UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

PROPRIEDADES NUTRICIONAIS E FUNCIONAIS DE FARINHA DE VÍSCERAS DE AVES OBTIDA POR HIDRÓLISE ENZIMÁTICA NA DIETA DE GATOS

Autora: Tânia Zóia Miltenburg
Orientador: Prof. Dr. Ricardo Souza Vasconcellos

MARINGÁ
Estado do Paraná
Fevereiro – 2021

PROPRIEDADES NUTRICIONAIS E FUNCIONAIS DE FARINHA DE VÍSCERAS DE AVES OBTIDA POR HIDRÓLISE ENZIMÁTICA NA DIETA DE GATOS

Autora: Tânia Zóia Miltenburg
Orientador: Prof. Dr. Ricardo Souza Vasconcellos

"Tese apresentada, como parte das exigências para obtenção do título de DOUTOR EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá - Área de concentração Produção Animal."

MARINGÁ
Estado do Paraná
Fevereiro – 2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

Miltenburg, Tânia Zóia

M662p Propriedades nutricionais e funcionais de farinha de vísceras de aves obtida por hidrólise enzimática na dieta de gatos / Tânia Zóia Miltenburg. -- Maringá, PR, 2021.
87 f.figs., tabs.

Orientador: Prof. Dr. Ricardo Souza Vasconcellos.
Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, 2021.

1. Farinha de origem animal. 2. Gato - Nutrição animal. 3. Farinha de vísceras (Aves). 4. Gato - Digestibilidade. 5. Reação de Maillard. I. Vasconcellos, Ricardo Souza, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 23.ed. 636.8

Rosana de Souza Costa de Oliveira - 9/1366

UNIVERSIDADE ESTADUAL DE MARINGÁ CENTRO DE CIÊNCIAS AGRÁRIAS

PROPRIEDADES NUTRICIONAIS E FUNCIONAIS DE FARINHA DE VÍSCERAS DE AVES OBTIDA POR HIDRÓLISE ENZIMÁTICA NA DIETA DE GATOS

Autora: Tânia Zóia Miltenburg
Orientador: Prof. Dr. Ricardo Souza Vasconcellos

TITULAÇÃO: Doutora em Zootecnia – Área de Concentração Produção Animal

APROVADA em 23 de fevereiro de 2021.

Profª Drª Paula Toshimi Matumoto Pintro

Prof. Dr. Luciano Trevizan

Porf Dr.Marcio Antonio Brunetto

Profª Drª Bruna Agy Loureiro

Prof. Dr. Ricardo Souza Vasconcellos Orientador

“Que seu remédio seja seu alimento, e que seu alimento seja seu remédio”.

Hipócrates

À

minha família pela força

Aos

amigos especiais pelo apoio

Ao

meu marido pelo companheirismo

DEDICO

AGRADECIMENTOS

A Deus, por me proteger todos os dias e por todas as bênçãos da minha vida.

Aos meus pais (Godofredo e Anelise), que nunca mediram esforços para que eu pudesse ter a melhor educação possível, por todo o amor e incentivo.

Ao meu irmão, Guilherme e minha cunhada, Isabela, por sempre estarem ao meu lado me apoiando em Maringá.

Ao meu orientador, Ricardo Vasconcellos, pelos ensinamentos e conselhos, pelo tempo e ajuda, apoio, humildade e paciência.

Aos membros da banca de defesa, obrigada por aceitarem o convite e contribuírem para o aperfeiçoamento do trabalho.

À equipe do CEENUFEL, por toda a ajuda, risadas e principalmente conhecimento durante o experimento. Também aos nossos queridos gatos.

Aos amigos de Holambra, da graduação e da pós-graduação, pelo companheirismo, momentos de descontração e apoio.

Ao meu marido, pela força e companheirismo,

Aos professores do departamento de Zootecnia-UEM, pelo aprendizado.

Aos funcionários da FEI, em especial Sr.Wilson, que ajudou durante o experimento.

À Capes, pela bolsa concedida durante o mestrado.

À Universidade Estadual de Maringá, por possibilitar a execução deste trabalho.

À PremieR PET, pelo financiamento do projeto.

BIOGRAFIA

TÂNIA ZÓIA MILTENBURG, filha de Godofredo Antonio Maria Miltenburg e Anelise Zóia Miltenburg, nasceu na cidade de Holambra, São Paulo, no dia 02 de agosto de 1992.

Em março de 2010, iniciou o curso de Zootecnia pela Universidade Estadual de Maringá – Paraná. Foi bolsista de iniciação científica (Pibic) e também do Programa de Extensão Tutorial (PET), concluindo o curso em dezembro de 2014.

Em março de 2015, iniciou o Programa de Pós-Graduação em Zootecnia, em nível de Mestrado, na Universidade Estadual de Maringá, na área de Produção Animal, realizando estudos com nutrição de animais de companhia (cães e gatos). O mesmo foi concluído em fevereiro de 2017.

Em março de 2017, iniciou seus estudos em nível de Doutorado no mesmo programa de pós-graduação e universidade, dando continuidade em seu trabalho com nutrição de cães e gatos.

Em novembro de 2019, foi aprovada no exame de qualificação do doutorado e iniciou o Doutorado Sanduíche pelo programa PDSE na Wageningen University and Research (Holanda), o qual foi finalizado em setembro de 2020.

ÍNDICE

LISTA DE TABELAS

LISTA DE FIGURAS

RESUMO

O objetivo do presente trabalho foi avaliar as propriedades anti-hipertensivas do hidrolisado de vísceras de aves em dieta extrusada para gatos, como também seus efeitos na digestibilidade da dieta, palatabilidade e formação, consumo e excreção dos produtos da reação de *Maillard* (PRM). No primeiro estudo (I), foram avaliados os efeitos da inclusão de farinha de vísceras de aves hidrolisada enzimaticamente em dieta extrusada para gatos sobre a atividade da enzima conversora de angiotensina (ECA) e níveis de aldosterona (ALD) séricos, como também na digestibilidade dos nutrientes e características fecais. Com base em um ensaio preliminar de atividade inibitória da ECA *in vitro*, uma farinha de vísceras de aves convencional (FVC) e uma hidrolisada enzimaticamente (FVHE), obtidas comercialmente, foram escolhidas para o estudo a seguir. Duas dietas isoenergéticas e isonitrogenadas foram formuladas: dieta convencional (25,7% de FVC) e dieta com hidrolisado (24,7% de FVHE). No ensaio 1, o efeito da dieta na atividade sérica da ECA e concentração de ALD foi avaliado usando 8 gatos saudáveis (4 fêmeas e 4 machos, 4,1 ± 0,38 kg de peso corporal) em um delineamento *cross-over*, com 5 dias de adaptação e coleta de sangue no dia 6. No ensaio 2, a digestibilidade aparente total e as características fecais foram avaliadas em 12 gatos (6 fêmeas e 6 machos, 4,0 ± 0,72 kg de peso corporal) em um delineamento inteiramente casualizado. A atividade da ECA e ALD foram analisadas usando um modelo misto, com dieta como o efeito fixo e gato como o efeito aleatório. Os dados do ensaio 2 foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey. A atividade inibitória da ECA *in vitro* da FVHE (90,4%) foi maior que da FVC

(52,0%). Os gatos alimentados com a dieta contendo a FVHE tenderam a ter menor atividade sérica da ECA do que aqueles alimentados com a dieta com FVC (126,1 versus 142,1 U / L, p = 0,09). A ALD sérica não foi influenciada pela dieta (p> 0,05). As dietas tiveram valores de digestibilidade semelhantes e os escores de consistência fecal tenderam a ser maiores (fezes mais firmes) em gatos alimentados com dieta com FVC do que em gatos alimentados com dieta com FVHE (4,6 versus 4,0, p = 0,09). A inclusão de HPM em dietas extrusadas pode reduzir a atividade da ECA no soro do gato e promover uma boa consistência fecal sem afetar a digestibilidade. Outras investigações são necessárias para explorar os benefícios potenciais para a saúde da FVHE em gatos hipertensos. No segundo estudo (II) foi avaliado os efeitos da hidrólise enzimática durante a produção de farinha de vísceras de aves na formação dos PRM, como também no consumo e excreção desses compostos em gatos. A digestibilidade e palatabilidade desses ingredientes também foram determinadas. Para isso, uma farinha de vísceras de aves convencional (FVC-l) e outra hidrolisada enzimaticamente (FVHE-l) foram produzidas em condições de laboratório. Uma dieta completa para gatos adultos foi formulada (dieta basal) e 30% desta foi substituída pela FVC-l, pelo método de substituição, para formar a dieta convencional (DC) ou pela FVHE-l para formar a dieta hidrolisada (DH). Dezoito gatos (9 machos, 9 fêmeas, 4,18 ± 0,87 kg de peso vivo) foram divididos em três grupos e cada grupo recebeu uma das dietas experimentais. No experimento 1, a digestibilidade aparente total (DAT) das dietas e das farinhas de vísceras de aves foi avaliada em um delineamento inteiramente casualizado. No experimento 2, o teor de PRM (carboximetilisina, CML e frutoselisina, FL) foi avaliado nas dietas e, em seguida, a ingestão e excreção dos PRM foram medidas durante 3 dias, após o teste de digestibilidade. No ensaio 3, o teste de palatabilidade foi realizado em 20 gatos (11 machos e 9 fêmeas, 4,52 ± 1,16 kg de peso corporal). O teste teve duração de 2 dias, um para adaptação e outro para coleta de dados. Não houve diferenças entre a digestibilidade das dietas (DC versus DH) e ingredientes (FVC versus FVHE) (p> 0,05). A DC apresentou maior quantidade de FL e CML quando comparado a DH. O consumo de FL e CML foi maior nos animais alimentados com a DC (p <0,05). No entanto, não houve diferenças na excreção de FL e CML tanto na urina quanto nas fezes entre as dietas (DC versus DH) (p> 0,05). A primeira escolha de alimento, tanto para o cheiro quanto para o sabor, foi maior para a DH. A proporção de ingestão foi significativamente maior (poder do teste = 0,95) para a DH. A farinha de vísceras de aves enzimaticamente hidrolisada em dietas extrusadas para gatos não melhora a

digestibilidade da proteína, porém aumenta a palatabilidade da dieta. O conteúdo e consumo de FL e CML, diferentemente do esperado, foi maior na dieta convencional quando comparada a dieta com hidrolisado.

