
Wet food	52.7	50.1	51.4	3.77		
Dry food	86.2	82.0	84.1	3.77		
Mean	69.7	66.1		2.66	0.214	<.001
K urine concentration (mg/dL)						
Wet food	187.4	151.7	169.6	22.58		
Dry food	530.5	532.8	531.6	23.28		
Mean	358.9	342.3		16.22	0.313	<.001
K renal excretion (mg/kg/d)						
Wet food	57.8	60.6	59.2	3.92		
Dry food	75.5	81.2	78.3	4.19		
Mean	66.6	70.9		2.87	0.149	<.001
S intake (mg/Kg/d)						
Wet food	65.92	86.74	76.33	4.25		
Dry food	72.93	84.29	78.62	4.25		
Mean	69.42	85.52		3.00	<.001	0.442
S urine concentration (mg/dL)						
Wet food	276.2	187.5	231.8	38.31		
Dry food	388.6	431.4	410.0	38.31		
Mean	332.4	309.39		27.09	0.402	<.001
S renal excretion(mg/kg/d)						
Wet food	79.4	69.2	74.3	10.53		
Dry food	51.4	74.3	62.9	10.86		
Mean	65.4	71.7		7.56	0.158	0.162
Uric Acid urine concentration (mg/dL)						
Wet food	4.63	5.00	4.81	0.86		
Dry food	6.09	7.69	6.89	0.89		
Mean	5.36	6.34		0.62	0.124	0.002
Uric Acid renal excretion (mg/kg/d)						
Wet food	1.45	1.81	1.63	0.26		
Dry food	0.88	1.37	1.12	0.26		
Mean	1.16	1.59		0.18	0.026	0.009
Urea urine concentration (mg/dL)*						
Wet food	2937.9[c]	3165.9[c]	3051.9	320.32		
Dry food	6616.5[b]	9515.2[a]	8064.1	330.18		
Mean	4777.2	6339.07		230.01	<.001	<.001
Urea renal excretion (mg/kg/d)						
Wet food	890.5	1254.5	1007.5	68.65		
Dry food	929.7	1386.3	1158.0	81.22		
Mean	910.1	1320.4		53.17	<.001	0.140
Ammonia urine concentration (mg/dL)						
Wet food	157.2	147.0	152.1	12.45		
Dry food	159.1	164.5	161.8	12.46		
Mean	158.1	155.7		8.80	0.787	0.280
Ammonia renal excretion (mg/kg/d)						
Wet food	49.2	60.0	54.6	5.44		
Dry food	24.4	28.2	26.3	5.44		
Mean	36.8	44.1		3.85	0.067	<.001

Table 5. Intake, urine concentration, and renal excretion of macroelements and metabolites of cats fed experimental diets with different starch to protein ratios and moisture contents.

[1] SEM - standard error of the mean (n = 9 cats per food)

[2] S:P - effect of the starch to protein ratio of the diet

* Starch to protein ratio * moisture content interaction (P<0.05)

[a, b, c, d] Inside a variable, means without a common superscript letter differs (P<0.05)

Item	Starch to protein ratio		Mean	SEM[1]	P value	
	High starch	High protein			S:P[2]	Moisture
RSS for calcium oxalate (Equil)						
Wet food	0.33	0.10	0.22	0.13		
Dry food	0.72	0.39	0.55	0.13		
Mean	0.53	0.25		0.09	0.005	0.001
RSS for calcium oxalate (Supersat)						
Wet food	0.03	0.04	0.03	0.10		
Dry food	0.33	0.32	0.32	0.10		
Mean	0.18	0.18		0.07	0.945	0.001
RSS for struvite*						
Wet food	0.02[c]	0.01[c]	0.02	0.01		
Dry food	0.06[b]	0.11[a]	0.09	0.01		
Mean	0.04	0.06		0.01	0.060	<.0001
Oxalate urine concentration (mg/dL)						
Wet food	2.24	1.50	1.88	0.28		
Dry food	2.09	1.18	1.63	0.28		
Mean	2.17	1.34		0.20	<0.001	0.234
Oxalate renal excretion (mg/kg/d)						
Wet food	0.70	0.69	0.69	0.07		
Dry food	0.21	0.11	0.16	0.07		
Mean	0.46	0.401		0.05	0.333	<.001
Citrate urine concentration (mg/dL)						
Wet food	21.5	22.1	21.8	0.74		
Dry food	23.3	24.8	24.1	0.76		
Mean	22.4	23.4		0.53	0.063	<.001
Citrate renal excretion (mg/kg/d)						
Wet food	6.92	9.12	8.02	0.50		
Dry food	3.42	4.55	3.98	0.50		
Mean	5.17	6.84		0.35	<0.001	<.0001

