UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

CONSUMO DE HIDROXIPROLINA E AMIDO E SUPERSATURAÇÃO URINÁRIA PARA OXALATO DE CÁLCIO EM GATOS

Fernanda Sanches Mendonça

Zootecnista

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Orientador: Prof. Dr. Aulus Cavalieri Carciofi

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DADOS CURRICULARES DO AUTOR

FERNANDA SANCHES MENDONÇA – Nascida em 13 de abril de 1991, em Osvaldo Cruz – SP. Graduada em Zootecnia pela Universidade Estadual de Maringá (UEM), em janeiro de 2014. Deu inicio no Programa de Mestrado em Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp) em março de 2014 na área Nutrição de Cães e Gatos, orientada pelo professor doutor Aulus Cavalieri Carciofi.

"Podes dizer-me, por favor, que caminho devo seguir para sair daqui? – perguntou Alice

Isso depende muito de para onde queres ir - respondeu o gato

Preocupa-me pouco aonde ir - disse Alice

Nesse caso, pouco importa o caminho que sigas - replicou o gato."

(Lewis Carrol – Alice no País das Maravilhas)

Dedico

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SUMÁRIO

UNIVERSIDADE ESTADUAL PAULISTA
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Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 008106/14 do trabalho de pesquisa intitulado **"Consumo de Hidroxiprolina e Supersaturação Urinária para Oxalato de Cálcio em Gatos",** sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 06 de junho de 2014.

Jaboticabal, 06 de junho de 2014.

Prof.ª Dr.ª Paola Castro Moraes
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CONSUMO DE HIDROXIPROLINA E AMIDO E SUPERSATURAÇÃO URINÁRIA PARA OXALATO DE CÁLCIO EM GATOS

RESUMO – O presente estudo avaliou o efeito do consumo de dietas secas extrusadas com diferentes quantidades de amido, proteína e hidroxiprolina sobre o balanço hídrico, excreção renal de oxalato, composição química da urina e a supersaturação relativa da urina para oxalato de cálcio em gatos. Três dietas foram formuladas: Dieta AMIDO com 25% de proteína, 47% de amido e 0,77% de hidroxiprolina; dieta SOJA com 50% de proteína, 19% de amido e 0,94% de hidroxiprolina; dieta HIDROX com 50% de proteína, 21% de amido e 2,98% de hidroxiprolina. Foram utilizados 24 gatos, totalizando oito repetições por tratamento. Os animais foram submetidos a dez dias de adaptação, seguido por sete dias de coleta total de fezes e urina e um dia para coleta de sangue. Os dados foram submetidos à análise de variância e médias comparadas pelo teste de Tukey. Variáveis não paramétricas foram submetidas aos testes de Kruskal-Wallis e Dunn. De acordo com o proposto pelo experimento, foi verificado diferença nas ingestões de amido, proteína e hidroxiprolina. O volume urinário ($P<0,001$), ingestão de água de bebedouro ($P<0,001$) e total de água ingerido ($P=0,006$) foi menor nos gatos alimentados com AMIDO. Houve aumento na excreção renal de Cl, S, ácido úrico e uréia para animais das dietas SOJA e HIDROX, comparados com os da dieta AMIDO ($P<0,05$). A excreção de K foi menor em animais recebendo a dieta SOJA quando comparados com o tratamento HIDROX ($P=0,02$). Os animais tiveram aumento na concentração urinária de K e Mg ($P<0,01$) quando alimentados com o tratamento AMIDO e a uréia aumentou para dieta HIDROX. A concentração de oxalato na urina foi maior nas dietas AMIDO e HIDROX do que na dieta SOJA ($P<0,05$) e a supersaturação relativa para oxalato de cálcio aumentou com o elevado consumo de amido ($P=0,0372$). A alta ingestão de proteína aumentou o volume de urina devido seu papel na diluição urinária. Animais recebendo a dieta AMIDO tiveram maior SSR urinária OxCa devido a baixa excreção de urina e como consequência alta concentração de oxalato. O mesmo aconteceu para dieta HIDROX, exceto para excreção de urina. Concluiu-se que elevado consumo de hidroxiprolina promove aumento da SSR urinária OxCa em gatos e valores consideráveis para excreção renal de oxalato. E o alto consumo de amido influencia no balanço hídrico de gatos levando a um aumento na SSR OxCa.

Palavras-Chave: carboidrato, felino, pH, urina, urólito

HYDROXYPROLINE AND STARCH CONSUMPTION AND URINARY SUPERSATURATION FOR CALCIUM OXALATE IN CATS

ABSTRACT – The present study evaluated the effect of dry extruded food intake with different amounts of starch, protein and hydroxyproline on the water balance, renal oxalate excretion, urine chemical composition and the relative supersaturation of urine for calcium oxalate in cats. Three diets were formulated: Diet STARCH with 25% of crude protein, 47% of starch and 0,77% of hydroxyproline; diet SOY with 50% of crude protein, 19% of starch and 0,94% of hydroxyproline; diet HYDROX with 50% of crude protein, 21% of starch and 2,98% of hydroxyproline. Twenty-four cats were used, with eight repetitions per treatment. Animals were submitted to an adaptation period of ten days, followed by seven days for total feces and urine collection and one day for blood collection. Data were submitted to analysis of variance and means compared by Tukey test. Non-parametric variables were submitted to Kruskal-Wallis and Dunn tests. Following the purpose of experiment, cats had different starch, crude protein and hydroxyproline intake. The urinary volume ($P<0.001$), water intake via drinking bowl ($P<0.001$) and total water intake ($P=0.006$) was lower in cats fed STARCH. There was an increase in renal excretion of Cl, S, uric acid and urea for animals receiving SOY and HYDROX diets compared to STARCH diet ($P<0.05$). The excretion of K was lower for animals receiving SOY diet when compared to HIDROX treatment ($P=0.02$). Animals presented higher urinary concentration of K and Mg ($P<0.01$) when fed the STARCH diet and higher urea for diet HYDROX. Oxalate concentration in urine was higher in STARCH and HYDROX diets than the SOY diet ($P<0.05$) and relative supersaturation for calcium oxalate increase with high consumption of starch ($P=0.0372$). High protein intake increased the urine volume due the role of protein in urinary dilution. Animals receiving STARCH diet had higher urinary RSS CaOx due to the lower urine water excretion and as a consequence higher oxalate concentration. The same was observed for HYDROX-fed cats except for urine water excretion. In conclusion, high hydroxyproline consumption promotes the increase in urinary RSS CaOx in cats and considerable values for oxalate renal excretion. The high starch intake influences the water balance of cats leading to an augment in the RSS CaOx.

Key Words: carbohydrate, feline, pH, urine, urolith

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CAPÍTULO 1 – Considerações Gerais

1. INTRODUÇÃO

Os conceitos atuais de nutrição não estão englobando somente a questão de sobrevivência e satisfação da fome, estes se expandiram e ampliaram o foco para utilização de alimentos que promovam bem estar, melhora da saúde e redução do risco de doenças aos animais (FAHEY, 2003), com isso, as indústrias *Pet Food* tem crescido. Segundo dados da Associação Brasileira da Indústria de Produtos para Animais de Estimação (ABINPET), o Brasil está em segundo lugar no mundo, com crescimento de 10% em 2014 sobre 2013 e faturamento de 16,7 bilhões, atrás apenas dos Estados Unidos.