ABSTRACT

The aim of this study was to evaluate the anti-hypertensive properties of an enzymatically hydrolyzed poultry byproduct meal in extruded diets for cats, as well the effects on the diets digestibility, palatability and *Maillard* reaction products, dietary intake and excretion. In the first study (I), we evaluated the effects of extruded diets containing enzymatically hydrolyzed poultry byproduct meal (HPM) on cat serum ACE activity and aldosterone (ALD) concentration, nutrient digestibility, and fecal characteristics. On the basis of a preliminary in vitro ACE inhibitory activity assay, we selected a commercial HPM and a commercial conventional poultry byproduct meal (CPM) for further investigation. Two isoenergetic and isonitrogenous diets were formulated: CPM diet (25.7% CPM) and HPM diet (24.7% HPM). In trial 1, the effect of diet on serum ACE activity and ALD concentration was evaluated using 8 healthy cats (4 female and 4 male, 4.1 ± 0.38 kg BW) in a crossover design, with 5 d of adaptation and blood collection on d 6. In trial 2, apparent total tract digestibility and fecal characteristics were evaluated using 12 cats (6 females and 6 males, 4.0 ± 0.72 kg BW) in a completely randomized design. Serum ACE and ALD were analyzed using a mixed model, with diet as the fixed effect and cat as the random effect. Data from trial 2 were subjected to analysis of variance, and means were compared by Tukey's test. In vitro ACE inhibitory activity of HPM (90.4%) was higher than that of CPM (52.0%). Cats fed the HPM diet tended to have lower serum ACE activity than those fed the CPM diet (126.1 versus 142.1 U/L, $p = 0.09$). Serum ALD was not influenced by diet ($p > 0.05$). Diets had similar digestibility values, and fecal consistency scores tended to be higher (firmer feces) in cats fed the CPM diet than in cats fed the HPM diet (4.6 versus

4.0, p = 0.09). Inclusion of HPM in extruded diets may reduce cat serum ACE activity and promote good fecal consistency without affecting digestibility. Further investigations are needed to explore the potential health benefits of HPM in hypertensive cats. In the second study (II), we investigated the effects of enzymatic hydrolysis during the poultry by-product meal production on MRP formation, intake and excretion in cats. The digestibility and palatability of this ingredients were also determined. A conventional (CPM) and an enzymatically (HPM) hydrolyzed poultry meal were produced in laboratory conditions. A complete diet for adult cats was formulated (basal diet) and 30% of this diet was replaced by CPM, by the substitution method, to form the conventional poultry by-product meal diet (CD) or by HPM to form the hydrolyzed diet (HD). Eighteen cats (9 males, 9 females, 4.18 ± 0.87 kg BW) were divided into three groups and each group were fed one of the experimental diets. In trial 1, the apparent total tract digestibility (ATTD) of the diets and poultry meals were evaluated in a completely randomized design. In trial 2, the MRP (carboxymethyllysine, CMF, and fructoselysine, FL) content were evaluated in diets and then the MRP intake and excretion were measured during 3 days, following the digestibility test. In trial 3, palatability test was performed using 20 cats (11 males, and 9 females, 4.52 ± 1.16 kg BW). The test lasted 2 days, one for adaptation and one for data collection. There were no differences between the ATTD of the diets (CD versus HD) and ingredients (CPM versus HPM) ($p > 0.05$). The CD had a greater amount of FL and CML when compared to the HD. The intake of FL and CML was greater in the cats fed the CD ($p < 0.05$). However, there were no differences in FL and CML excretion both in urine and feces between the diets (CD versus HD) ($p > 0.05$). The first food choice, both for smell and taste, was higher for HD. The intake ratio was significantly higher (test power = 0.95) for the HD. Enzymatically hydrolyzed poultry by-product meal in extruded diets for cats do not improve the protein digestibility, but enhances the dietary palatability. Fructoselysine and carboxymethyllysine amount and intake, different to expected, were greater in the CD when compared to the HD.

I. INTRODUÇÃO GERAL

A farinha de vísceras de aves (FVA) é uma das principais fontes de proteína utilizada pela indústria *pet food*, visto que possui um bom balanço de aminoácidos, é relativamente barata em relação aos outros ingredientes proteicos, além de ser uma opção ambientalmente sustentável. O processo convencional de obtenção da FVA consiste, brevemente, no tratamento térmico do material cru (intestino, moela, pescoço, cabeça, pés e carcaças) em altas temperaturas, prensagem para separação do óleo e moagem da fase sólida restante. Atualmente, novos métodos de processamento com a utilização de enzimas estão sendo estudados no intuito de melhorar a qualidade final do ingrediente e obter propriedades funcionais (Brandelli et al., 2015).

Diversos hidrolisados proteicos oriundos de subprodutos da indústria avícola demonstraram possuir peptídeos com características bioativas, como por exemplo, inibidores da enzima conversora de angiotensina (ECA) (Fontoura et al., 2014; Mane and Jamdar, 2017; Mas-Capdevila et al., 2018). A ECA é responsável pela conversão da angiotensina I em angiotensina II, um potente vasoconstritor e estimulador da secreção de aldosterona pertencente ao sistema renina-angiotensina-aldosterona, o qual está envolvido no controle da pressão arterial. Drogas anti-hipertensivas como enalapril e benazepril atuam inibindo a ECA, porém efeitos adversos são reportados em humanos

(Laurent, 2017). Nesse sentido, hidrolisados proteicos de vísceras de aves, se provado o efeito fisiológico benéfico em gatos, é um potencial ingrediente funcional.

Além da possível propriedade funcional, a hidrólise enzimática também pode ter um efeito positivo na digestibilidade da proteína das farinhas de vísceras, pelo fato de gerar pequenos peptídeos e aminoácidos livres. No entanto, estes podem se complexar com maior facilidade a açúcares redutores quando expostos a tratamentos térmicos, formando os produtos da reação de Maillard (PRM). Uma vez que a produção de farinha de vísceras, como também o processo de extrusão e secagem das dietas, envolvem altas temperaturas, é possível que haja maior formação desses compostos e consequentemente, menor biodisponibilidade de alguns aminoácidos essenciais, como a por exemplo, a lisina (van Rooijen et al., 2014). Além disso, elevados níveis de PRM nos tecidos estão associados a várias doenças relacionadas a idade tanto em humanos como em cães, como a diabetes, catarata, osteoartrite, entre outras (van Rooijen et al., 2016). Sendo assim, é de suma importância entender a formação desses compostos durante o processamento dos ingredientes e dietas para *pet food*, como também seu metabolismo em gatos, visto a escassez de informação nessa área.

Deve-se ressaltar que embora os PRM possam gerar efeitos negativos sobre a saúde do animal em grandes quantidades, por outro lado, eles exibem uma grande importância para os alimentos nos quesitos cor e sabor, influenciando positivamente na palatabilidade e sendo foco de muitos estudos por fábricas de palatabilizantes e rações para *pet* (van Rooijen et al., 2013).

Considerando o exposto acima, nesta tese foram estudados os efeitos comparativos de uma farinha de vísceras de aves convencional e outra produzida por meio da hidrólise enzimática, sobre a digestibilidade das dietas, palatabilidade, formação e balanço orgânico de PRM, bem como seus efeitos sobre a atividade sérica da ECA em gatos. Também, determinou-se a digestibilidade e energia metabolizável de ambos os ingredientes.

II. REVISÃO DE LITERATURA

1. Farinha de vísceras de aves convencional: composição, processamento e qualidade

A FVA é um dos principais ingredientes utilizados em formulações para *pet food* devido ao seu alto teor de proteína, bom equilíbrio de aminoácidos essenciais, disponibilidade de minerais (especialmente cálcio e fósforo) e boa fonte de gordura, com um custo relativamente baixo em relação a qualidade nutricional apresentada. Além disso, é uma matéria-prima considerada ambientalmente sustentável, visto que é obtida a partir de sub-produtos da indústria avícola e não compete por recursos da alimentação humana (Lasekan et al., 2013; Meeker and Meisinger, 2015).

O material que compõe a FVA varia de acordo com a fábrica e lote de processamento, sendo permitido, de acordo com a AAFCO (2017) a inclusão de moela, intestino, pescoço, cabeça, pés, ovos não desenvolvidos, aparas, carcaças danificadas para consumo humano e resíduo de carne mecanicamente separada (CMS). A inclusão de penas não é permitida, exceto em quantidades que possam ocorrer de forma inevitável durante o processamento das aves.

O processamento convencional, também chamado de renderização, e é composto por várias etapas. A primeira, consiste no tratamento térmico do material cru em digestores utilizando altas temperaturas (115 a 180ºC), por um tempo médio de 90 min.

A seguir, é realizada a separação da gordura da massa sólida através de uma prensa do tipo Expeller, a mais comum. A gordura é então encaminhada para tanques de armazenamento e a massa segue para moagem, obtendo assim a farinha de vísceras de aves (Meeker and Hamilton, 2006).

Em relação a qualidade da FVA, vários são os fatores que a influenciam. Como mencionado acima, o conteúdo do material cru é muito variável e tem um impacto expressivo na composição final do ingrediente. Quando uma maior quantidade de tecidos conjuntivos e fragmentos contendo ossos são adicionados, há uma diluição dos aminoácidos essenciais e um aumento significativo na matéria mineral (Meeker and Meisinger, 2015). O período de transporte e espera da matéria prima até o processamento também é um ponto crítico. Uma vez que as aves são abatidas e evisceradas, os tecidos imediatamente começam a se decompor e deteriorar, e se o tempo de espera for longo, bactérias se proliferam e compostos indesejáveis (como por exemplo, aminas biogênicas) são formados em maior quantidade (Lázaro et al., 2015). Além disso, os parâmetros de processo, como tempo e temperatura, também desempenham um papel importante. Embora sejam necessários para extração da gordura, evaporação da maior parte da umidade e pela inativação de bactérias, vírus, protozoários e parasitas, quando em excesso, danificam proteínas e seus componentes, como também ácidos graxos, os quais são oxidados em maior proporção (Meeker and Hamilton, 2006; Ribeiro et al., 2019).

Uma vez que não existe padrão em relação a porcentagem de inclusão de cada resíduo (ex: vísceras, cabeças, CMS, etc) e o tempo permitido entre a geração do resíduo no frigorífico e seu processamento pode ser elevado (até 24 horas permitido por Lei), as condições de processamento no digestor são extremanete variáveis e empíricas, gerando ingredientes com a composição química e qualidade extremamente variáveis, atualmente. No entanto, a busca por ingredientes com maior qualidade e padronização pela maioria das empresas de *pet food* está fazendo com que o desenvolvimento tecnológico na produção das FVA cresça. Novas instalações, com equipamentos mais modernos e novos métodos de processamento estão sendo estudados e aplicados.

2. Hidrólise enzimática de proteínas

A hidrólise enzimática consiste na clivagem das ligações peptídicas das proteínas pela ação das proteases, resultando na formação de peptídeos de diferentes tamanhos moleculares e aminoácidos livres (Clemente, 2000). As proteases são classificadas em endo e exoproteases de acordo com a posição em que clivam as ligações peptídicas, sendo a primeira responsável por hidrolisar as ligações na região interna das proteínas, produzindo peptídeos relativamente grandes, e a segunda por remover os aminoácidos da posição terminal N ou C. De acordo com o propósito da hidrólise, pode-se aplicar apenas uma das enzimas, ou ambas, em conjunto ou em processos sequenciais (Hou et al., 2017). A escolha da enzima a ser aplicada depende da fonte proteica (matéria prima) utilizada, o grau de hidrólise e as características adicionais (por exemplo, propriedades biativas) desejadas no produto final.

As enzimas podem ser obtidas de diversas fontes, sendo de animais (ex: pancreatina, tripsina, pepsina, carboxipeptidases e aminopeptidases), de vegetais (ex: papaína e bromelina), de origem fúngicas (*Aspergillus niger*, *Aspergillus melleus*, *Aspergillus oryzae,* entre outros) e bacterianas (*Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus cereus*, *entre outras*) (Rao et al., 1998). Devido a maior facilidade de produção, rápido crescimento, pequeno espaço tempo necessário para o cultivo e grande variedade de atividade catalítica, as proteases bacterianas são as mais utilizadas pelas indústrias químicas e alimentícias (Giongo, 2006).