Table 6. Relative Supersaturation (RSS) for calcium oxalate and struvite, and oxalate and citrate concentration and renal excretion of cats fed experimental diets with different starch to protein ratios and moisture contents.

[1] SEM - standard error of the mean (n = 9 cats per food)

[2] S:P - effect of the starch to protein ratio of the diet

* Starch to protein ratio * moisture content interaction (P<0.05)

[a, b, c] Inside a variable, means without a common superscript letter differs (P<0.05)

Diets presented adequate chemical composition (Table 1), with crude protein amounts close to planned. Starch amount was also similar for the high starch wet and dry formulations, however the wet food high protein formulation presented lower starch value than planned. Amino acid and mineral composition varied according to raw material source and crude protein content.

The body weight and BCS of the cats was similar among high starch and high protein formulations, but cats fed the dry foods were heavier (Table 2). Cats appropriately accepted the four diets, all the animals remained healthy, with no episodes of gastrointestinal disturbances or altered behavior during the study. The body weight of the cats remained constant along the study (data not shown). The dry matter intake was lower for cats fed wet foods ($P<0.01$), but it did not differ between high starch and high protein formulations. The differences on dry matter intake could be related to the physical form and moisture content of diets or is may only reflecting the higher crude fat and energy content of the wet food formulations. The starch and protein intake differed between groups, following the chemical composition and dry matter intake of the treatments ($P<0.05$).

In general, the total tract apparent digestibility of nutrients was high, indicating good raw material sources and food processing (Table 3). No effect of starch to protein ratio was observed for nutrient digestibility coefficients or feces characteristics. Differences among diet types was observed, with higher protein digestibility for the high starch wet food, and lower crude fat and crude energy digestibility for the high protein wet food ($P<0.05$). Feces amount (as-is) was also high for cats fed the high protein wet food ($P<0.05$), may be explained by the lower digestibility of this diet in comparison with the others.

Urine pH was similar between treatments, according to the formulation objective, in order to avoid a possible interference of urine pH on RSS results (Table 4). Interaction starch to protein ratio *versus* food type was observed for urine density, water intake from food, metabolic water, total water intake, and urine excretion ($P<0.05$). Urine density was higher for cats fed dry in comparison to wet foods ($P<0.01$), and among cats fed dry foods higher for high protein than high starch ($P<0.05$). Water intake from food and total water intake was higher for cats fed wet foods ($P<0.01$), and inside this diet type higher for high protein than high starch formulation ($P<0.05$). On the contrary, drinking water was higher for cats fed dry foods ($P<0.01$), without effect of starch to protein ratio. Cats fed wet foods presented more than a double of urine excretion than cats fed dry foods (P<0.01), and inside the wet foods urine production was higher for cats fed the high protein than the high starch formulation (P<0.05).