Os alimentos secos ganharam espaço no comércio em geral, por apresentarem características vantajosas como permitirem bom tempo de armazenamento, facilidade no transporte, conveniência no oferecimento aos animais, boa aceitação e apresentarem menor custo comparado a alimentos úmidos. Isto faz com que os proprietários, em sua maioria, prefiram o alimento seco. Apesar de não existirem dados oficiais, acredita-se que no Brasil mais de 95% do total produzido de rações para gatos sejam rações secas.

As rações secas, no entanto, têm apreciável quantidade de amido e umidade muito reduzida, normalmente inferior a 9%. O alto consumo de amido e a ingestão de pouca água via alimento têm sido estudados e incriminados como possíveis participantes da formação de urólitos de oxalato de cálcio em gatos (CARCIOFI et al., 2005). Estudos epidemiológicos antigos já relacionaram o consumo de alimentos secos como fator de risco para o aparecimento de doenças do trato urinário inferior em gatos (JACKSON, 1971; WALKER et al., 1977).

Rações secas para cães e gatos são produzidas por extrusão. Durante o processo de fabricação as fontes de amido são moídas e cozidas, etapas fundamentais para aumentar a digestibilidade do alimento para carnívoros (MURRAY et al., 2001). Felinos são capazes de digerir ingredientes amiláceos, com digestibilidade aparente superior a 95%, contanto que estes sejam processados adequadamente (DE-OLIVEIRA et al., 2008). No entanto, como a dieta natural dos

gatos é rica em proteínas e gorduras, com baixo carboidrato (ZORAN, 2002), verifica-se que o amido não é nutriente da dieta natural de felinos, o que tem criado polêmica à respeito de sua ingestão (KIENZLE, 1993, DIJCKER et al., 2011).

São muitos os fatores da dieta que representam possível risco para ocorrência de doença do trato urinário inferior em felinos, mais especificamente para a formação de urólitos. Estes incluem o teor de matéria seca, macrominerais, aminoácidos sulfurados, hidroxiprolina, amido, proteínas, fibras e oxalato (BARTGES; KIRK, 2012), que influenciam características importantes da urina como pH, volume, densidade, concentração de oxalato, citrato, cálcio, magnésio, fósforo e, desta forma sua supersaturação relativa (JEREMIAS, 2013). Verifica-se que muitos outros fatores do alimento são potencialmente importantes, além da umidade (ração seca x úmida) e teor de carboidratos.

Produtos de origem animal com alto colágeno possuem elevadas quantidades do aminoácido hidroxiprolina, considerado em estudos como fator de risco na formação de urólitos de oxalato de cálcio em felinos. Estudo com ratos mostrou que a excreção urinária de oxalato e glicolato foram aumentadas quando administrada alta dose de L-hidroxiprolina aos animais, pois esta é precursora do glioxilato que, nas mitocôndrias, pode ser oxidado gerando oxalato (TAKAYAMA et al., 2003). Assim, o teor e fonte de proteínas podem também ser importantes, especificamente sua composição em prolina, hidroxiprolina, glicina, serina, fenilalanina, tirosina e triptofano, aminoácidos com potencial participação na formação endógena e excreção urinária de oxalato (DIJCKER et al, 2011).

2. REVISÃO DE LITERATURA

2.1. Urolitíase por Oxalato de Cálcio

A Doença do trato urinário inferior em felinos (DTUIF) é um conjunto de sintomas clínicos com causa multifatorial. Dentre as principais causas estão urolitíase, cistite idiopática felina (CIF) e plug uretral (FORRESTER; ROUDEBUSH, 2007). As causas relacionadas à formação de cristais e urólitos representam de 15-45 % dos casos, consequentes à precipitação de minerais no trato urinário (BARTGES; KIRK, 2012).

A urolitíase é um processo multifacetado que inicia com a formação de microcristais na urina e termina com o urólito maduro no trato urinário (OSBORNE; KRUGER, 1984). É raramente diagnosticada em animais com menos de um ano, sendo a idade de quatro anos a de maior incidência. Em geral, animais com urólitos de estruvita tendem a ser mais jovens e com urolitos de oxalato de cálcio mais velhos (CANNON et al., 2007). 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).

Fator relativamente melhor elucidado em relação à sua ocorrência é a composição mineral da dieta, pois o equilíbrio de minerais influencia significativamente o pH pós-prandial da urina e pode, portanto, predispor gatos a desenvolver a forma de DTUIF relativa à formação de cristais ou urólitos (KIENZLE, et al., 1991; JEREMIAS et al., 2013). Os urólitos, compostos de partículas minerais macroscópicas e pequenas quantidades de matriz, são formados em duas fases complementares: iniciação, com a formação de microcristais; crescimento, com a agregação dos cristais (ALLEN; KRUGER, 2000; OSBORNE; LULICH; ULRICH, 2010). Estes são nomeados de acordo com sua composição mineral, normalmente um tipo de mineral é predominante, porém alguns urólitos podem ser formados por mistura de minerais (FORRESTER et al., 2010). Os tipos de urólitos mais comuns em gatos são de estruvita (fosfato amônio magnesiano) e oxalato de cálcio (HOUSTON et al., 2003). Em geral, 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 1981 a maior parte de urólitos observados (78%) era de estruvita, enquanto apenas 2% era de oxalato de cálcio. Porém, com a utilização de métodos de prevenção dos urólitos de estruvita, como a formulação de alimentos que promovem acidificação do pH urinário, aumentou a proporção relativa de oxalato de cálcio (FORRESTER et al., 2010).

2.2. Supersaturação Relativa (SSR) Urinária

A SSR da urina é um método de se prever a chance ou risco de agregação mineral em uma amostra de urina com base na sua composição química, mais

especificamente a concentração de minerais e metabólitos envolvidos na agregação dos urólitos, bem como o pH urinário.

A técnica de avaliação da saturação urinária foi inicialmente desenvolvida para seres humanos e posteriormente adaptada para cães e gatos. O valor da supersaturação relativa (SSR) incorpora todos os parâmetros que influenciam a probabilidade da formação de um tipo particular de urólito e as possíveis interações entre eles (HURLEY; STEVENSON; WATSON, 2003).

O estudo da supersaturação urinária é essencial quando se trata de urolitíase, já que muitas pesquisas relacionam a saturação da urina com formação de cristais (ROBERTSON et al., 2002; HOUSTON et al., 2011). O grau de saturação urinária é influenciado por muitos fatores, como a concentração de solutos presentes na urina, pH, força iônica, temperatura, ingestão e excreção de água e presença de complexos químicos pré-formados (ALLEN; KRUGER, 2000). O aumento da concentração de substâncias calculogênicas, a exemplo do oxalato e do cálcio, com consequente aumento da supersaturação urinária, unindo-se a pH urinário fora da faixa ideal, são os eventos mais importantes na formação dos cálculos (ALLEN; KRUGER, 2000; HOSTUTLER; CHEW; DIBARTOLA, 2005).