Na alimentação humana, a utilização de proteínas hidrolisadas já é consolidada. São várias suas aplicações: no tratamento clínico de pacientes com distúrbios específicos da digestão, absorção ou metabolismo de aminoácidos; pacientes com desnutrição associada ao câncer, trauma, queimaduras e encefalopatias hepáticas; em fórmulas infantis hipoalergênicas; na nutrição esportiva; em dietas para controle de peso; produtos geriátricos e bebidas energéticas (Clemente, 2000; Manninen, 2009). Na nutrição de animais de companhia, sua aplicação não é tão ampla, sendo utilizada principalmente em dietas hipoalergênicas e em palatabilizantes. Também pode ser recomendada para pacientes com doença inflamatória intestinal (Cave, 2006).

Atualmente, além do emprego já conhecido dos hidrolisados proteicos, novas propriedades funcionais de peptídeos obtidos a partir da hidrólise enzimática estão sendo estudadas, como por exemplo atividade antioxidante, antimicrobiana, imunomoduladora, atividade inibitória da dipeptidil peptidase IV e inibitória da ECA (Brandelli et al., 2015; Kang et al., 2020). Nesse aspecto, diversas fontes proteicas estão

sendo avaliadas em relação a sua capacidade de, após a hidrólise, fornecer peptídeos bioativos.

As vísceras obtidas da indústria avícola são, normalmente, processadas de maneira convencional para obtenção da farinha de vísceras de aves. No entanto, a hidrólise enzimática desse material é uma alternativa para produção de um ingrediente com maior leque de aplicações, como também no fornecimento de peptídeos bioativos. Diferentemente do modo convencional, o processamento consiste na drenagem do material cru e trituração, transporte até o reator, aplicação da enzima e posterior hidrólise enzimática, separação das fases (fase solúvel, resíduo não solúvel e gordura), secagem por desidratação da fase solúvel, resfriamento e moagem.

3. Peptídeos bioativos

Peptídeos bioativos são sequências específicas de aminoácidos que, além do seu valor nutricional, possuem a capacidade de regular diversos processos fisiológicos afetando a saúde de maneira benéfica (Möller et al., 2008). São constituídos de aproximadamente 2 – 20 aminoácidos e possuem estruturas similares, como reduzido número de aminoácidos com a maior proporção deles sendo os hidrofóbicos, além da presença de arginina, lisina e prolina (Bechaux et al., 2019; Hou et al., 2017).

Os peptídeos bioativos são inicialmente inativos dentro da proteína de origem e tornam-se ativos após sua liberação, a qual pode ser realizada internamente através das proteases do trato gastrointestinal ou externamente pelo prévio tratamento ácido, alcalino ou enzimático da fonte proteica. Visto que a atividade das enzimas endógenas é incontrolável, possui sítios específicos de clivagem e fornece uma quantidade limitada de peptídeos bioativos, outras formas de obtenção de PB são requeridas (Lafarga and Hayes, 2014). Em relação aos tratamentos ácido e alcalino, a aplicação de proteases possui vantagens, como: maior especificidade do sítio de clivagem e controle do grau de hidrólise, condições de processo (exemplo: pH e temperatura) menos agressivos com menor chance de danos aos aminoácidos e fácil e rápida desativação das enzimas após hidrólise (exemplo: 85ºC por 3 min), sendo o método mais utilizado atualmente (Bechaux et al., 2019; Hou et al., 2017).

3.1. Atividade inibitória de peptídeos bioativos sobre a enzima conversora de angiotensina (ECA)

A enzima conversora de angiotensina faz parte do sistema renina-angiotensina-aldosterona (SRAA), um dos quais regulam a pressão sanguínea. A ECA é responsável por remover o peptídeo His-Leu da extremidade C-terminal da angiotensina I (Ang I), transformando-a em angiotensina II (Ang II) (Hou et al., 2017). A Ang II é o principal peptídeo efetor do SRAA e exerce suas funções principalmente através do receptor AT1, o qual é encontrado de forma abundante nos vasos, rins, coração, fígado e cérebro. A Ang II atua nas arteríolas causando vasoconstrição; estimula a adrenal a secretar aldosterona, aumentando a reabsorção renal de sódio e a excreção de potássio e estimula a hipófise posterior a secretar o hormônio antidiurético, aumentando a reabsorção renal de água (Atlas, 2007). Além de atuar no sistema SRAA, a ECA também degrada a bradicinina, à qual pertence ao sistema kallikreina-kinina. A bradicinina, quando liberada, estimula a vasodilatação, porém quando degradada pela ECA, não exibe seu efeito (Couture and Lindsey, 2000). Como consequência da atuação da ECA em ambos os sistemas, há um aumento da pressão arterial sistêmica (Atlas, 2007; Ryan et al., 2011).

Em humanos, as principais drogas utilizadas para regularizar a pressão (por exemplo, captopril, benazepril e enalapril) atuam inibindo a ECA, porém efeitos adversos são relatados, sendo eles angioedema, tosse seca, distúrbios do paladar, reações cutâneas, entre outros (Sica, 2004). Esses efeitos colaterais, juntamente com a alta incidência de pacientes hipertensos e o fato da hipertensão ser um fator de risco conhecido para derrames e doenças cardiovasculares, contribuíram para a busca de peptídeos anti-hipertensivos derivados de alimentos (Ryan et al., 2011).

Os peptídeos bioativos podem inibir a ECA de duas maneiras, ligando-se ao seu sítio ativo ou modificando sua conformação ao ligar-se a um sítio inibidor impedindo a ligação da Ang I ao local ativo (Ryan et al., 2011). Diversos estudos demonstraram a capacidade de inibição da ECA *in vitro* por hidrolisados de diferentes subprodutos de aves: da crista e da “papada” (Bezerra et al., 2019), dos pés (Mas-Capdevila et al., 2018), de vísceras (dos Santos Aguilar et al., 2019; Mane and Jamdar, 2017), da pele (Onuh et al., 2015; Yusop et al., 2016), do colágeno comercial obtido de aves (Soladoye et al., 2015), do extrato de osso (NAKADE et al., 2008), entre outros. No entanto, estudos *in vitro,* embora sejam uma ferramenta interessante para avaliar a atividade dos

peptídeos bioativos, não necessariamente confirmam seu efeito fisiológico (Mas-Capdevila et al., 2018).

Para exercer efeito benéfico *in vivo*, os peptídeos bioativos devem ser resistentes aos diferentes pH e enzimas do trato gastrointestinal, devem ser absorvidos pelos enterócitos para o soro, chegarem completos ao sítio de ligação desejado e em quantidade suficiente (Lopez-Barrios et al., 2014). Mane and Jamdar (2017) avaliaram a resistência de três peptídeos bioativos obtidos de vísceras de frango a degradação por enzimas gastrointestinais (pepsina, tripsina e quimotripsina) e observaram atividade residual de 82% em relação a atividade inicial. Anna et al. (2016) avaliaram o efeito da digestão gastrointestinal *in vitro* sobre a atividade inibitória da ECA de peptídeos obtidos de proteína hidrolisada de frango e observaram um aumento de 2,23 vezes na inibição da ECA após a digestão. Diversos estudos também demonstraram a capacidade de absorção dos peptídeos bioativos pelo organismo (Ding et al., 2016; Fan et al., 2018; Gallego et al., 2016; Sangsawad et al., 2018).

Além dos fatores citados acima, a forma que o ingrediente contendo os peptídeos bioativos é fornecido ao animal também é importante. Uma vez que, para dietas secas para gatos, as matérias-primas são misturadas, extrusadas e secas, diversos fatores podem influenciar na atividade inibitória da ECA do produto final. Durante o processamento, altas temperaturas são aplicadas, podendo danificar os peptídeos ou induzir sua complexação com outros nutrientes, como por exemplo, os açúcares redutores, formando os produtos da reação de Maillard (Udenigwe and Fogliano, 2017). Shi et al. (2019) avaliaram os efeitos do processo térmico (25 a 100ºC por 1 h) e o tempo submetido ao aquecimento (20 a 180 min a 100ºC) sobre a capacidade inibitória da ECA de peptídeos obtidos de carne de pato hidrolisada e observaram que o processo térmico, independente da temperatura, por 1h não influenciou a atividade inibitória da ECA, porém após 3 horas submetidos a temperatura de 100ºC, esta foi reduzida. Resultado similar foi encontrado por López-Sánchez et al. (2016) avaliando o efeito da temperatura (40 - 120ºC por 1h) sobre a atividade inibitória da ECA, o qual observou que até 100ºC não houve influência no resultado, porém o tratamento a 120ºC diminui a atividade.

Nenhum estudo *in vivo* avaliando alimentos com peptídeos bioativos inibidores da ECA em dietas extrusadas para gatos foi encontrado. No entanto, diversos trabalhos foram realizados avaliando ingredientes utilizando ratos como modelo animal. Mas-

Capdevila et al. (2018) observaram 21% de redução na atividade sérica da ECA de ratos hipertensos alimentados com 55 mg/kg de hidrolisado de pé de frango. Huang et al. (2016) alimentaram ratos hipertensos com 200 mg/kg de hidrolisado proteico de sardinha e observaram 51% de queda na atividade sérica da ECA em relação ao grupo controle. Dessa maneira, se comprovado seu efeito benéfico em gatos, o hidrolisado de vísceras de aves é um potencial ingrediente a ser utilizado em dietas para gatos hipertensos.

4. Produtos da reação de *Maillard* (PRM)

A reação de *Maillard* pode ser definida como uma série de reações não enzimáticas de escurecimento em que um grupo amino de um aminoácido complexa-se com um grupo carbonila de um açúcar redutor (Nie et al., 2017). Tanto aminoácidos livres como aminoácidos pertencentes a peptídeos e proteínas estão envolvidos na reação, no entanto, o primeiro possui o grupo α-amino (-NH_2) e uma cadeia lateral expostos para tal, enquanto o segundo possui apenas a cadeia lateral livre, visto que o grupo α-amino está ligado ao grupo α-carboxila de outro aminoácido (van Rooijen et al., 2013).

A reação de Maillard pode ser dividida em três fases: inicial, avançada e final. A fase inicial consiste na condensação de um grupo carbonila de um açúcar redutor com um grupo amino de um aminoácido, resultando na formação da base de Schiff. A base de Schiff, através do rearranjo de Amadori, forma os compostos de Amadori. Tanto a base de Schiff quanto os compostos de Amadori são reversíveis, no entanto, não é completamente elucidado o mecanismo pelo qual isso ocorre e quanto é relevante para a reação de Maillard (Van Rooijen et al., 2013). Na fase avançada, os compostos de Amadori podem reagir por várias vias, como rearranjo, condensação, oxidação, hidratação e desidratação, e formar os compostos intermediários da reação de Maillard. Diversos compostos com alto potencial oxidativo são formados nessa etapa, sendo eles metilglioxal, glioxal e 1- e 3-desoxiglucosona. Esses compostos mais o composto de Amadori são precursores de outros produtos avançados da reação de Maillard, como o hidroximetilfurfural (HMF), carboximetilisina (CML), pentosidina e pirralina, sendo estes os principais marcadores da extensão da reação de Maillard (Erbersdobler and Somoza, 2007). Na fase final, estes compostos reagem com outro grupo amino

formando as melanoidinas, as quais são responsáveis pela cor marrom dos alimentos submetidos ao tratamento térmico.