Values of macromineral intake, urine concentration and renal excretion are present in Table 5. Ca intake was higher for cats fed the high starch and dry foods, but it urine concertation and renal excretion higher for cats fed dry foods and high protein formulations (P<0.01). P intake was higher for cats fed high starch formulation (P<0.01), but similar among food types. P urine concentration was higher for cats fed high starch dry food (P<0.05), and similar between the other treatments. Although the similar intake, the renal P excretion was higher for cats fed wet than dry foods (P<0.01). Mg intake was higher for cats fed high starch formulations and dry foods, and it urine concentration and renal excretion higher for cats fed dry foods and high protein formulations (P<0.05), For Na, higher intake was observed for cats fed wet foods and high starch formulations, its urine concentration higher for cats fed dry foods and high starch formulations, and its renal excretion higher for cats fed wet food and high protein formulations (P<0.05). For Cl a starch to protein ratio versus food type interaction was verified, and its intake was higher for cats fed high starch dry food, then for animals fed high protein dry food, then high protein wet food, and lower for cats fed high starch wet food (P<0.05). On urine, Cl concentration differed only among food types, it was higher for cats fed dry than wet foods (P<0.01). Cl renal excretion was higher for cats fed dry foods and high protein formulations (P<0.05). For K only food type effect was observed, and its intake, urine concentration, and renal excretion was higher for cats fed dry than wet food (P<0.01). Sulfur intake was higher for cats fed high protein formulations (P<0.01) and similar among food types. Its urine concentration was higher for cats fed dry foods (P<0.01), and its renal excretion similar between treatments.

The urine concentration of uric acid was higher for cats fed dry foods (P<0.01) and similar between high starch and high protein formulations. However, its renal excretion was higher for cats fed high protein formulations and wet foods (P<0.01). A food type versus starch to protein ratio interaction was observed for urea urine concentration (P<0.05), that was higher for cats fed the high protein dry food, intermediary for animals fed the high starch dry food, and lower for animals fed the both wet food formulations (P<0.05). Urea renal excretion, on the other hand only differed among the formulation type, been higher for cats fed the high protein than the high starch diets (P<0.01). Ammonia urine concentration was similar between treatments, and it renal excretion higher for cats fed the wet foods (P<0.01).

The results of urine RSS for calcium oxalate differed between the Equil and Supersat estimations (Table 6). Using Equil, cats fed dry foods and high starch formulations presented higher RSS for calcium oxalate (p<0.01). Utilizing Supersat, no effect of starch to protein ration was observed, only a diet type effect with higher RSS for calcium oxalate in cats fed the dry foods (P<0.01). A food type versus starch to protein ratio interaction was observed for urine RSS for struvite (P<0.05), that was higher for cats fed the high protein dry food, intermediary for high starch dry food, and lower for cats fed both wet food formulations (P<0.05). Oxalate urine concentration presented only an effect of starch to protein ratio; it was higher for cats fed the high starch foods (P<0.01). Oxalate renal excretion, on the other hand, was higher for cats fed wet than dry foods (P<0.01), regardless of starch to protein ratio. Citrate on urine was higher for cats fed the dry foods (P<0.05), and it renal excretion higher for animals fed wet diets and high protein formulations (P<0.01).

Discussion

The obtained results in general confirmed the hypothesis stablishe for the study, with increased urine production in cats fed wet foods and high protein formulations, and high oxalate and urine RSS for calcium oxalate in cats fed the high starch formulations. It was interesting that the RSS for struvite was lower in cats fed the high starch than high protein food, showing that the prevention of these two types of stones requires different dietary approaches.

The cats used were not familiar to eat wet diets as the sole meal. Despite this, animals accepted the diets well and there was no weight loss or difference in body condition score. No differences were also observed on fecal score or moisture, showing good acceptance to the wet foods used.

It is suggested that high protein diets induces low urine pH in cats due to the acid load arising from the metabolization of sulfur amino acids[25]. However, the present results showed clearly that food base excess, consequent of diet macromineral composition is the predominant factor as diets, regardless of their protein content induced urine production with similar pH [9,33]. in a safe range for the occurrence of struvite and calcium oxalate stones[34]. This confirms that it is possible to formulate diets with high protein and neutral urinary pH with the excess base equation[35,36].