Para mensuração da SSR urinária, é necessária a obtenção das concentrações urinárias de cálcio, fósforo, potássio, magnésio, cloro, sódio, enxofre, ácido úrico, oxalato, citrato e pH da urina. Estas informações abastecem um software que aponta o risco de formação ou não de urólitos, possibilitando definir a SSR da urina em três zonas. 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, porém, estes 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 da urina altamente propícia à formação, agregação e crescimento de cristais homogêneos. Os valores de SSR correspondentes com a 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).

2.3. Carboidratos como precursores de oxalato

Segundo Dijcker et al. (2011), a via metabólica do glioxalato é a principal responsável pela síntese endógena de oxalato. Esta síntese ocorre predominantemente no fígado e é dependente do glioxilato presente nos hepatócitos. Quando o glioxalato está disponível e não sofre redução a glicolato pela enzima glioxilato redutase/hidroxipiruvato redutase (GR/HPR), ou então não é transaminado a glicina pela ação da enzima alanina:glioxilato aminotransferase (AGT1), será 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). Como o oxalato é produto residual final, sem nenhuma função específica no organismo e prejudicial quando em excesso, a transaminação do glioxilato a glicina é extremamente importante. Seguindo esta via ocorrerá formação do aminoácido glicina ao invés do produto final oxalato. Essa via é dependente da enzima AGT1, que em felinos está presente somente na mitocôndria. Isso pode ser explicado devido ao hábito alimentar dos carnívoros. Diferentemente dos herbívoros, que necessitam dessa via bem desenvolvida para evitar a oxidação à oxalato, devido ao baixo consumo de carboidratos e glicolato pelos felinos, durante o processo evolutivo provavelmente estes dispensaram a existência de sistema de detoxificação do glioxilato nos peroxissomos (DANPURE et al., 1990; DIJCKER et al., 2011).

Assim, se especula que o consumo de carboidratos pelos felinos alimentados com rações secas possa aumentar a produção e excreção renal de oxalato, representando fator de risco à formação de urólitos de oxalato de cálcio nestes animais. Isto se baseia no fato dos carboidratos serem precursores do glioxilato, que por sua vez será convertido em glicolato que terá aumento de concentração no citoplasma do hepatócito dos gatos. Este glicolato em excesso no citoplasma pode ser reduzido a glioxilato e oxidado a oxalato pela L-LDH ou ainda pode entrar no peroxissomos e sofrer a ação da glicolato desidrogenase (GD) e ser convertido a oxalato por não haver meios para promover sua detoxificação nesta organela

(RIBAYA-MERCADO; GERSHOFF, 1984; NGUYEN et al., 1995; DIJCKER et al., 2011).

2.4. Hidroxiprolina como precursora de oxalato

A hidroxiprolina é produto resultante da hidroxilação da prolina, componente estrutural importante do colágeno (MOTTA, 2003). Segundo Neuman e Logan (1950), o teor de hidroxiprolina no colágeno é de 10 a 14%. Os principais locais de degradação da hidroxiprolina são os rins e fígado, principalmente nas mitocôndrias onde estão as enzimas envolvidas na conversão de hidroxiprolina para glioxilato. Este processo de degradação envolve a ação passo a passo de hidroxiprolina oxidase (HPOX), Δ1-pirrolina-5-carboxilato desidrogenase (1P5CDH), aspartato aminotransferase (Aspat) e 4-hidroxi-2-oxoglutarato aldolase (HOGA). Por meio de clivagem tipo aldol ocorre à formação do glioxilato e piruvato (VALLE et al., 1979; ADAMS; FRANK, 1980; PHANG; HU; VALLE, 2001; TAKAYAMA et al., 2003). Este glioxilato formado pela degradação da hidroxiprolina, em condições normais participará da gliconeogenese sendo transaminado pela ação da AGT1 em L-Glicina, L-serina, hidroxipiruvato e açúcares (D-Galactose, D-Frutose, D-Glicose), ou, quando em excesso pode extravasar a mitocôndria e servir como substrato para a glioxilato redutase e L-LDH, resultando na formação de oxalato que será, posteriormente, excretado na urina (TAKAYAMA et al., 2003; JIANG et al., 2012).

Desta forma, a ingestão de hidroxiprolina, presente em proteínas de origem animal de baixa qualidade e ricas em colágeno, é potencialmente importante fator contribuinte para geração endógena e excreção urinária do composto. Em estudo comparando dietas com colágeno, carne de cavalo e proteína isolada de soja para gatos foi verificado aumento na excreção de oxalato nos animais que consumiram a dieta com colágeno, tendo como explicação a elevada quantidade de hidroxiprolina deste ingrediente (ZENTEK; SCHULZ, 2004). Outro estudo mais recente, também com gatos reforçou esta hipótese ao avaliar a influência da ingestão de oxalato e hidroxiprolina, tendo verificado que o oxalato alimentar é muito pouco disponível sendo excretado pelas fezes, enquanto o consumo de hidroxiprolina foi capaz de elevar a excreção urinária de oxalato dos gatos (DIJCKER et al., 2012). Isso pode ser explicado devido à possibilidade da hidroxiprolina oriunda de produtos de origem

animal com alto colágeno ser altamente disponível para absorção intestinal (ZENTEK et al., 2004; DIJCKER et al., 2012).

3. OBJETIVO GERAL

Diante do exposto, o presente estudo avaliou, em dietas secas extrusadas para gatos, diferentes teores de amido, proteína e hidroxiprolina sobre o balanço hídrico, excreção urinária de oxalato e a supersaturação relativa da urina para oxalato de cálcio. Para isto foram comparadas três dietas, uma com elevado amido e baixas proteína e hidroxiprolina, uma com elevada proteína e baixa hidroxiprolina e amido e outra com elevadas proteína e hidroxiprolina e baixo amido.

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CAPÍTULO 2 – Hydroxyproline and starch consumption and urinary supersaturation for calcium oxalate in cats[1]

[1] Escrito de acordo com as normas da Revista Journal of Animal Science

Running head: Hydroxyproline, starch, and oxalate in cats

Hydroxyproline and starch consumption and urinary supersaturation for calcium oxalate in cats[1, 2]

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ABSTRACT: The present study evaluated the effect of dry extruded food intake with different contents of starch, protein and hydroxyproline on the water balance, renal oxalate excretion, urine chemical composition and the relative supersaturation of urine for calcium oxalate (RSS CaOx) in cats. Three diets were formulated with different proportions of starch, protein and hydroxyproline. Diet STARCH with 25% of crude protein, 47% of starch and 0.77% of hydroxyproline; diet SOY with 50% of crude protein, 19% of starch and 0.94% of hydroxyproline; diet HYDROX with 50% of crude protein, 21% of starch and 2.98% of hydroxyproline. Twenty-four cats were used, with eight repetitions per treatment. Animals were submitted to an adaptation period of ten days, followed by seven days for total feces and urine collection and one day for blood collection. Data were submitted to analysis of variance and means compared by Tukey test. Non-normal data were analyzed using non-parametric tests, Kruskal-Wallis and Dunn. Cats had different starch, crude protein and hydroxyproline intake. The urinary volume (P<0.001), water intake via drinking bowl (P<0.001) and total water intake (P=0.006) reduced when cats were fed higher starch and increased when fed higher hydroxyproline. There was an increase in renal excretion of Cl, S, uric acid and urea for animals receiving SOY and HYDROX diets compared to STARCH diet (P<0.05). The excretion of K was lower for animals receiving SOY diet when compared to HIDROX treatment (P=0.02). Animals presented higher urinary concentration of K and Mg (P<0.01) when fed STARCH diet and higher urea for HYDROX diet. Oxalate concentration was higher in STARCH and HYDROX diets than the SOY diet (P<0.05) and RSS CaOx increase with high consumption of starch (P=0.0372). High protein intake increased the urine volume due the role of protein in urinary dilution. Animals receiving STARCH diet had higher urinary RSS CaOx due to the lower urine water excretion and as a consequence higher oxalate concentration. The same was observed for HYDROX-fed cats except for urine water