Diversos fatores interferem na formação dos produtos da reação de Maillard, como por exemplo composição dos ingredientes, temperatura e tempo de processamento, pH, atividade de água, presença de metais de transição, entre outros (Poulsen et al., 2013). Em relação a reatividade dos açúcares redutores, os mais reativos em ordem são pentoses, hexoses e dissacarídeos, sendo as aldoses mais reativas que cetoses (Steele, 2004). Dentre os aminoácidos, a lisina é a mais reativa (Baynes, 2005), porém arginina, histidina e triptofano, em menor proporção, também estão envolvidos na reação de Maillard (van Boekel, 2006). Além disso, aminoácidos na sua forma livre são mais susceptíveis à complexação, visto que possuem o grupo α-amino não ligado a outro aminoácido (van Rooijen et al., 2013). Em relação a temperatura, a reação de Maillard pode ocorrer em temperaturas amenas (ambiente e corporal) porém aumenta com o aquecimento. Segundo Ledl and Schleicher (1990) a taxa de reação de Maillard pelo menos dobra a cada 10ºC aumentado. Conforme o tempo de processamento ou tempo de armazenagem (no caso de alimentos acabados), os PRM aumentam linearmente, em maior ou menor extensão variando com a temperatura em que são submetidos. No que se refere ao pH, a reação é mínima em meio ácido, porém evolui com o aumento do pH até atingir um valor máximo de 10 (Poulsen et al., 2013). A reação de Maillard aumenta exponencialmente com o aumento do teor de umidade devido a mobilidade dos nutrientes, até atingir um ponto máximo em uma faixa de umidade intermediária (aw 0,4 – 0,7), após essa faixa é observada uma diminuição na reação devido a diluição dos reagentes (O'Brien et al., 1989). De acordo com Wu et al. (2010), tanto Fe^{3+} quanto Fe^{2+} aceleram a reação de Maillard, porém Ca^{2+} e Mg^{2+} a inibem.

4.1. Efeitos dos PRM na qualidade das dietas

- Valor nutritivo: a lisina é o aminoácido mais reativo, ou seja, que mais contribui na formação dos PRM’s. Uma vez complexada, torna-se indisponível para utilização pelos animais. Segundo van Rooijen et al. (2013), os compostos formados até podem ser absorvidos, no entanto, não possuem valor nutritivo. Tendo em vista que a lisina é um aminoácido essencial e um dos mais limitantes,

é de suma importância quantificar a porcentagem de lisina “perdida” nas reações de Maillard. As dietas para gatos são formuladas para atender as exigências de aminoácidos de acordo com a fase da vida e fisiológica dos animais, no entanto, não leva-se em consideração as perdas de aminoácidos por complexação. Dessa maneira, diversos alimentos originalmente balanceados, podem se tornar deficientes, principalmente em lisina, após o processo de extrusão, secagem, como também armazenagem. Filhotes de gatos alimentados com uma mistura de caseína-dextrose aquecida contendo 34% de lisina complexada apresentaram menor ganho de peso (2,7 g/dia) em relação aos filhotes alimentados com a dieta controle contendo caseína não aquecida e apenas 0,4% de lisina complexada (14,9 g/dia de ganho de peso). Ao suplementar as dietas aquecidas com 4,0, 5,5, e 7,0 g de lisina sintética, o ganho de peso diário aumentou para 5,4, 11,2 e 20,1, respectivamente (Larsen et al., 2002; Larsen et al., 2010). Esse estudo demonstra como a complexação da lisina diminui sua biodisponibilidade. Além disso, a formação dos PRM’s pode afetar a digestibilidade da proteína da dieta. Hulshof et al. (2016) avaliaram a digestibilidade ileal estandardizada (DIS) e total (DIT) da proteína em leitões alimentados com farelo de soja (FS) e farelo de colza (FC) tostados ou não e observaram uma redução na DIS do FS de 83,9 para 71,6% no FS tostado, e 74,9 para 64,6% no FC tostado. Para a DIT, a redução foi de 88,8 para 81,3% para FS e de 76,5 para 72,1% para FC. A menor redução da DIT em relação a DIS pode ser explicada devido a metabolização de produtos da reação de Maillard intermediários e avançados no intestino grosso pela microbiota, os quais são resistentes a ação das enzimas nas etapas anteriores.

- Palatabilidade: embora a formação dos PRM pode afetar de forma negativa o valor nutritivo da dieta, a reação de Maillard desempenha um papel importante na geração de compostos que realçam o aroma e sabor dos alimentos processados (Eric et al., 2013). O PRM são conhecidos por gerarem sabores como o de carne/caldo, umami e kokumi, sendo que a fonte proteica e de açúcar selecionados, como também o tratamento térmico, são alguns dos fatores mais importantes que afetam a taxa de reação e as características aromatizantes (Martins et al., 2001; Liu et al., 2015). De acordo com Liu et al., (2015), altas temperaturas (>100°C) podem aumentar notavelmente o aroma de carne em modelos utilizando peptídeos provenientes de frango e xylose, enquanto os

sabores umami e kokumi são produzidos com temperaturas relativamente menores em um período de tempo maior. Ainda, segundo o autor, as pirazinas parecem ser os principais contribuintes para a formação do aroma de carne e "nozes", os quais são formados principalmente por peptídeos de baixo peso molecular (<500 Da). Por último, foi demonstrado que PRM's entre 1000 – 3000 Da, formados pelo *cross-linking* e em temperaturas menores, podem fazer parte dos compostos com sabor kokumi, enquanto os >3000 Da foram responsáveis pelo sabor amargo. Ainda segundo (Bradshaw et al., 1996), a complexação da arginina durante o processamento pode aumentar a palatabilidade do alimento, visto que esta, quando não complexada e em seu estado livre, tende a ser evitada pelos gatos devido ao seu sabor amargo (Bradshaw et al., 1996). Em um estudo com cães, (Chen et al., 2017) identificaram 23 aromas relacionados ao aumento da palatabilidade em 7 palatabilizantes, dentre eles metil pyrazina e 2,5-dimetil pyrazina, os quais são associados a reação de Maillard. Para validar o achado, os autores adicionaram o composto 2,5-dimetil pyrazina como palatabilizante na dieta e observaram que a relação de consumo foi de 76,4% para o alimento suplementado com o composto.

5. Digestibilidade de hidrolisados proteicos

O valor nutritivo de uma fonte proteica depende do seu conteúdo de aminoácidos essenciais como também de sua digestibilidade, a qual é influenciada pelo tipo de proteína e pelo método de processamento, tanto do ingrediente como da dieta (Tjernsbekk et al., 2016). Dessa maneira, a digestibilidade da proteína dos ingredientes e/ou dietas é comumente realizada como forma de avaliar a qualidade proteica, embora a biodisponibilidade não possa ser mensurada através desse parâmetro.

O processo de digestão da proteína dietética se inicia no estômago através da ação do ácido clorídrico e pepsina, mas ocorre principalmente no intestino delgado, através da ação das enzimas pancreáticas (tripsina, quimiotripsina, carboxipeptidase e elastase) e das peptidases presentes na borda em escova. Através desse processo, é formado então, peptídeos de diversos pesos moleculares e aminoácidos livres, os quais são absorvidos através das células epiteliais intestinais (Guyton and Hall Textbook of Medical Physiology). A digestibilidade aparente total é então comumente definida como

a porcentagem de proteína ingerida, digerida e absorvida, que não é excretada nas fezes (NRC, 2006).

A digestibilidade de uma fonte proteica varia de acordo com a origem da proteína e sua estrutura, como também com o processo que foi submetida. Proteínas estruturais ou do tecido conjuntivo, como por exemplo, colágeno e queratina, possuem uma digestibilidade menor, devido à sua conformação (Donadelli et al., 2019). Além disso, ingredientes proteicos submetidos a tratamentos térmicos intensos podem ter sua digestibilidade proteica diminuída, uma vez que esse processo pode danificar a proteína e possibilita sua complexação com outros nutrientes (Van Rooijen et al., 2013).

Atualmente, tem crescido o interesse na utilização de proteínas hidrolisadas na alimentação de cães e gatos. Geralmente, os hidrolisados proteicos são utilizados como palatabilizantes e como ingredientes hipoalergênicos. No entanto, estudos vem sendo realizados a fim de mensurar um possível efeito funcional, como também uma melhora na digestibilidade da proteína (Tjernsbekk et al., 2017).

De acordo com (Martínez-Alvarez et al., 2015), uma vez que as proteínas são previamente hidrolisadas em pequenos peptídeos e aminoácidos, eles são facilmente absorvidos no intestino delgado sem digestão gastrointestinal prévia e potencialmente aumentam o crescimento e desenvolvimento do animal. Além disso, a absorção de aminoácidos lábeis e insolúveis, como cisteína, glutamina e tirosina, em forma de pequeno peptídeo, aumenta a disponibilidade desses aminoácidos para o corpo.

Embora a hidrólise enzimática possa ter um efeito positivo na digestibilidade proteica do ingrediente, deve-se considerar a forma em que este será utilizado e fornecido aos animais. Uma vez que a maior parte das dietas para gatos são extrusadas, deve-se considerar a digestibilidade proteica do hidrolisado após a extrusão da dieta, visto que o processo e outros ingredientes podem influenciar nesse parâmetro. Em trabalho realizado por (Tjernsbekk et al., 2017), foi avaliado a digestibilidade proteica da proteína hidrolisada de salmão antes do processo de extrusão e após ser inclusa em uma dieta extrusada completa para cães. O autor observou que, antes da extrusão, a digestibilidade da proteína do hidrolisado foi maior que da farinha de vísceras e carne de frango mecanicamente separada (91,3; 80,9 e 88,2%, respectivamente). No entanto, após a extrusão, não houve diferença estatística entre a digestibilidade proteica dos ingredientes (79,0 - hidrolisado; 80,3 – farinha de vísceras e 81,3 – carne

mecanicamente separada). Em gatos, não foi encontrado nenhum estudo avaliando a digestibilidade de uma fonte proteica de origem animal hidrolisada enzimaticamente.

Uma possível explicação para a diminuição da digestibilidade da proteína do hidrolisado após a extrusão, é a complexação dos aminoácidos e peptídeos livres com outros nutrientes, formando os produtos da reação de Maillard. Em estudo realizado por van Rooijen (2015), a extrusão diminuiu significativamente a digestibilidade *in vitro* da proteína de uma dieta contendo proteína hidrolisada de peixe de 94,1 para 93,1%. Também reduziu a digestibilidade *in vitro* da lisina total e reativa.

6. Referências bibliográficas

Anna, T. et al. 2016. Effect of in vitro Gastrointestinal Digestion on Bioactivity of Poultry Protein Hydrolysate. Current Research in Nutrition and Food Science Journal 4: 77-86.

Atlas, S. A. 2007. The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. Journal of managed care pharmacy 13: 9-20.

Baynes, J. W. 2005. The Maillard Reaction: Chemistry, Biochemistry and Implications By Harry Nursten (The University of Reading, Reading, U.K.). Royal Society of Chemistry: Cambridge. 2005. xii + 214 pp. $199.00. ISBN 0-85404-964-9. Journal of the American Chemical Society 127: 14527-14528.