Cats have a desert origin and thereby a high capacity to concentrate urine[37]. Its renal physiology is composed of juxtamedullary nephrons, which are longer and allow greater water absorption[11]. In nature, their prey is composed up to 75% of water and when fed with high-moisture diets, they can go for periods without ingesting water from other sources[32]. Wet diets led the animals to ingest a smaller volume of water by drinker ($P<0.001$), according to Carciofi[18], the more water content of the food, the lower the water intake by drinker, even so, the total water consumed was higher than the dry diets.

High-humidity diets led to lower urinary density values. According to Buckey[17], cats fed diets with at least 73.3% of humidity have urine volume produced significantly higher and led to lower urinary density values compared to dry diets (1.036 vs 1.054). In this study, animals fed wet diets had even lower values (1.020 in wet diets vs 1.051 in dry diets) and water excretion was 118% higher.

High protein: high protein consumption led to greater total water consumption and 30% higher urine excretion than high starch diets. However, the density was higher. As a final product of the catabolism of its amino acids, the protein generates urea, which promotes an increase in the tonicity of the glomerular filtrate, generating water retention with a greater volume of urine, but also with an increase in specific density[38]. There was a higher concentration of urea in the dry and dry high protein: high protein diets ($P<0.05$). Other studies corroborate these findings[39].

Hypercalciuria is a well-documented risk factor for CaOx urolithiasis in dogs and humans[40]. Lulich[27] evaluated calcium oxalate urolith-forming cats and they all had hypercalciuria. Results of several studies in humans suggest that increased protein consumption increases the risk for CaOx uroliths by promoting mobilization of calcium from bone, increasing glomerular filtration of calcium, and reducing renal tubular resorption of calcium[38,41]. Studies in felines diverge. Pablack[39] showed increased renal Ca concentration and excretion with reduced citrate in cats fed a higher protein diet (35 a 57% DM). Other authors did not detect differences in calcium and in citrate in high protein diets [41,42]. In this study, the use of high protein actually increased renal calcium excretion, but appear not affect the results obtained and did not change citrate content. However, in dry diets, the calcium intake concentration and renal excretion was higher and may have contributed to the results obtained ($P>0.05$).

The high-starch dry diet had the acidifying phosphoric acid as an extra ingredient, which reflected in greater phosphorus consumption, that was also higher in wet diets ($P<0.001$), and reflected in higher urinary concentration in high starch diets ($P<0.001$). Diets formulated to prevent struvite urolithiasis are low in phosphorus and magnesium contentes[44]. Phosphorus renal excretion was higher in wet diets but even so, it did not seem to contribute to the increase in struvite RSS. Results from a human study indicated that increased urinary phosphorus excretion reduced the risk of CaOx urolithiasis by increasing the urinary concentration of pyrophosphate, a crystal inhibitor of CaOx[40].

Supplemental magnesium has been reported to prevent CaOx urolithiasis in humans[45], the addition of reduced CaOx supersaturation of magnesium by combination with oxalic acid. There was higher urinary magnesium concentration and renal excretion in the wet and low-starch diets, in which we had lower saturation for calcium oxalate. Excessive magnesium consumption, on the other hand, can also be a risk factor for the formation of CaOx uroliths, causing hypercalciuria. High magnesium intake has been associated with increased urinary calcium excretion in humans, dogs, and cats[46]. Our results coincide with these findings; dry diets had the highest consumption of magnesium and had the highest excretion and concentration of calcium.

Increasing dietary sodium may seem inappropriate because sodium promotes urinary calcium excretion[47]. However, in one study, when cats consumed diets containing more sodium (0.91 g compared to 0.43 g/100 g of diet), CaOx supersaturation decreased although calcium excretion increased due to its dilution in urinary concentration[30]. In the present study, there was a higher sodium intake in wet and high-starch diets ($P<0.001$) but no increase in urinary calcium content was detected in these diets.