49 excretion. In conclusion, high hydroxyproline consumption promotes the increase in urinary
50 RSS CaOx in cats and considerable values for oxalate renal excretion. The high starch intake
51 influences the water balance of cats leading to an augment in the RSS CaOx.

52 **Keywords:** carbohydrate, feline, pH, urine, urolith

INTRODUCTION

Urolithiasis is a multifaceted process, which starts up with microcrystals and subsequent mature urolith formation in the urinary tract (Osborne et al., 2010). Calcium oxalate and struvite are the most common uroliths in cats, and the incidence of calcium oxalate uroliths has been increasing over the struvite in the last years (Houston et al., 2003; Forrester et al., 2010). Interest exist on the comprehension of the reasons of this proportional increase and on nutritional alternatives to reduce the chance of calcium oxalate formation (Lekcharoensuk et al., 2001). Oxalate is a final product of metabolism excreted in the urine. When glyoxylate is available on hepatocytes and is not reduced to glycolate or transaminated to glycine, it is oxidized to oxalate (Behnam et al., 2006). Starch and hydroxyproline are the most probable precursors of glyoxylate in feline hepatocytes (Dijcker et al., 2011).

Most cats nowadays ingest dry extruded foods, which has variable amounts of starch. It has been speculated a relation between dry diet intake and calcium oxalate stones (Walker et al., 1977). The main postulated causes might be a reduced water intake and urine output (Carciofi et al., 2005; Buckley et al., 2011), and increased sugars intake (Ribaya-Mercado and Gershoff, 1984). It is possible that an increased starch consumption with high glucose absorption increases glycolate and oxalate production in feline hepatocyte (Dijcker et al., 2011). Hydroxyproline is a non-essential amino acid found on collagen rich animal proteins (Neuman and Logan, 1950). Collagen rich protein intake was also associated with increased glyoxylate and oxalate hepatic production (Takayama et al, 2003) with higher renal oxalate excretion in cats (Zentek et al., 2004; Dijcker et al., 2012). Considering this, the present study evaluated extruded diets with different proportions of starch, protein, and hydroxyproline on water balance, renal oxalate excretion, and urine supersaturation for calcium oxalate in cats.

MATERIALS AND METHODS

The Ethics Committee on Animal Use of the Faculdade de Ciências Agrárias e Veterinárias, UNESP - Univ Estadual Paulista, Campus Jaboticabal, approved all experimental procedures (CEUA, protocol n°008106/14).

Animals and experimental design

Twenty-four mixed-breed, neutered, male or female cats, with 10.44 ± 2.1 years old, 5.17 ± 1.01 kg of body weight, and body condition score (Laflamme, 1997) of 5.56±1.4 were used. Before study, physical and hematologic examination, serum biochemical profiles (albumin, phosphatase alkaline, alanine aminotransferase, urea, and creatinine), and complete urine analysis indicate good health. Animals belonged to Laboratory of Research in Nutrition and Nutritional Diseases of Dogs and Cats, at Faculdade de Ciências Agrárias e Veterinárias, UNESP - Univ Estadual Paulista, Campus Jaboticabal, Jaboticabal, Brazil.

The experiment was conducted in a completely randomized design with 3 treatments and 8 repetitions (cats) per treatment. Animals received the experimental diets for 17 days, the first 10 days for adaptation, followed by 7 days for total feces and urine collection. On the last day blood samples were taken from all cats. During the adaptation period, cats were individually kept during 16 hours (from 1600 to 0800) restrained in 0.9 x 0.8 x 0.9 m individual stainless steel cages. From 0800 to 1600 animals remained in a collective cattery for exercise and socialization. During the fecal collection period, cats were restricted all day on their individual cages. Cages were daily washed with distillated water and dried.

Food were individually provided when animals were in the cages. The ME content of the foods were estimated from their chemical composition, and the delivered amount calculated as 100 kcal x kg $BW^{0.67}$ (NRC, 2006). The amount of offered food and leftovers

were daily weighted and the intake calculated. Distillated water was available *ad libitum*. Cats were kept with 12:12 light: dark cycle under artificial and natural illumination.

Experimental diets

Three diets were formulated to meet the nutrient requirements for adult cats (NRC, 2006). Each diet had different proportions of starch, protein and hydroxyproline. The high starch diet (**STARCH**) was formulated with corn and poultry by-product meal as the main ingredients, with low amounts of protein and hydroxyproline. The high protein low hydroxyproline diet (**SOY**) was formulated with soy-isolated protein, a vegetable protein source with low hydroxyproline, which replaced corn in the formulation reducing starch content. The high hydroxyproline diet (**HYDROX**) was formulated with swine-isolated protein, an animal protein source rich in collagen, that replaced corn reducing starch content (Table 1). The chemical compositions of diets are presented on Table 2.

Foods were prepared at the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ. Estadual Paulista. All diets were mixed and ground with a hammer mill (Moinhos Tigre, São Paulo, Brasil) fitted with a 0.6-mm screen sieve size before being extruded in a single-screw extruder with a processing capacity of 250 kg/h (MEX 250, Manzoni, Campinas, Brasil). During the extrusion process the extruder motor amperage were constantly monitored and recorded each ten minutes. After the extrusion system reached the stability, bulk density (g/L) was determined every 10 minutes. The preconditioner temperature was kept over 90ºC through direct steam injection. In order to have good processed foods, water and steam additions in the extruder screw were adjusted according to each diet. The feed rate and extruder screw speed were kept constant. After extrusion, kibbles were dried in a forced air dryer at 105°C and coated with fat and palatability enhancers.

Urinary parameters and relative supersaturation for calcium oxalate

During 5 consecutive days (120 h), urine was quantitatively collected at least 3 times a day. Urine was collected in plastic bottles with 100 mg of thymol as a preservative, and kept under refrigeration (4ºC). Each 24h-pooled urine sample of a cat was homogenized and its volume, density, and pH determined. Density was measured using a refractometer (ATACO, Japan) and pH using a pH-meter (Digimed DM20, Digicrom Analítica, São Paulo, Brasil). The pooled urine sample was further divided in 2 aliquots for storage: acidified urine sample – composed by the addition of 1 mL of 6N HCl for each 50 mL of urine, and stored at -20ºC; and non acidified urine – the composed sample was frozen at -20ºC. Additional 0.5 ml aliquot of each urine was storage at -80ºC for oxalate and citrate analysis. Before analysis, urine samples were thawed to room temperature and homogenized by cat. The concentration of calcium, phosphorus, magnesium and oxalate were determined in the acidified urine sample. Sodium, potassium, chloride, sulfur, citrate, creatinine, uric acid and urea were analyzed in the non-acidified urine sample.