Bechaux, J., P. Gatellier, J.-F. Le Page, Y. Drillet, and V. Sante-Lhoutellier. 2019. A comprehensive review of bioactive peptides obtained from animal byproducts and their applications. Food & Function 10: 6244-6266.

Bezerra, T. K. A. et al. 2019. Identification of Angiotensin I-Converting Enzyme-Inhibitory and Anticoagulant Peptides from Enzymatic Hydrolysates of Chicken Combs and Wattles. Journal of Medicinal Food.

Bradshaw, J. W. S., D. Goodwin, V. Legrand-Defre', and H. M. R. Nott. 1996. Food Selection by the Domestic Cat, an Obligate Carnivore.

Brandelli, A., L. Sala, and S. J. Kalil. 2015. Microbial enzymes for bioconversion of poultry waste into added-value products. Food research international 73: 3-12.

Cave, N. J. 2006. Hydrolyzed Protein Diets for Dogs and Cats. Veterinary Clinics: Small Animal Practice 36: 1251-1268.

Chen, M., X. Chen, J. Nsor-Atindana, K. G. Masamba, J. Ma, and F. Zhong. 2017. Optimization of key aroma compounds for dog food attractant. Animal Feed Science and Technology. 225:173–181. doi:10.1016/j.anifeedsci.2016.12.005.

Clemente, A. 2000. Enzymatic protein hydrolysates in human nutrition. Trends in Food Science & Technology 11: 254-262.

Couture, R., and C. J. Lindsey. 2000. Chapter VII Brain kallikrein–kinin system: from receptors to neuronal pathways and physiological functions. In: R. Quirion, A. Björklund and T. Hökfelt (eds.) Handbook of Chemical Neuroanatomy No. 16. p 241-300. Elsevier.

Ding, L., L. Wang, Z. Yu, T. Zhang, and J. Liu. 2016. Digestion and absorption of an egg white ACE-inhibitory peptide in human intestinal Caco-2 cell monolayers. International Journal of Food Sciences and Nutrition 67: 111-116.

Donadelli, R. A., C. G. Aldrich, C. K. Jones, and R. S. Beyer. 2019. The amino acid composition and protein quality of various egg, poultry meal by-products, and vegetable proteins used in the production of dog and cat diets. Poultry Science. 98:1371–1378. doi:10.3382/ps/pey462.

dos Santos Aguilar, J. G., A. K. S. de Souza, and R. J. S. de Castro. 2019. Enzymatic Hydrolysis of Chicken Viscera to Obtain Added-Value Protein Hydrolysates with Antioxidant and Antihypertensive Properties. International Journal of Peptide Research and Therapeutics.

Erbersdobler, H. F., and V. Somoza. 2007. Forty years of furosine - forty years of using Maillard reaction products as indicators of the nutritional quality of foods. Molecular nutrition & food research 51: 423-430.

Eric, K., L. V. Raymond, M. Huang, M. J. Cheserek, K. Hayat, N. D. Savio, M. Amédée, and X. Zhang. 2013. Sensory attributes and antioxidant capacity of Maillard reaction products derived from xylose, cysteine and sunflower protein hydrolysate model system. Food Research International. 54:1437–1447. doi:10.1016/j.foodres.2013.09.034.

Fan, H., Q. Xu, H. Hong, and J. Wu. 2018. Stability and Transport of Spent Hen-Derived ACE-Inhibitory Peptides IWHHT, IWH, and IW in Human Intestinal Caco-2 Cell Monolayers. Journal of Agricultural and Food Chemistry 66: 11347-11354.

Fontoura, R. et al. 2014. Production of feather hydrolysates with antioxidant, angiotensin-I converting enzyme-and dipeptidyl peptidase-IV-inhibitory activities. New biotechnology 31: 506-513.

Gallego, M. et al. 2016. Transepithelial transport of dry-cured ham peptides with ACE inhibitory activity through a Caco-2 cell monolayer. Journal of Functional Foods 21: 388-395.

Giongo, J. L. 2006. Caracterização e aplicação de proteases produzidas por linhagens de Bacillus sp.

Guyton and Hall Textbook of Medical Physiology.

Hou, Y., Z. Wu, Z. Dai, G. Wang, and G. Wu. 2017. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. Journal of Animal Science and Biotechnology 8: 24.

Huang, J. et al. 2016. Angiotensin-I-Converting Enzyme Inhibitory Activities and In Vivo Antihypertensive Effects of Sardine Protein Hydrolysate. J Food Sci 81: H2831-H2840.

Hulshof, T. G., P. Bikker, A. F. van der Poel, and W. H. Hendriks. 2016. Assessment of protein quality of soybean meal and 00-rapeseed meal toasted in the presence of lignosulfonate by amino acid digestibility in growing pigs and Maillard reaction products. Journal of animal science 94: 1020-1030.

Kang, N. J. et al. 2020. New approaches towards the discovery and evaluation of bioactive peptides from natural resources. Critical Reviews in Environmental Science and Technology 50: 72-103.

Lafarga, T., and M. Hayes. 2014. Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Science 98: 227-239.

Larsen, J. A., A. J. Fascetti, C. C. Calvert, and Q. R. Rogers. 2010. Bioavailability of lysine for kittens in overheated casein is underestimated by the rat growth assay method. J Anim Physiol Anim Nutr (Berl) 94: e102-108.

Larsen, J. A., C. C. Calvert, and Q. R. Rogers. 2002. Processing of Dietary Casein Decreases Bioavailability of Lysine in Growing Kittens. The Journal of Nutrition 132: 1748S-1750S.

Lasekan, A., F. Abu Bakar, and D. Hashim. 2013. Potential of chicken by-products as sources of useful biological resources. Waste Management 33: 552-565.

Laurent, S. 2017. Antihypertensive drugs. Pharmacological Research 124: 116-125.

Lázaro, C. A. et al. 2015. Biogenic amines as bacterial quality indicators in different poultry meat species. LWT - Food Science and Technology 60: 15-21.

Ledl, F., and E. Schleicher. 1990. New aspects of the Maillard reaction in foods and in the human body. Angewandte Chemie International Edition in English 29: 565-594.

Liu, J., M. Liu, C. He, H. Song, and F. Chen. 2015. Effect of thermal treatment on the flavor generation from Maillard reaction of xylose and chicken peptide. LWT - Food Science and Technology. 64:316–325. doi:10.1016/j.lwt.2015.05.061.

Lopez-Barrios, L., J. A. Gutierrez-Uribe, and S. O. Serna-Saldivar. 2014. Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients. J Food Sci 79: R273-283.

López-Sánchez, J., E. Ponce-Alquicira, R. Pedroza-Islas, A. de la Peña-Díaz, and J. Soriano-Santos. 2016. Effects of heat and pH treatments and in vitro digestion on the biological activity of protein hydrolysates of Amaranthus hypochondriacus L. grain. Journal of Food Science and Technology 53: 4298-4307.

Mane, S., and S. N. Jamdar. 2017. Purification and identification of Ace-inhibitory peptides from poultry viscera protein hydrolysate. Journal of Food Biochemistry 41: e12275.

Manninen, A. H. 2009. Protein hydrolysates in sports nutrition. Nutrition & Metabolism 6: 38.

Martínez-Alvarez, O., S. Chamorro, and A. Brenes. 2015. Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. Food Research International. 73:204–212. doi:10.1016/j.foodres.2015.04.005.

Martins, S. I. F. S., W. M. F. Jongen, and M. A. J. S. van Boekel. 2001. A review of Maillard reaction in food and implications to kinetic modelling.

Mas-Capdevila, A., Z. Pons, A. Aleixandre, F. Bravo, and B. Muguerza. 2018. Dose-Related Antihypertensive Properties and the Corresponding Mechanisms of a Chicken Foot Hydrolysate in Hypertensive Rats. Nutrients 10: 1295.

Meeker, D., and C. Hamilton. 2006. An overview of the rendering industry. Essential rendering: 1-16.

Meeker, D., and J. Meisinger. 2015. COMPANION ANIMALS SYMPOSIUM: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. Journal of animal science 93: 835-847.

Möller, N. P., K. E. Scholz-Ahrens, N. Roos, and J. Schrezenmeir. 2008. Bioactive peptides and proteins from foods: indication for health effects. European Journal of Nutrition 47: 171-182.

NAKADE, K. et al. 2008. Identification of an antihypertensive peptide derived from chicken bone extract. Animal Science Journal 79: 710-715.

Nie, X., D. Xu, L. Zhao, and X. Meng. 2017. Antioxidant activities of chicken bone peptide fractions and their Maillard reaction products: Effects of different molecular weight distributions. International Journal of Food Properties 20: S457-S466.

O'Brien, J., P. A. Morrissey, and J. M. Ames. 1989. Nutritional and toxicological aspects of the Maillard browning reaction in foods. Critical Reviews in Food Science and Nutrition 28: 211-248.

Onuh, J. O., A. T. Girgih, S. A. Malomo, R. E. Aluko, and M. Aliani. 2015. Kinetics of in vitro renin and angiotensin converting enzyme inhibition by chicken skin protein hydrolysates and their blood pressure lowering effects in spontaneously hypertensive rats. Journal of Functional Foods 14: 133-143.

Poulsen, M. W. et al. 2013. Advanced glycation endproducts in food and their effects on health. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 60: 10-37.

Rao, M. B., A. M. Tanksale, M. S. Ghatge, and V. V. Deshpande. 1998. Molecular and Biotechnological Aspects of Microbial Proteases. Microbiology and Molecular Biology Reviews 62: 597-635.

Ribeiro, L. B. et al. 2019. Oxidative stability and nutritional quality of poultry by-product meal: An approach from the raw material to the finished product. Animal Feed Science and Technology 255: 114226.

Ryan, J. T., R. P. Ross, D. Bolton, G. F. Fitzgerald, and C. Stanton. 2011. Bioactive Peptides from Muscle Sources: Meat and Fish. Nutrients 3: 765-791.

Sangsawad, P. et al. 2018. Transepithelial transport and structural changes of chicken angiotensin I-converting enzyme (ACE) inhibitory peptides through Caco-2 cell monolayers. Journal of Functional Foods 45: 401-408.

Shi, C. et al. 2019. Effect of processing conditions and simulated gastrointestinal digestion on the activity of angiotensin I-converting enzyme (ACE) inhibitory peptide derived from duck meat hydrolysate. CyTA - Journal of Food 17: 393-399.

Sica, D. A. 2004. Angiotensin-converting enzyme inhibitors side effects--physiologic and non-physiologic considerations. Journal of clinical hypertension (Greenwich, Conn.) 6: 410-416.

Soladoye, O. P., J. Saldo, L. Peiro, A. Rovira, and M. Mor-Mur. 2015. Antioxidant and angiotensin 1 converting enzyme inhibitory functions from chicken collagen hydrolysates. Journal of Nutrition & Food Sciences 5: 1.

Steele, R. 2004. Understanding and measuring the shelf-life of food. Woodhead Publishing.

Tjernsbekk, M. T., A. H. Tauson, O. F. Kraugerud, and Ahlstrøm. 2017. Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility. Journal of Animal Physiology and Animal Nutrition. 101:e323–e331. doi:10.1111/jpn.12608.