The goals of increasing water intake is produce greater amounts of urine, resulting in a reduction in the relative supersaturation (RSS) of urolith-forming minerals. According to Lekcharoensuk et al[41], cats fed wet diets are 1/3 likely to develop oxalate stones when compared to dry diets, which has also been observed in other species such as dogs and humans. The use of wet diets did indeed reduce the supersaturation for calcium oxalate and struvite. Wet diets also showed lower values of Hydroxyproline,calcium oxalate precursor. In high starch: low protein diets there was a higher RSS OxCa value ($P<0.05$), which coincides with previous studies[30], these diets had higher urinary concentration of calcium oxalate. An epidemiological study evaluating effects of dietary factors on the risk of CaOx uroliths in cats revealed that high-protein diets were less than half as likely to be associated with CaOx urolith formation as diets low in protein[41]. Citrate is protective for calcium oxalate formation, as it binds to calcium, leaving it unavailable to bind to oxalate[46]. Citrate values concentration were higher in dry diets ($P<0.001$).

The SSR values for struvite were higher for the dry diets and among them, the high protein dry diets ($P<0.001$). This coincides with the results found for magnesium concentration and urinary excretion, known to be a precursor of urolith, which were higher in such diets. Bijsmans[34] evaluated the influence of isonutrient diets with increased levels of acidifier. Struvite RSS decreased in diets with more acidic urinary pH (5.9) and CaOx RSS was not different ($P<0.05$). Despite the dry diet having the highest SSR value, it is still in the under saturated zone and the ph values obtained contributed to the low SSR struvite values.

Based on results it is concluded that elvated protein to starch ratio was effective to increase urine volume and even though increased Ca renal excretion it reduced oxalate and the RSS of calcium oxalate of the urine. Foods with more tham 80% moisture was effective to increase water intake and urine volume, reducing the density and urinary RSS of calcium oxalate and struvite

Material and Methods

Animals and experimental design

The experiment was performed at the Research Laboratory in Nutrition and Nutritional Diseases of Dogs and Cats “Prof. Dr. Flávio Prada”, Faculty of Agrarian and Veterinarian Sciences, São Paulo State University (UNESP), Jaboticabal . The experimental procedures were submitted to prior approval by the Ethics Committee on Animal Use (CEUA) (protocol number 3501/20).

Thirty-six mixed-breed cats, neutered, dewormed, immunized with polyvalent and anti-rabies vaccines, with 4.1±1.2 years old, and a mean body condition score (BCS) of 5.3±0,6 in a scale of 9 points[48], belonging to the cat colony of the Laboratory were used. To assess their health, before the study each cat was physically examined by a veterinarian, and hematological, serum biochemical profiles (albumin, phosphatase alkaline, alanine aminotransferase, urea and creatinine), and complete urine analysis was conducted, indicating good health.

The experiment was conducted in a completely randomized block design, with cats divide in 3 blocks of 12 animals, 3 cats eating each food on each block, totaling 9 cats per food. The blocking factor was period, due to the impossibility to test all cats at the same time. On each block, cats received the experimental diets for 18 days, distributed as follows: 10 days for adaptation, followed by 8 days of total collection of feces and urine to conduct water balance, determine the apparent digestibility of nutrients, and to obtain urine samples for chemical analysis. During the adaptation period, cats were restricted on individual cages during 16 hours (from 16:00 to 8:00) and kept free in a cattery for 8 hours to exercise and socialization (from 8:00 to 16:00). Cages were stainless steel with apparatus for separate collection of feces and urine (dimensions 0.65x0.80x0.65m). All cats interacted with people at least three times per day: to be housed in the cages and fed; to be released of the cage and to be brushed for 15 minutes. During the feces and urine collection period, the animals were restricted to their individual cages. Cages were daily washed, rinsed with distillated water and dried. To control food consumption, diets were only available when the animals were in theirs individual cages. Distillated water was provided ad libitum.

The food amount was individually calculated. To this the metabolizable energy (ME) content of the foods were estimated from their chemical composition and cats initially fed 75 kcal/kg$^{0.67}$/day[31]. The amount of offered food and leftovers were daily weighted and the intake calculated and recorded along the all period. The animals were weekly weighted at morning, before the first meal, and the amount of food offered adjusted to cats present constant body weight along the study.