Commercial kits were used to determine calcium, phosphorus, magnesium, creatinine, uric acid, chloride and urea (respectively Ref. 90; Ref. 12; Ref. 50; Ref. 96; Ref. 140; Ref. 49; Ref. 104; Labquest, Labtest Diagnóstica S.A, Lagoa Santa, Brazil), using a spectrophotometer (Labquest, Labtest Diagnóstica S.A, Lagoa Santa, Brazil). The urinary concentrations of sodium and potassium were obtained by ion-selective electrodes (Roche Diagnostics, 9180, Indianapolis, USA). Sulfur was measured by the turbidimetric method AOAC (2010) after sample digestion with nitro-perchloric solution, in a spectrophotometer (model B442, Micronal, São Paulo, SP, Brazil).

Urinary citrate was analyzed by citrate lyase, an enzymatic method (Citrate, Biovision, South Milpitas Boulevard, Milpitas, CA, USA). Oxalate was obtained

149 through the enzymatic and colorimetric determination of oxalic acid (OXALATE Enzymatic
150 Assay kit, Libios, Bully, France). The measured parameters were used to calculate the index
151 of relative supersaturation of calcium oxalate (**RSS CaOx**) in the urine using the EQUIL-93
152 software (Department of Biochemistry and Molecular Biology, University of Florida, USA).
153 Macroelements intake and renal excretion was calculated considering, respectively, the diet
154 composition and ingested amount, and the daily urine production and chemical composition.
155
156 ***Oxalate fractional excretion***
157 The fractional excretion of oxalate was calculated using the values of oxalate and
158 creatinine obtained in the urine and blood plasma. Blood samples were taken in the last day
159 (day 17) by direct jugular puncture after local antisepsis, using Na-heparin as anticoagulant.
160 Samples were centrifuged at 2.200 G at 4ºC for ten minutes (Centrifuge 5810R, Eppendorf,
161 Hamburg, Germany) and plasma was obtained. Plasma samples was aliquoted in acidified
162 plasma (10 µL of 6N HCl for 1 mL of plasma; pH 2.3 – 2.7) for oxalate determination or non-
163 acidified plasma for creatinine analysis, and frozen at -80 ºC. Oxalate and creatinine in blood
164 plasma were determined as described for the urine samples.
165 Based on obtained resulted, the fractional excretion of oxalate was calculated
166 according to the equation (Lefebvre et al., 2008):

167 $$\text{Fractional excretion (\%)} = \frac{U_{ox} \text{ X } S_{cr}}{S_{ox} \text{ X } U_{Cr}} \text{ X } 100$$

168 Where: U_{ox} is the urinary concentration of oxalate, S_{cr} is the plasma concentration of
169 creatinine, S_{ox} is the plasma concentration of oxalate, and U_{cr} is the urinary concentration of
170 creatinine.
171 ***Digestibility protocol, nitrogen balance and water balance***

The digestibility assay was carried out through the quantitative collection of feces and urine method, following the recommendations of AAFCO (2013). During 7 days (168 h) feces were collected at least twice a day, weighed and frozen (-20°C). Fecal samples were 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 (Carciofi et al, 2008).

For nitrogen balance determination, urine was collected twice a day for 2 days (48 h) in plastic bottles containing 1 mL of H_2SO_4 (1N). Urine volume was recorded, and urine filtered and frozen (-20ºC). This urine collection occurred on days 6 and 7 of the collection period, after the first 5 days of urine collection for RSS CaOx calculation.

At the end of the collection period, feces and urine were thawed, homogenized, and pooled by cat. Before analysis, fecal samples were dried in a forced-air oven (Fanem, São Paulo, Brazil) at 55 ºC for 72 h. Feces and diets were ground in a cutting mill (MOD 340, ART LAB, São Paulo, Brazil) fitted with a 0.8-mm screen sieve size, and analyzed according to AOAC (2010) for dry matter with oven-drying of the sample (934.01), converting to ash with muffle furnace incineration (942.05), measuring the crude protein with the Kjeldahl method (954.01), and measuring the acid-hydrolyzed fat (954.02). The organic matter (OM) was calculated by difference (OM = DM – ash). Dietary fiber was measured on foods by using a combination of enzymatic and gravimetric procedures (method 991.43, AOAC, 2010). Starch was determined according to Hendrix (1993). Gross energy (**GE**) of diets, urine and feces were determined by an adiabatic bomb calorimeter (1281, PARR Instruments, USA). All samples were analyzed in duplicate and repeated when the variation was greater than 5%

between replicates. With the obtained results, the total tract apparent digestibility coefficients of DM, OM, CP, acid-hydrolyzed fat (**AHF**), GE, and starch, as well the food ME content were calculated (Pond et al., 2005). Nitrogen balance was calculated using the ingestion and excretion data obtained during the digestibility trial, by the difference between total nitrogen intake and nitrogen excreted in feces and urine.

Diets were also analyzed for macroelements and amino acids. Extracts from food were prepared by wet digestion with nitro-perchloric solution. The extract of chloride was obtained by dry digestion (AOAC, 2010). Ca, Mg, chloride, Na and K were analyzed through an atomic-absorption spectrophotometer (Model GBC-932 AA, Scientific Equipment PTY LTD, Melbourne, Australia). Sulfur, as sulfate, was analyzed using turbidimetric method, and phosphorus was determined through vanadate-molibdate method, both using a spectrophotometer (model B442, Micronal, Brazil) (AOAC, 2010). Total amino acids was evaluated in the laboratory of Ajinomoto do Brasil by high-performance liquid chromatography (Chromatography model Shimadzu LC-10A, Shimadzu Corporation, Kyoto, Japan) using fluorescence after ninhydrin staining according to Compendium of Animal Nutrition (Compêndio Brasileiro de Alimentação Animal, 2009).

During the collection phase, the offered water and its leftovers after 24 h were weighed, allowing the calculation of daily water intake of each cat. One drinking bowl with water was kept in the same room of the cats to quantify the evaporative water loss, which was discounted in the calculation of the daily water intake. The water balance calculation included the water intake via cat food, the water intake via drinking bowl, the calculation of metabolic water, the total water intake (sum of the first three mentioned parameters), the water excretion via feces, the water excretion via urine, the insensible water losses, and the total water excretion (sum of the three previously mentioned parameters). The produced metabolic water

was calculated by multiplying the amount of digestible protein consumed by 0.396, the digestible starch by 0.566 and the digestible fat by 1.071 (Buffington and Chew, 1999). The insensible losses consisted of the sum of skin, breathing and salivary losses, calculated indirectly by the difference among the total water intake and the sum of feces and urine water excretion (Carciofi et al., 2005).