Tjernsbekk, M. T., A.-H. Tauson, † C F Matthiesen, and Ø. Ahlstrøm. 2016. protein and amino acid bioavailability of extruded dog food with protein meals of different quality using growing mink (Neovison vison) as a model 1,2. J. Anim. Sci. 94:3796–3804. doi:10.2527/jas2016-0526.

Udenigwe, C. C., and V. Fogliano. 2017. Food matrix interaction and bioavailability of bioactive peptides: Two faces of the same coin? Journal of Functional Foods 35: 9-12.

van Boekel, M. A. J. S. 2006. Formation of flavour compounds in the Maillard reaction. Biotechnology Advances 24: 230-233.

van Rooijen, C. et al. 2013. The Maillard reaction and pet food processing: effects on nutritive value and pet health. Nutrition research reviews 26: 130-148.

van Rooijen, C. et al. 2014. Quantitation of Maillard reaction products in commercially available pet foods. Journal of agricultural and food chemistry 62: 8883-8891.

Wu, H., Z. Wang, C. Han, Z. Peng, and Y. Chen. 2010. Factors affecting the Maillard reaction. Modern Food Science and Technology 26: 441-444, 440.

Yusop, S. M. et al. 2016. Production of Antihypertensive Elastin Peptides from Waste Poultry Skin. International Journal of Food Engineering 2.

III. OBJETIVO GERAL

O objetivo do estudo foi avaliar as propriedades funcionais do hidrolisado de vísceras de aves em dieta extrusada para gatos, como também seus efeitos na digestibilidade da dieta, palatabilidade e formação dos produtos da reação de Maillard.

IV. Effects of enzymatically hydrolysed poultry byproduct meal in extruded diets on serum angiotensin-converting enzyme activity and aldosterone in cats

Archives of Animal Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gaan20

Effects of enzymatically hydrolysed poultry byproduct meal in extruded diets on serum angiotensin-converting enzyme activity and aldosterone in cats

Tânia Zóia Miltenburg , Mayara Uana da Silva , Guido Bosch & Ricardo Souza Vasconcellos

To cite this article: Tânia Zóia Miltenburg , Mayara Uana da Silva , Guido Bosch & Ricardo Souza Vasconcellos (2020): Effects of enzymatically hydrolysed poultry byproduct meal in extruded diets on serum angiotensin-converting enzyme activity and aldosterone in cats, Archives of Animal Nutrition, DOI: 10.1080/1745039X.2020.1849899

To link to this article: https://doi.org/10.1080/1745039X.2020.1849899

Published online: 13 Dec 2020.

Effects of enzymatically hydrolyzed poultry byproduct meal in extruded diets on serum angiotensin-converting enzyme activity and aldosterone in cats

(Published: Archives of Animal Nutrition)

ABSTRACT

Several peptides found in hydrolyzed poultry byproduct meal can inhibit angiotensin-converting enzyme (ACE) activity, a property that indicates potential antihypertensive and health-promoting effects. This study aimed to assess the effects of extruded diets containing enzymatically hydrolyzed poultry byproduct meal (HPM) on cat serum ACE activity and aldosterone (ALD) concentration, nutrient digestibility, and fecal characteristics. On the basis of a preliminary in vitro ACE inhibitory activity assay, we selected a commercial HPM and a commercial conventional poultry byproduct meal (CPM) for further investigation. Two isoenergetic and isonitrogenous diets were formulated: CPM diet (25.7% CPM) and HPM diet (24.7% HPM). In trial 1, the effect of diet on serum ACE activity and ALD concentration was evaluated using 8 healthy cats (4 female and 4 male, 4.1 ± 0.38 kg BW) in a crossover design, with 5 d of adaptation and blood collection on d 6. In trial 2, apparent total tract digestibility and fecal characteristics were evaluated using 12 cats (6 female and 6 male, 4.0 ± 0.72 kg BW) in a completely randomized design. Serum ACE and ALD were analyzed using a mixed model, with diet as the fixed effect and cat as the random effect. Data from trial 2 were subjected to analysis of variance, and means were compared by Tukey's test. In vitro ACE inhibitory activity of HPM (90.4%) was higher than that of CPM (52.0%). Cats fed the HPM diet tended to have lower serum ACE activity than those fed the CPM diet (126.1 versus 142.1 U/L, $p = 0.09$). Serum ALD was not influenced by diet ($p > 0.05$). Diets had similar digestibility values, and fecal consistency scores tended to be

higher (firmer feces) in cats fed the CPM diet than in cats fed the HPM diet (4.6 versus 4.0, $p = 0.09$). Inclusion of HPM in extruded diets may reduce cat serum ACE activity and promote good fecal consistency without affecting digestibility. Further investigations are needed to explore the potential health benefits of HPM in hypertensive cats.

KEYWORDS: antihypertensive, bioactive peptide, cat, enzyme, hydrolysate, poultry byproduct

1. INTRODUCTION

Poultry byproduct meal (PBM) is the main protein source used by pet food industries, as it provides high-quality protein with good amino acid balance and is a sustainable, low-cost ingredient (Meeker and Meisinger, 2015). PBM is conventionally obtained by high-temperature treatment of byproducts, followed by pressing, drying, and grinding (Meeker and Hamilton, 2006). Currently, new processing methods involving the use of proteases are being investigated to improve final product quality and enhance bioactivity (Brandelli et al., 2015; Mas-Capdevila et al., 2019).

Enzymatic hydrolysis of proteins results in the release of small peptides, some of which have the capacity to inhibit angiotensin-converting enzyme (ACE). ACE plays a role in the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor and stimulator of aldosterone (ALD) secretion belonging to the renin–angiotensin–ALD system. Antihypertensive drugs such as enalapril and benazepril act by inhibiting ACE activity; however, the drugs cause side effects in humans, motivating the search for natural sources of ACE inhibitors (Mas-Capdevila et al., 2018).

Hypertension is common in elderly cats, affecting 13% of healthy cats aged 9 years and older (Conroy et al., 2018). Because this condition is frequently associated with an underlying disease, its prevalence reaches 87% among cats with concurrent disorders (Acierno et al., 2018). Enzymatically hydrolyzed PBM is a potentially functional ingredient that could be used by pet food industries for the formulation of clinical diets for hypertensive animals.

In addition to their potential functional properties, hydrolyzed proteins are easily digested and absorbed and increase amino acid availability after passage through the upper gastrointestinal tract compared with intact proteins (Kleinnijenhuis et al., 2019;

Koopman et al., 2009). It is hypothesized that cat food containing hydrolyzed PBM has higher protein digestibility than food containing conventional PBM. However, it must be considered that protein hydrolysates may increase osmolarity compared with intact proteins and that diets containing hydrolyzed PBM might influence the moisture content of digesta and, ultimately, the quality of feces (Cave, 2006).

This study aimed to investigate the effects of extruded diets containing enzymatically hydrolyzed PBM on cat serum ACE activity and ALD concentration, diet nutrient digestibility, and fecal characteristics.

2. MATERIALS AND METHODS

In a preliminary investigation, we evaluated four PBM: a conventional and an enzymatically hydrolyzed formulation prepared at the pilot processing facilities of the State University of Maringá, Brazil, and a conventional and an enzymatically hydrolyzed formulation obtained commercially. PBM samples were screened in vitro for ACE inhibitory activity, and the most active conventional and hydrolyzed PBM (commercial products) were selected for further investigation in vivo. Two trials were conducted, the first to assess ACE activity and ALD serum concentration and the second to determine diet digestibility and fecal characteristics.

2.1. Preparation of PBM

Two experimental PBM were prepared by similar procedures, except that one included an enzyme treatment step prior to digestion. Chicken viscera, offal (head and bones removed), and meat were obtained from a local slaughterhouse (Abatedouro Coroaves Ltd., Maringá, Brazil) and separated into two equal batches. To the first batch was added a commercial proteolytic enzyme preparation from *Bacillus licheniformis*

(Protezyn APP 3000, Prozyn, São Paulo, Brazil) at 0.03% (fresh matter basis). The mixture was homogenized and heated at 55 °C for 45 min in a digestor. After this step, the temperature was set at 105 °C for 2 h. The second batch was cooked at 105 °C for 2 h without pre-digestion. Cooked batches were pressed using a laboratory-scale screw press to expel the poultry fat, and the resulting cakes were dried in a forced ventilation oven at 80 °C for 24 h. After drying, both materials were ground in a hammer mill equipped with a 2.5 mm sieve.

The processing procedures of conventional (low-ash poultry byproduct meal, BRF S.A., Concórdia, Brazil) and enzymatically hydrolyzed (chicken protein hydrolysate, BRF S.A., Concórdia, Brazil) PBM obtained commercially were not fully disclosed by the manufacturer. In general, conventional PBM is produced from viscera, offal, cartilage, and meat, which are cooked in a digestor, pressed, and ground. Hydrolyzed PBM is generally prepared from viscera, liver, and meat previously hydrolyzed in an enzymatic reactor; after hydrolysis, the soluble phase is separated from the fat fraction and insoluble residues, then dried, and ground.

The chemical composition (dry matter, N, acid-hydrolyzed fat, and ash contents) of meals was determined according to AOAC procedures (2006). Total amino acids, except tryptophan, were analyzed by high-performance liquid chromatography (Agilent 1200 Series, Santa Clara, United States) on a LUNA 00G-4252-E0 C18 (100 Å, 5 µm, 250 × 4.6 mm) column, according to White et al. (1986). The absorbance was read at 254 nm. All analyses were performed in duplicate. Amino acid contents were converted to a crude protein basis (N × 6.25). For this, the analyzed amino acid value (on a dry matter basis) was divided by the crude protein value of the respective ingredient.

2.2. In vitro ACE inhibitory activity

ACE inhibitory activity was evaluated by the method of Cushman and Cheung (1971), with some modifications. Five grams of PBM was added to a beaker containing 100 mL of distilled water, and the mixture was vortexed for 1 min. Then, a 10 mL aliquot was collected and centrifuged at 4000 rpm. The supernatant (20 µL) was added to 100 µL of a buffered substrate solution containing 5 mM *N*-hippuryl-histidyl-leucine (Hip-His-Leu, H1635, Sigma–Aldrich, St. Louis, USA), 50 mM HEPES-HCl buffer (H7006, Sigma–Aldrich, St. Louis, USA), and 0.3 M NaCl (pH 8.3) at 37 °C. Then, 40 µL of ACE solution (0.1 U/mL, A6778, Sigma–Aldrich, St. Louis, USA) was added and vortexed for 15 s. The reaction was carried out at 37 °C for 30 min and stopped by adding 150 µL of 1 M HCl. Then, the hippuric acid released by the reaction was extracted by adding 1 mL of ethyl acetate, vortexing for 30 s, and centrifuging at 1484.7 *g* (Z 323 K, Hermle LaborTechnik, Wehingen, Germany) for 10 min. The organic phase (750 µL) was transferred to a glass tube and heat-evaporated at 90 °C for 40 min. The residue was dissolved in 800 µL of distilled water and analyzed spectrophotometrically at 228 nm. ACE inhibitory activity was calculated according to Geng et al. (2016), as follows: Inhibitory activity (%) = $1 - (A - B)/(C - D) \times 100$, where A is the absorbance of the solution containing ACE and inhibitor, B is the absorbance of the solution containing inhibitor, C is the absorbance of the solution containing ACE without inhibitor, and D is the absorbance of the solution without ACE or inhibitor. Samples were analyzed in duplicate. Conventional and hydrolyzed PBM prepared in-house had ACE inhibitory activities of 47.8 and 69.6%, respectively, whereas, for conventional and hydrolyzed PBM obtained commercially, these values were 52.0 and 90.4%, respectively. As commercial ingredients had higher inhibitory activity, they were selected for in vivo experiments.