Experimental Diets

The treatments were organized in a 2 x 2 factorial arrangement, constituted by 2 starch to protein ratios and 2 food moisture content, totaling 4 experimental diets. Four treatments were formulated to meet the nutrient requirements of adult cats[33]. Two treatments were formulated to present high starch (approximately 25%, in a dry matter basis) and moderate crude protein (approximately 36%) content, resulting in an elevated starch to protein ratio. The other two treatments were formulated to present high protein (approximately 52%), low starch (less than 15%) and low starch to protein ratio. Each formulation was further divided into two raw material sources and processing type: extruded dry food or canned high moisture pouches (wet food). The high protein dry food and the two wet diets were manufactured at ADIMAX Company (Salto de Pirapora, São Paulo, Brazil). The high protein dry food were manufactured at the Extrusion Laboratory of the Faculty of Agrarian and Veterinarian Sciences, São Paulo State University (UNESP), Jaboticabal campus.

Due to the strong influence of urine pH on RSS[9], before formulation the ingredients were purchased, stored, sampled, and analyzed for dry matter, crude protein, starch, Na, K, Ca, Mg, Cl, P and S. These analyzes were performed according to procedures recommended by AOAC[51]. Based on the obtained results, foods were formulated to meet the protein and starch objectives, and to present similar food base excess targeting an urine pH of the cats close to 6.2[11,27]. Food base excess was calculated considering the analyzed results of each raw material, according to the following equation: EB (mEq/kg of DM) = (49.9 x Ca) + (82.3 x Mg) + (43.5 x Na) + (25.6 x K) - (64.6 x P) - (62.4 x S) - (28.2 x Cl).

Foods were analyzed for dry matter, ash, crude protein, acid-hydrolyzed fat, starch, and crude fiber following recommended procedures of AOAC[49] The amino acid content of the diets was analyzed with the methods of White et al., (1986)[50] and Hagen et al., (1989)[51] by high-performance liquid chromatography and tryptophan analyzed according to Lucas & Sotelo (1980)[52] by and enzymatic method. The analyzed chemical composition of the experimental diets, as well as their ingredient list is presented in Table 1.

Digestibility and water balance

Total collection of feces and urine was carried out during 8 consecutive days to calculate water balance and the coefficients of total tract apparent digestibility of nutrients and energy. Feces were quantitative collected at least twice a day, weighed and kept frozen (-20°C). At the end of the collection period, feces were pooled, thawed and homogenized compounding a single sample per cat. Fecal samples and wet diets were dried in a forced-air oven at 55 ºC for 72 h. Pre-dried feces and diets were then ground in a cutting mill fitted with a 1mm screen sieve size for analysis. Moisture, crude protein, acid hydrolyzed fat, and starch were determined according to AOAC[49] proposed methodologies. The crude energy content was analyzed by a bomb calorimeter (IKA calorimeter, C200; IKA-Werke GmbH & Co.KG, Staufen, Germany).

Digestibility was calculated according recommendations of FEDIAF[31]. During collection, fecal samples were also scored according to the following system: 0 = watery liquid, which can be poured; 1 = soft, unformed; 2 = soft, malformed stool, which assumes shape of container; 3 = soft, formed, and moist, which retains shape; 4 = well-formed and consistent stool, which does not adhere to the floor; and 5 = hard, dry pellets, which are small and hard mass[18].

The water balance was determined computing water intake at the drinker, water intake via food, metabolic water generated in the metabolism, water excretion via feces, and water excretion via urine. Insensible water losses were estimated by the difference of the total water intake (drink, via food, and metabolic water) and the loses via urine and feces[14]. To this, the offered water and its leftovers were weighed, allowing the calculation of daily water intake. To quantify the water loss by evaporation at the drinker, one drinking bowl with water was kept in the same room of the cats, but without access of the animals, and the evaporative water estimated form the difference in weight at the start and end of the period. Water evaporative rate was discounted in the calculation of the daily water intake. The metabolic water was calculated multiplying the amount of digestible protein consumed by 0.396, digestible starch by 0.566 and digestible fat by 1.071 [14].