Statistical analysis

The data were analyzed as a completely randomized design. The experimental unit was 1 cat. Data were tested for homogeneity of variances and normality of errors, and then, submitted to analysis of variance. The model sums of squares were separated into diet and animal effects and the interactions. When differences were detected on F test, means were compared by the Tukey test. Data that not comply with the analysis of variance assumptions (starch digestibility, urine density, plasma oxalate and sodium concentration on urine) were submitted to Kruskal-Wallis and Dunn test. Values of $P < 0.05$ were considered significant and trend at $P < 0.1$. All statistical analysis were conducted using the R software Version 3.1.2 (R Core Team 2014, Vienna, Austria).

RESULTS

Cats had an adequate food intake throughout the experiment, all animals remained health with no adverse reaction to experimental diets consumptions. There were no changes in body weight (data not shown) nor differences in food DM intake among diets ($P > 0.05$). Following the purpose of experiment, cats had different starch, protein, and hydroxyproline intake according to diet (Table 3). With the exception of starch, whose digestibility was similar, all nutrients digestibility were

lower for the STARCH diet than the other diets ($P < 0.01$), resulting in higher ME for the SOY and HYDROX diets ($P < 0.001$). Fat digestibility, however, was an exception as it digestibility was higher only for the HYDROX ($P < 0.001$), with the others presenting similar values. The nitrogen balance was positive with no differences among treatments (P=0.556).

The urine pH was higher for SOY-fed cats compared to the other two treatments (P=0.004), but urine density was similar among diets (Table 4). There was no difference on feces output and DM content, but the fecal score was lower for the HYDROX diet (P=0.039).On the water balance, cats fed STARCH ingested less drinking water, resulting in lower total water intake for this diet ($P < 0.01$). Cats fed STARCH diet also produced less urine than the cats fed the other diets ($P < 0.001$).

No difference among diets was verified for the urine concentration, intake and renal excretion of Ca (Table 5). The urinary concentration and intake of Mg were higher for animals fed STARCH diet ($P<0.01$) and there was a trend for lower Mg renal excretion for the SOY diet ($P = 0.050$). Urine concentration of Cl and S did not change among treatments, but renal excretion on both increased in high protein diets ($P<0.01$) and the intake of S was lower for STARCH diet. Cats fed HYDROX diet presented high Cl consumption and the STARCH diet had the lowest value ($P<0.001$). SOY-fed cats had higher intake and excretion of Na, with intermediary values for HYDROX-fed cats ($P < 0.001$); however there were no differences among diets for Na urine concentration. The excretion of K was lower for animals fed SOY than HIDROX diet ($P = 0.022$), the urine concentration increased for STARCH diet without differences on intake. The renal excretion of P was lower for cats fed STARCH in comparison with SOY diet ($P = 0.024$), but its consumption was higher for SOY diet compared with HYDROX diet .

The RSS CaOx was higher for animals fed STARCH and HYDROX diets and lower for cats fed SOY diet (P = <0.001; Table 6). Oxalate urine concentration was higher for STARCH and HYDROX than for cats fed SOY (P < 0.001). There was a trend for higher oxalate renal excretion on cats fed HYDROX in comparison with SOY (P = 0.109). No difference among diets was verified for citrate (P>0.05). Urea urine concentration and renal excretion increased on animals fed with high protein (P < 0.01). Urinary concentration of uric acid did not change among treatments, but the renal excretion increased with high protein intake (P<0.001).

Oxalate plasma concentration was similar among diets with values of 3.0±0.38 mg/dL, 3.3±0.94 mg/dL and 3.2±1.16 mg/dL for cats fed STARCH, SOY and HYDROX, respectively (P=0.611). A trend for higher oxalate fractional excretion was verified for cats fed STARCH diet (0.050±0.044), than SOY (0.024±0.021) or HYDROX (0.021±0.009) foods (P = 0.072).

DISCUSSION

Considering the hypothesis of the present study, that the consumption of dry extruded diets with high hydroxyproline and starch content may increase the endogenous formation of oxalate, three foods were formulated with different content of starch, protein and hydroxyproline. The amino acid composition of high starch and low protein diet was in accordance with the recommended values for adult cats by NRC (2006). Animals did not show any differences on nitrogen balance among experimental diets that evidences the proper amino acids composition in all treatments.

Diets with high protein content (SOY and HYDROX) had higher apparent digestibility values compared to the high-starch diet, which may be explained by better digestibility of

soy-isolated protein and swine-isolated protein for cats. However, the chemical composition of diets was not determinant for the differences among treatments, since the starch digestibility was equal among them. Protein digestibility were higher for SOY and HYDROX diets due to the high protein intake in these treatments, even though there were no differences for DM intake. When animal has lower nutrient intake, the endogenous losses become more significant compared to an animal that has higher nutrient intake. The true digestibility was not evaluated in the experiment, only the apparent digestibility; because of this, the nutrient endogenous losses could not be measured.

Protein has been considered as an important diuretic agent for cats in some studies, because it promotes an augment in the urea endogenous production, which in the glomerular filtrate induces increase of tonus, urine osmolality and water retention leading to higher volume of urinary excretion (Hawthome et al., 2004; Paßlack et al., 2014). With the present study, the role of protein in urinary dilution could be confirmed, once animals that consumed the high protein diets had higher urea production and urine volume. In contrast, STARCH-fed cats had lower water ingestion via drinking bowl and total water intake; as they had lower excreted urine volume, turning urine more concentrated with some minerals and oxalate.

The chemical composition of diets was different considering the nutrients and minerals. Renal excretion of Na, Cl, P, S, uric acid and urea had lower or higher values among treatments depending on their consumption. In addition to the lower urine volume from animals receiving STARCH diet, the urinary concentration of K and the oxalate metabolite increased, resulting in higher urinary RSS. The urinary RSS with the calculogenic substances, as oxalate and calcium, is one of the major factors to be considered for crystals formation (Allen and Kruger, 2000; Hostutler et al., 2005). Cats fed the HYDROX diet had intermediate values for urinary RSS, which is explained by the higher concentration of

315 oxalate and lower urinary pH. The lower pH can promote the increase of urinary saturation
316 for calcium oxalate. However, the urine in STARCH and HYDROX diets remained in the
317 undersaturated zone, presenting no risk of new crystals formation.

318 The concentration of oxalate also increased for HYDROX diet, even with the higher
319 urine volume, which led towards to the hypothesis of the study, the higher concentration of
320 oxalate is due to the endogenous oxalate formation. This is reinforced by the tendency of
321 higher oxalate renal excretion in the HYDROX diet. The degradation of hydroxyproline
322 occurs in the liver and kidneys, mainly in the mitochondria where the enzymes involved in the
323 conversion of hydroxyproline to glyoxylate are placed. This metabolic process is catalyzed by
324 hydroxyproline oxidase (HPOX), Δ1-pyrrolini-5-carboxylate dehydrogenase (1P5CDH),
325 aspartate aminotransferase (Aspat) and 4-hydroxy-2-oxoglutarate aldolase (HOGA); through
326 aldol-type cleavage (Valle et al., 1979; Adams et al., 1980; Phang et al., 2001; Takayama et
327 al., 2003). In normal conditions, the glyoxylate participates in gluconeogenesis, it is converted
328 to L-glycine by the alanine:amino-transferase-1, and then, to L-serine, hydroxypyruvate and
329 carbohydrates. When it is in excess the glyoxylate over pass the mitochondria and it is
330 substrate for glyoxylate-reductase and L-lactate dehydrogenase resulting in oxalate formation
331 and excreted in urine (Takayama et al., 2003; Jiang et al., 2012). In a study comparing diets
332 with collagen, horsemeat and soy-isolated protein, collagen-fed cats had higher oxalate
333 excretion, which was explained by the hydroxyproline concentration in this ingredient
334 (Zentek et al, 2004). This hypothesis was strengthened in other study with cats where the
335 influence of oxalate or hydroxyproline intake was evaluated; the feed oxalate was few
336 available for cats and it was excreted in feces, while the hydroxyproline was able to increase
337 the urinary excretion of oxalate in cats (Dijcker et al., 2012). One of the explanation is that the

hydroxyproline from animal origin products with high collagen is highly available for intestinal absorption (Zentek et al., 2004; Dijcker et al., 2012).