2.3. Diets

Two isoenergetic and isonitrogenous diets were formulated to meet the recommendations for nutrients defined by FEDIAF (2018). The CPM diet contained 25.75% of conventional PBM, and the HPM diet contained 24.72% of hydrolyzed PBM (Table 1). The metabolizable energy of the diets was estimated from the gross energy content and digestibility coefficient, which in turn were calculated from the digestible energy value (NRC, 2006).

Both diets were extruded using a single-screw extruder (Imbra 120, Inbramaq, Ribeirão Preto, Brazil). Extrusion parameters were preconditioner water flow rate of 250–300 mL/min, preconditioning temperature of 90–95 °C, motor load of 20–30 A, and kibble density of 330–380 g/L. After extrusion, kibbles were dried in a crawler-type dryer (G 3.130, Ferraz, Ribeirão Preto, Brazil). Poultry fat and palatants were sprayed on the kibbles under continuous mixing immediately after drying.

2.4. Animals, housing, and feeding

All animal experimental procedures were approved by the Animal Ethics Committee of the State University of Maringá, Paraná, Brazil (protocol no. 1738040618). The study was conducted at the Laboratory of Nutrition and Metabolism of Domestic Cats of the State University of Maringá.

All cats were mixed breed, neutered, aged 5 to 6 years, vaccinated, dewormed, and free of ectoparasites. Their body condition score ranged from 5 to 6 (Laflamme, 1997). Each animal was fed according to its maintenance energy requirement (MER), calculated as MER [kcal] = 75 × kg body weight $(BW)^{0.67}$. Food was weighed daily and provided in two equal meals. Water was provided *ad libitum*. Leftover food was weighed every day for determination of food intake.

The first trial, aimed at determining the effects of diets on serum ACE activity and ALD concentration, lasted 12 days. Cats were housed in a cattery (49 m^2) with access to an outdoor area (49 m^2) for socialization and physical activities. The space was environmentally enriched with shelves at various heights, toys, and tree trunks (outside). At the time of feeding (8:00 and 14:00), cats were housed in individual metabolic cages (0.5 × 0.5 × 0.6 m) for 120 min.

The second trial, conducted to assess diet digestibility, lasted 10 days (5 days for adaptation and 5 days for sample collection). During the first 3 days, animals were housed under the same conditions as mentioned above. On the last 2 days of adaptation and during the 5 days of collection, the animals were housed full time in individual metabolic cages.

2.5. Trial 1: Serum ACE activity and ALD concentration

Eight cats (4 male and 4 female, 4.1 ± 0.38 kg BW) were randomly assigned by sex to two groups and fed the diets in a crossover design. The experimental period was 12 days (5 days for adaptation to each diet and one day for blood collection). Blood collection was performed at the jugular vein 6 h after morning feeding, which occurred at 08:00. For this procedure, the animals were maintained in lateral recumbency. Blood (3 mL) was collected by using a 25 × 0.5 mm needle and immediately transferred to coagulation tubes. Samples were centrifuged at 1750 *g* for 10 min, and sera were stored at −20 °C until analysis.

Serum ACE was measured following the fluorometric method reported by Yang and Neff (1972), with some modifications. Briefly, triplicate serum aliquots (10 µL) were incubated for 30 min at 37 °C with 490 µL of ACE substrate (5 mM Hip-His-Leu buffer). Then, 1.2 mL of 0.34 N NaOH was added to stop the reaction. In the next step,

100 µL of 2% phthaldialdehyde (P0657, Sigma–Aldrich, St. Louis, USA) in methanol was added to the solution and incubated for 10 min. The reaction was stopped by adding 200 µL of 3 N HCl. Tubes were centrifuged at 3000 rpm for 10 min, and the generated product, His-Leu, was measured fluorometrically (Shimadzu, RF-1501, Kyoto, Japan) in the supernatant using 365 nm excitation and 495 nm emission filters. Commercial His-Leu solutions at different concentrations were used to obtain a calibration curve. Results are expressed in units (U) of ACE per liter of serum and are the mean of at least three replications.

Serum ALD was measured by a chemiluminescence method (Stabler & Siegel, 1991) by a private laboratory specialized in veterinary analyses (VetLab, Análises Clínicas Veterinárias, Rio de Janeiro, Brazil).

2.6. Trial 2: Apparent total tract digestibility, metabolizable energy, and fecal characteristics

The coefficients of apparent total tract digestibility of nutrients were determined by the method of total feces collection (without urine collection), according to AAFCO (2014). Metabolizable energy (ME) was determined on a dry matter basis by using a correction factor for cats, as shown in the equation: ME [kcal/kg] = [(total ration consumed × gross energy of ration) – (total feces produced × gross energy of feces) – (0.86 × digestible protein consumed)]/total ration consumed.

Twelve cats (6 male and 6 female, 4.0 ± 0.72 kg BW) were divided by sex into two groups, one assigned to the CPM diet and the other to the HPM diet. On the first day of feces collection, all feces produced before 08:00 were collected and discarded. Feces excreted thereafter were collected for 5 consecutive days. Fecal samples were scored for consistency, weighed, placed in a separate plastic bag for each animal, and

frozen (−15 °C). Fecal consistency was scored from 1 (watery feces) to 5 (hard, dry pellets) (Carciofi et al., 2008). At the end of the collection period, feces were thawed and dried in a forced-air oven (MA035, Marconi, Piracicaba, Brazil) at 55 °C for 72 h. Each sample was milled separately through a 1 mm Wiley mill screen (MA340, Marconi, Piracicaba, Brazil) and homogenized for subsequent analyses. Foods were subjected to the same milling procedure.

Dry matter, crude ash, N, acid-hydrolyzed fat, and crude fiber contents were determined in diets and feces according to AOAC procedures (2006). Gross energy was determined by using an adiabatic calorimetric pump (6200 Isoperibol, Parr Instrument Company, Moline, USA). Apparent total tract nutrient digestibility [%] was calculated as the difference between nutrient intake [g/day] and nutrient excreted in feces [g/day] divided by nutrient intake [g/day] and multiplied by 100.

2.7. Statistical analyses

For ACE and ALD variables, the data were analyzed using a mixed model with diet as fixed effect and cat as random effect. Analyses were performed in SAS software version 9.4 (SAS Institute, Cary, NC, USA). When significant differences were found by the *F*-test, means were compared by the Tukey–Kramer test. Data from digestibility and fecal assays were subjected to analysis of variance followed by Tukey's test for mean comparisons in the R environment (R version 3.2.4, R Core Team, 2016). Probability values lower than 5% were considered to be significant, and those between 5 and 10% were treated as a tendency.

3. RESULTS

3.1. Chemical composition of PBM formulations

Hydrolyzed PBM had higher crude protein content and lower ash and crude fat contents than conventional PBM (Table 2). However, conventional PBM proteins were richer in total indispensable and dispensable amino acids than hydrolyzed PBM proteins. Lysine and taurine concentrations were higher in hydrolyzed PBM.

3.2. Trial 1: Serum ACE activity and ALD

Serum ACE activity tended to be lower in cats fed the HPM diet (126.1 U/L) than in those fed the CPM diet (142.1 U/L) ($p = 0.094$). Serum ALD concentrations did not differ between dietary treatments ($p = 0.507$) (Fig. 1).

3.3. Trial 2: Nutrient and gross energy intake, apparent total tract digestibility, metabolizable energy, and fecal characteristics

Diets had no effect on nutrient or gross energy intake ($p \geq 0.408$, Table 3), apparent total tract digestibility coefficients ($p \geq 0.327$), metabolizable energy ($p = 0.929$), fecal dry matter ($p = 0.417$), or daily fecal output ($p = 0.280$). Fecal score tended to be lower in the HPM diet group ($p = 0.099$).

4. DISCUSSION

In recent years, there has been an increasing number of studies on the bioactive properties of peptides from hydrolyzed dietary proteins (Corrêa et al., 2014; Geng et al., 2016; Mas-Capdevila et al., 2019; Neves et al., 2017). Many of these peptides exhibit antihypertensive properties for their capacity to inhibit ACE. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor and stimulator of ALD secretion whose end result is the increase in blood pressure (Mentz et al., 2013) especially by its capacity to improve water reabsorption in the nefrons. Several antihypertensive drugs act by

inhibiting ACE but cause unwanted side effects (Spiller, 2014). Natural sources of ACE inhibitors are, therefore, being investigated as alternatives to alleviate hypertension in humans.

In cats, systemic hypertension is well documented and can cause significant morbidity and mortality (Rishniw, 2012). Feline hypertension is often associated with underlying diseases (≥80%, based on current data), such as renal disease and hyperthyroidism. Unlike in humans, obesity is not a main risk factor for hypertension in cats (Acierno et al., 2018; Jordan et al., 2008). Treatment consists in the administration of antihypertensive drugs combined with other treatments for associated conditions, when necessary. The most common drugs used for feline hypertension are calcium channel blockers. ACE inhibitors are used as a secondary treatment in animals that are refractory to high doses of first-line drugs (Acierno et al., 2018; Rishniw, 2012). Therefore, hydrolyzed proteins with ACE inhibitory activity could be an interesting ingredient to include in diets for animals with hypertension or other diseases that may lead to the condition. Here, we investigated this potential by assessing in vitro ACE inhibitory activity as well as in vivo effects of a diet containing hydrolyzed PBM on serum ACE activity and ALD concentration in healthy cats.

The in vitro ACE inhibitory activity of hydrolyzed PBM has been confirmed in previous reports (Bezerra et al., 2019; dos Santos Aguilar et al., 2019; Mane and Jamdar, 2017; Soladoye et al., 2015; Yusop et al., 2016). In the current study, preliminary in vitro analysis showed that hydrolyzed commercial and experimental PBM inhibited ACE activity by 90.4 and 69.6%, respectively. This effect was much higher than that obtained by conventionally processed PBM (50% ACE inhibition). Values were in line with those for hydrolyzed poultry viscera (70.5 to 83.7%; dos Santos Aguilar et al., 2019) and ultra-filtered elastin from broiler skin (88.2%; Yusop et

al., 2016). The capacity of peptides to inhibit ACE is related to their length and amino acid composition. The most effective peptides share common structural properties, including relatively short length (usually 2–12 amino acids), hydrophobicity, and presence of arginine, lysine, and proline. Protein source, enzyme, and processing conditions play a key role in the bioactivity of peptides (Bechaux et al., 2019; Mine et al., 2010). The results of this study show that enzymatic hydrolysis was crucial in obtaining bioactive peptides, as hydrolyzed PBM formulations clearly differed in in vitro ACE inhibitory capacity.