Urinary parameters and relative supersaturation for calcium oxalate and struvite

Urine was quantitative collected in plastic bottles placed under the metabolic cages during 8 consecutive days. In the first 5 days of collection 100 mg of thymol (Synth, LABSYNTH, Diadema – SP, Brasil) was added as a preservative at the bottles. Urine produced within 24 hours was homogenized and its volume measured, density determined in a digital refractometer (T2-Ne Clinical; ATAGO CO., LTD.; Fujita Yorii-cho Osato gun, Saitama, Japan) and pH in a pH meter (Digimed DM20; Digicrom Analítica, São Paulo, Brazil). The pooled urine samples were further divided into two aliquots for storage: an acidified urine sample, prepared by the addition of 1 mL of 6 N HCl for each 50 mL of urine and stored at -20ºC; and a non-acidified urine, which was frozen at -20ºC. In addition, a 1 mL aliquot of each urine sample (acidified and non-acidified) was stored at -80ºC for oxalate and citrate analyses. The concentration of calcium, phosphorus, magnesium, urea and oxalate were determined in the acidified urine sample. Sodium, potassium, chloride, sulfur, citrate and uric acid were analyzed in the non-acidified urine sample

Commercial kits were used to determine calcium (Cálcio Liquiform Ref. 90), phosphorus (Fósforo UV Liquiform Ref. 12), magnesium (Magnésio Ref. 50), uric acid (Ácido Úrico Liquiform Ref. 140), chloride (Cloretos Liquiform Ref. 115) and urea (Ureia Ref. 104). The kits were produced by Labtest Diagnóstica S.A (Lagoa Santa, Brazil), and the analysis conducted in a spectrophotometer (Labquest, Labtest Diagnóstica S.A, Lagoa Santa, Brazil). The urine concentrations of sodium and potassium were obtained by ion-selective electrodes (Roche Diagnostics, 9180, Indianapolis, USA). Sulfur was measured by the turbidimetric method AOAC[49] after sample digestion with nitro-perchloric solution, in a spectrophotometer (model B442, Micronal, São Paulo, SP, Brazil). Citrate and oxalate were analyzed by enzymatic and colorimetric determination (Citric Acid and Oxalic Acid, LTA, Bussero, Milan, Italy) in a semi-automatic spectrophotometer (Labquest, Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil).

To analyze ammonia on urine, during the last 3 days of total urine collection 1 mL of 1N H_2SO_4 solution was added to the collection bottles, and urine collected at least 3 times a day. The daily volume of urine was recorded, and urine filtered and frozen (-20ºC). The pooled urine sample was diluted in 1:4 (v:v) formic acid solution and stored at -20ºC for further analysis. Ammonia concentration was determined by the Kjedhal method AOAC[49] with titration in 2% boric acid solution.

Based on the results, the urine RSS for calcium oxalate was calculated using the software EQUIL-93 (Department of Biochemistry and Molecular Biology, University of Florida, USA) and the RSS for calcium oxalate and struvite using the software SUPERSAT (RSS calculator for pets, Mars, Incorporated and its Affiliates, updated version of the program developed by Robertson (1969).

Statistical Analysis

For statistical analysis, the error normality hypothesis was evaluated by the Cramer-von Mises test, and the homoscedasticity of variance was determined by the Levene test. The experimental unit was considered one cat. The data were analyzed in a randomized block design, with 3 blocks of 12 cats, 3 cats per food in each block and 9 cats (repetitions) per food in total. Treatments was organized in a factorial arrangement with 2 starch to protein ratios and 2 food moisture content, totaling 4 treatments. Data was submitted to analysis of variance, and the model sums of squares separated into the effects of starch to protein ratio, moisture content and their interactions. When differences were detected by F-test, means were compared with the Tukey test. The MIXED procedure of SAS software was used (version 9, SAS Inst. Inc., Cary, NC, USA). Values of $P<0.05$ was considered significant.

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