In conclusion, high starch intake (or low protein consumption) reduces urine production increasing urine oxalate concentration and relative supersaturation for calcium oxalate, without increasing oxalate renal excretion. High hydroxyproline consumption increase oxalate urine concentration and collagen rich protein sources should be considered with for cats.

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433 **Table 1.** Ingredient composition of experimental diets for cats with different inclusions of
434 starch, protein and hydroxyproline

Ingredients (%)	Diets[1]		
	STARCH	SOY	HYDROX
Corn, grain	52.96	19.20	21.22
Soy-isolated protein (84% CP) [2]	0.00	34.33	0.00
Swine-isolated protein (62% CP) [3]	0.00	0.00	35.20
Poultry by-product meal	26.15	26.15	26.50
Poultry fat	8.50	8.00	5.06
Broken rice	5.00	5.00	5.00
Palatant, liquid [5]	2.00	2.00	2.00
Sugarcane fiber [4]	2.50	2.50	2.50
Palatant, powder [5]	1.00	1.00	1.00
Potassium chloride	0.40	0.40	0.40
Common salt	0.35	0.35	0.35
Vitamin-mineral premix [6]	0.30	0.30	0.00
Choline chloride	0.27	0.27	0.27
Fish oil	0.20	0.20	0.20
Taurine	0.15	0.15	0.15
Mold Inhibitor [7]	0.10	0.10	0.10
Antioxidant [8]	0.05	0.05	0.05
L-lysine HCl	0.05	0.00	0.00
DL-Methionine	0.02	0.00	0.00

435 [1] STARCH – High starch and low protein and hydroxyproline diet; SOY – High protein and
436 low hydroxyproline and starch diet; HYDROX – High hydroxyproline and protein, and low
437 starch diet.
438 [2] Solae, Esteio, RS, Brazil.
439 [3] Donated by Premier Pet, Dourado, SP, Brazil
440 [4] Vit2be Fiber, Dilumix, Leme, SP, Brazil, Mean geometric diameter =188±1.8microns
441 [5] SPF do Brasil, Descalvado, SP, Brazil
442 [6] Provided per kg of diet: vitamin A, 22,000 IU; vitamin D, 2,200 IU; vitamin E, 90 IU;
443 vitamin B1, 3 mg/kg; vitamin B2, 7 mg/kg; pantothenic acid, 22 mg/kg; niacin, 14 mg/kg;
444 vitamin B6, 4 mg/kg; folic acid 0.3 mg/kg; vitamin B12, 33 µg/kg; zinc, 140 mg/kg; iron, 80
445 mg/kg; copper 9,5 mg/kg; iodine 1,5 mg/kg; selenium, 0,25 mg/kg.
446 [7] Mold Zap: Ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do
447 Brasil Agroindustrial Ltda, Curitiba, 81170-610 PR, Brazil.

448 [8] Banox: BHA, BHT, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial
449 Ltda, Curitiba, 81170-610 PR, Brazil.
450

451 **Table 2.** Analyzed chemical composition of experimental diets for cats with different
452 inclusions of starch, protein and hydroxyproline

Item	Diets[1]		
	STARCH	SOY	HYDROX
Moisture (%)	6.5	5.8	5.4
	----------(DM-basis)----------		
Crude Protein (%)	24.8	49.9	50.5
Starch (%)	46.9	19.2	20.9
Amino acids (%)			
Lysine	1.35	2.90	2.72
Threonine	0.94	1.90	1.59
Methionine	0.52	0.75	0.82
Methionine + Cysteine	0.78	1.25	1.14
Arginine	1.58	3.40	3.66
Histidine	0.53	1.13	0.89
Isoleucine	0.86	2.11	1.42
Leucine	1.96	3.84	3.02
Serine	1.06	2.38	2.01
Phenylalanine	1.04	2.41	1.76
Phenylalanine + Tyrosine	1.78	4.04	2.83
Valine	1.09	2.26	1.95
Hydroxyproline	0.82	1.00	3.15
Acid-hydrolyzed fat (%)	11.7	12.1	12.7
Total dietary fiber (%)	10.8	10.9	11.0
Ash (%)	6.4	7.9	7.3
Ca (%)	1.79	1.59	1.44
P (%)	1.37	1.45	1.15
K (%)	0.62	0.51	0.62
Mg (%)	0.11	0.08	0.08
Na (%)	0.50	0.94	0.67
Cl (%)	0.21	0.33	0.47
S (%)	0.54	0.75	0.66

453 [1] STARCH – High starch and low protein and hydroxyproline diet; SOY – High protein and
454 low hydroxyproline and starch diet; HYDROX – High hydroxyproline and protein, and low
455 starch diet .

Table 3. Nutrient intake, apparent total tract digestibility coefficients, diet ME content, and nitrogen balance of cats fed experimental diets with different inclusions of starch, protein and hydroxyproline

Item	Diets[1]			SEM[2]	*P* value
	STARCH	SOY	HYDROX		
Nutrient intake during digestibility (g • kg BW^{-1} • d^{-1})					
Dry Matter	10.9	11.9	11.8	0.37	0.522
Protein	2.7 ^{b}	5.9 ^{a}	5.9 ^{a}	0.35	<0.001
Hydroxyproline	0.08 ^{b}	0.11 ^{b}	0.35 ^{a}	0.025	<0.001
Starch	5.1 ^{a}	2.3 ^{b}	2.5 ^{b}	0.30	<0.001
Acid-hydrolyzed fat	1.3 ^{b}	1.6 ^{ab}	1.7 ^{a}	0.06	0.002
Apparent total tract digestibility (%)					
Dry Matter	80.1 ^{b}	83.5 ^{a}	84.5 ^{a}	0.63	0.005
Organic Matter	84.2 ^{b}	87.1 ^{a}	88.0 ^{a}	0.54	0.004
Crude Protein	82.3 ^{b}	90.5 ^{a}	91.7 ^{a}	0.95	<0.001
Acid-hydrolyzed fat	86.7 ^{b}	89.3 ^{b}	92.5 ^{a}	0.70	<0.001
Starch	99.9	99.9	99.9	0.03	0.345
Gross Energy	83.4 ^{b}	87.5 ^{a}	88.4 ^{a}	0.62	<0.001
Metabolizable Energy (kJ/g, DM)	15.8 ^{b}	17.6 ^{a}	17.4 ^{a}	0.20	<0.001
Nitrogen Balance (mg • kg^{-1} • day^{-1})	229.5	395.8	180.9	80.49	0.556

[1] STARCH – High starch and low protein and hydroxyproline diet; SOY – High protein and low hydroxyproline and starch diet; HYDROX – High hydroxyproline and protein, and low starch diet.

[2] SEM = standard error of the mean, n=8 per diet

[a,b] Within a row, means without a common superscript differ ($P < 0.05$).

Table 4. Water balance and characteristics of the urine and feces of cats fed experimental extruded diets with different inclusions of starch, protein and hydroxyproline

Item	Diets[1]			SEM[2]	*P* value
	STARCH	SOY	HYDROX		
Urine characteristics					
Density	1,065	1,064	1,062	0.003	0.749
pH	6.57 [b]	6.97 [a]	6.59 [b]	0.060	0.004
Fecal characteristics					
Output ($g \bullet kg^{-1} \bullet day^{-1}$, As-is)	7.5	5.5	5.9	0.39	0.070
Feces DM (%)	37.9	37.5	36.2	1.25	0.861
Score	3.3 [ab]	3.6 [a]	2.5 [b]	0.39	0.039
Water intake ($ml \bullet kg^{-1} \bullet day^{-1}$)					
Drinking water	16.8 [b]	29.0 [a]	25.3 [a]	0.65	<0.001
Food water	0.76	0.73	0.68	0.347	0.365
Metabolic water	5.2	5.1	4.6	0.17	0.351
Total water intake	22.8 [b]	34.8 [a]	30.6 [ab]	1.69	0.006
Water excretion ($ml \bullet kg^{-1} \bullet day^{-1}$)					
Urine water excretion	9.2 [b]	18.7 [a]	19.6 [a]	1.32	<0.001
Fecal water excretion	4.0	3.4	3.7	0.28	0.721
Insensible losses	9.6	12.7	7.3	0.93	0.060

[1] STARCH – High starch and low protein and hydroxyproline diet; SOY – High protein and low hydroxyproline and starch diet; HYDROX – High hydroxyproline and protein, and low starch diet.

[2] SEM = standard error of the mean, n=8 per diet

[a,b] Within a row, means without a common superscript differ ($P < 0.05$)

469 **Table 5.** Urine concentration, intake, and renal excretion of macroelements of cats fed
470 experimental extruded diets with different inclusions of starch, protein and hydroxyproline

Item	Diets[1]			SEM[2]	*P* value
	STARCH	SOY	HYDROX		
Na					
Urine concentration (mg/dL)	373	505	346	21.5	0.131
Intake (mg•kg^{-1}•day^{-1})	54.6 ^{c}	112 ^{a}	79.0 ^{b}	0.01	<0.001
Renal excretion (mg•kg^{-1}•day^{-1})	36.6 ^{c}	75.5 ^{a}	60.7 ^{b}	3.84	<0.001
K					
Urine concentration (mg/dL)	639 ^{a}	309 ^{b}	370 ^{b}	37.3	<0.001
Intake (mg•kg^{-1}•day^{-1})	67.7	60.6	73.1	0.00	0.105
Renal excretion (mg•kg^{-1}•day^{-1})	62.2 ^{ab}	54.0 ^{b}	68.0 ^{a}	2.12	0.022
Cl					
Urine concentration (mg/dL)	935	723	751	43.8	0.087
Intake (mg•kg^{-1}•day^{-1})	22.9 ^{c}	39.2 ^{b}	55.4 ^{a}	0.00	<0.001
Renal excretion (mg•kg^{-1}•day^{-1})	91.4 ^{b}	127.9 ^{a}	138.4 ^{a}	6.38	0,002
Ca					
Urine concentration (mg/dL)	1.9	1.4	1.6	0.13	0.282
Intake (mg•kg^{-1}•day^{-1})	196	189	170	0.01	0.241
Renal excretion (mg•kg^{-1}•day^{-1})	0.20	0.24	0.30	0.022	0.139
P					
Urine concentration (mg/dL)	291	242	207	15.2	0.087
Intake (mg•kg^{-1}•day^{-1})	150 ^{ab}	172 ^{a}	136 ^{b}	0.01	0.027
Renal excretion (mg•kg^{-1}•day^{-1})	28.5 ^{b}	40.1 ^{a}	38.2 ^{ab}	2,15	0.024
Mg					
Urine concentration (mg/dL)	13.3 ^{a}	4.6 ^{b}	7.8 ^{b}	1.12	<0.001
Intake (mg•kg^{-1}•day^{-1})	12.0 ^{a}	9.5 ^{b}	9.4 ^{b}	0.00	0.011
Renal excretion (mg•kg^{-1}•day^{-1})	1.29	0.84	1.39	0.099	0.050
S					
Urine concentration (mg/dL)	221	214	177	11.4	0.277
Intake (mg•kg^{-1}•day^{-1})	59.0 ^{b}	89.1 ^{a}	77.8 ^{a}	0.00	<0.001
Renal excretion (mg•kg^{-1}•day^{-1})	21.3 ^{b}	37.0 ^{a}	32.6 ^{a}	1.69	<0.001

471 [1] STARCH – High starch and low protein and hydroxyproline diet; SOY – High protein and
472 low hydroxyproline and starch diet; HYDROX – High hydroxyproline and protein, and low
473 starch diet.

474 [2] SEM = standard error of the mean, n=8 per diet

475 [a,b] Within a row, means without a common superscript differ ($P < 0.05$)

Table 6. Urinary supersaturation of calcium oxalate (RSS OxCa), urine concentration and renal excretion of oxalate, citrate, urea and uric acid of cats fed experimental extruded diets with different inclusions of starch, protein and hydroxyproline

Item	Diets[1]			SEM[2]	*P* value
	STARCH	SOY	HYDROX		
RSS CaOx	0.69 [a]	0.20 [b]	0.45 [a]	0.060	<0.001
Oxalate					
mg/dl	2.9 [a]	1.1 [b]	1.9 [a]	0.22	<0.001
mg• kg^{-1}•day^{-1}	0.33	0.22	0.37	0.035	0.109
Citrate					
mg/dl	0.34	0.37	0.40	0.042	0.865
mg• kg^{-1}•day^{-1}	0.04	0.07	0.08	0.011	0.212
Urea					
mg/dl	6779 [b]	9613 [a]	9101 [a]	436.2	0.010
mg• kg^{-1}•day^{-1}	684 [b]	1738 [a]	1688 [a]	122.8	<0.001
Uric acid					
mg/dl	82.8	82.3	71.2	4.28	0.484
mg• kg^{-1}•day^{-1}	8.2 [b]	15.1 [a]	13.2 [a]	0.87	<0.001

[1] STARCH – High starch and low protein and hydroxyproline diet; SOY – High protein and low hydroxyproline and starch diet; HYDROX – High hydroxyproline and protein, and low starch diet.

[2] SEM = standard error of the mean, n=8 per diet

[a,b] Within a row, means without a common superscript differ ($P < 0.05$)