For in vivo functionality, PBM peptides must be resistant to extrusion and drying, common processes used to manufacture dry cat foods. Cian et al. (2011) observed that in vitro ACE inhibitory activity of hydrolyzed bovine hemoglobin decreased from 70 to 40% when the material was mixed (5 g/kg) and extruded with maize. The reduction in activity might, however, have been caused by dilution of bioactive peptides following mixture with maize rather than by extrusion. After ingestion, peptides must then resist to variations in pH and the action of gastrointestinal enzymes, be absorbed by enterocytes, transported to the blood, and arrive intact at the target site in sufficient quantities (Lopez-Barrios et al., 2014). Mane and Jamdar (2017) investigated the resistance of three antihypertensive peptides from poultry viscera hydrolysate to gastrointestinal enzymes (pepsin, trypsin, and chymotrypsin) and observed that peptides had 82% residual activity, indicating their in vivo potential as antihypertensive agents.

The in vivo ACE inhibitory activity of dietary peptides has mainly been investigated in rats (Deng et al., 2018; Geng et al., 2016; Mas-Capdevila et al., 2019; Mas-Capdevila et al., 2018; Onuh et al., 2015), with no studies in cats. In our study, we found that the HPM diet tended to decrease serum ACE activity in healthy cats

compared with the CPM diet (126.1 versus 142.1 U/L). In spontaneously hypertensive rats, chicken foot hydrolysate administered by gastric intubation decreased plasma ACE activity by 21% (Mas-Capdevila et al., 2018). Further studies should explore inhibition of ACE activity in hypertensive cats, measure other parameters linked to hypertension, and evaluate the long-term effects of diets.

It was hypothesized that serum ALD concentrations would decrease in cats fed hydrolyzed PBM because of the decrease in ACE activity; however, this was not observed in the present study. Steele et al. (2002) found that the ACE inhibitory drug enalapril (0.5 mg/kg BW daily for 7 days) did not affect serum ALD concentrations in hypertensive cats. According to the authors, it is possible that neurohormonal mechanisms prevent the decrease of hormones such as ALD, resulting in the maintenance of intravascular volume. Such effects can be attributed to alternative angiotensin II-generating pathways (e.g., via chymase and tonin), which are not affected by ACE inhibitors (Resende and Mill, 2002), or to basal and pulsatile ALD secretion, which is not influenced by the renin–angiotensin–ALD axis (Fliser et al., 1998). Thus, inhibition of ACE alone was not sufficient to cause a reduction in serum ALD concentrations.

We expected the apparent total tract digestibility coefficient of HPM proteins to be higher than that of CPM proteins, as hydrolyzed proteins are more easily absorbed. Furthermore, HPM proteins contained lower amounts of hydroxyproline and glycine, suggesting lower concentrations of collagen-rich connective tissues that are difficult to digest (Donadelli et al., 2019). However, CPM and HPM diets showed similar and high (>90%) digestibility coefficients for protein and other parameters. This is the first investigation of the digestibility of diets containing enzymatically hydrolyzed PBM in cats. In mink (*Neovison vison*), used as a model for dogs, the apparent total tract

digestibility of crude protein of an extruded food containing salmon protein hydrolysate was similar to that of food containing PBM at 25% inclusion (79.0 and 80.3%, respectively; Tjernsbekk et al., 2017). These results were explained by differences in the origin and processing of ingredients and the effects of extrusion on proteins (Tjernsbekk et al., 2017). For instance, hydrolyzed proteins are more susceptible to cross-linking of amino acids and Maillard reactions during heat treatment, which can decrease protein digestibility (van Rooijen, 2014). It is important to point out that cat endogenous proteases are capable of hydrolyzing most types of proteins and that the source of conventional PBM (i.e., intact proteins) used in the present and other studies was of high quality. A substantial difference in digestibility would probably be observed between intact and hydrolyzed forms of difficult-to-digest proteins, such as keratin (Hekman, 2003). Not only digestibility but also the rate of digestion can differ between conventional and hydrolyzed PBM. For example, hydrolyzation increased the rate of absorption of egg white and whey in portal blood of healthy rats without affecting total tract protein digestibility (Matsuoka et al., 2019).

Finally, hydrolyzed protein might increase digesta osmolarity, leading to soft stools or diarrhea. Cats fed the HPM diet had good fecal scores (score of 4), and no episodes of diarrhea were observed. Verlinden et al. (2006) found that the fecal score of dogs was not affected by ingestion of commercial dry food containing hydrolyzed chicken liver and meat. According to Cave (2006), it is difficult to predict the effect of diets containing hydrolyzed PBM on intestinal luminal osmolarity, as the parameter may be affected by other ingredients. Gastric emptying rate is influenced by many factors, including chyme osmolarity. When hypotonic or hypertonic stomach fluids enter the duodenum, hormonal feedback inhibits gastric emptying, avoiding fast flow of nonisotonic fluids into the small intestine (Hall and Guyton, 2011).

5. CONCLUSIONS

Inclusion of enzymatically hydrolyzed PBM in extruded diets for cats may reduce serum ACE activity and promote good fecal consistency without affecting digestibility. However, further investigations are needed to assess the effectiveness of hydrolyzed PBM in promoting health benefits in hypertensive cats and its long-term effects.

Acknowledgments

The authors thank Danês Alimentos, Kemin Industries Inc., and Prozyn BioSolutions for kindly providing and processing ingredients and diets.

Author contributions

TZM and RSV delineated the study; TZM and MU carried out the research; TZM and MU contributed with the analysis; RSV provided laboratory facilities; TZM, RSV, and GB contributed to data collection and statistical analyses; TZM, RSV, and GB wrote the paper. All authors consider themselves equally responsible for the content of the manuscript.

Disclosure statement

The authors state that there is no conflict of interest.

Funding

This study was supported by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES, grant no. 88882.181511/2018-01), and Premier Pet, Dourado, Brazil.

6. REFERENCES

Acierno, M. J. et al. 2018. ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. Journal of Veterinary Internal Medicine 32: 1803-1822. doi: 10.1111/jvim.15331

Association of American Feed Control Officials (AAFCO). 2014. Official publication. Oxford, IN: AAFCO.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 17th ed. Gaithersburg, MD: Association of Official Analytical Chemists.

Bechaux, J., P. Gatellier, J.-F. Le Page, Y. Drillet, and V. Sante-Lhoutellier. 2019. A comprehensive review of bioactive peptides obtained from animal byproducts and their applications. Food & Function 10: 6244-6266. doi: 10.1039/c9fo01546a.

Bezerra, T. K. A. et al. 2019. Identification of angiotensin I-converting enzyme-inhibitory and anticoagulant peptides from enzymatic hydrolysates of chicken combs and wattles. Journal of Medicinal Food. doi: 10.1089/jmf.2019.0066

Bollag, W. B. 2014. Regulation of aldosterone synthesis and secretion. Comprehensive Physiology 4: 1017-1055. doi: 10.1002/cphy.c130037

Brandelli, A., L. Sala, and S. J. Kalil. 2015. Microbial enzymes for bioconversion of poultry waste into added-value products. Food Research International 73: 3-12. doi: 10.1016/j.foodres.2015.01.015

Carciofi, A. C. et al. 2008. Effects of six carbohydrate sources on dog diet digestibility and post-prandial glucose and insulin response. Journal of Animal Physiology and Animal Nutrition 92: 326-336. doi: 10.1111/j.1439-0396.2007.00794.x

Cave, N. J. 2006. Hydrolyzed protein diets for dogs and cats. Veterinary Clinics: Small Animal Practice 36: 1251-1268. doi: 10.1016/j.cvsm.2006.08.008

Cian, R. E., P. Luggren, and S. R. Drago. 2011. Effect of extrusion process on antioxidant and ACE inhibition properties from bovine haemoglobin concentrate hydrolysates incorporated into expanded maize products. International Journal of Food Sciences and Nutrition 62: 774-780. doi: 10.3109/09637486.2011.582034

Conroy, M., Y. M. Chang, D. Brodbelt, and J. Elliott. 2018. Survival after diagnosis of hypertension in cats attending primary care practice in the United Kingdom. Journal of Veterinary Internal Medicine 32:1846–1855. doi:10.1111/jvim.15307.

Corrêa, A. P. F. et al. 2014. Hydrolysates of sheep cheese whey as a source of bioactive peptides with antioxidant and angiotensin-converting enzyme inhibitory activities. Peptides 61: 48-55. doi: 10.1016/j.peptides.2014.09.001

Cushman, D., and H. Cheung. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochemical Pharmacology 20: 1637-1648. doi: 10.1016/0006-2952(71)90292-9

Deng, Z. et al. 2018. Antihypertensive effects of two novel angiotensin I-converting enzyme (ACE) inhibitory peptides from *Gracilariopsis lemaneiformis* (Rhodophyta) in spontaneously hypertensive rats (SHRs). Mar Drugs 16. doi: 10.3390/md16090299

Donadelli, R. A., C. G. Aldrich, C. K. Jones, and R. S. Beyer. 2019. The amino acid composition and protein quality of various egg, poultry meal by-products, and

vegetable proteins used in the production of dog and cat diets. Poultry Science. 98:1371–1378. doi:10.3382/ps/pey462.

dos Santos Aguilar, J. G., A. K. S. de Souza, and R. J. S. de Castro. 2019. Enzymatic hydrolysis of chicken viscera to obtain added-value protein hydrolysates with antioxidant and antihypertensive properties. International Journal of Peptide Research and Therapeutics. doi: 10.1007/s10989-019-09879-3

Fliser, D., J. D. Veldhuis, R. Dikow, H. Schmidt-Gayk, and E. Ritz. 1998. Effects of acute ACE inhibition on pulsatile renin and aldosterone secretion and their synchrony. Hypertension 32:929-934. doi: 10.1161/01.HYP.32.5.929

Geng, X. et al. 2016. A *Tricholoma matsutake* peptide with angiotensin converting enzyme inhibitory and antioxidative activities and antihypertensive effects in spontaneously hypertensive rats. Scientific Reports 6: 24130. doi: 10.1038/srep24130

Hall, J. E., and A. C. Guyton. 2011. Guyton and Hall Textbook of Medical Physiology. Saunders/Elsevier.

Hekman, M. 2003. Research into causes of diarrhoea associated with the Hill's prescription diet Canine Z/D [trade-mark symbol] Ultra Allergen Free: a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science at Massey University, Palmerston North, New Zealand, Massey University.

Jordan, E., S. Kley, N. A. Le, M. Waldron, and M. Hoenig. 2008. Dyslipidemia in obese cats. Domestic Animal Endocrinology 35: 290-299. doi: 10.1016/j.domaniend.2008.05.008

Kleinnijenhuis, A. J. et al. 2019. Non-targeted and targeted analysis of collagen hydrolysates during the course of digestion and absorption. Analytical and Bioanalytical Chemistry. doi: 10.1007/s00216-019-02323-x

Koopman, R. et al. 2009. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. The American Journal of Clinical Nutrition 90: 106-115. doi: 10.3945/ajcn.2009.27474

Laflamme, D. 1997. Development and validation of a body condition score system for cats: a clinical tool. Feline Practice 25: 13-18.

Lopez-Barrios, L., J. A. Gutierrez-Uribe, and S. O. Serna-Saldivar. 2014. Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients. Journal of Food Science 79: R273-283. doi: 10.1111/1750-3841.12365

Mane, S., and S. N. Jamdar. 2017. Purification and identification of ACE-inhibitory peptides from poultry viscera protein hydrolysate. Journal of Food Biochemistry 41: e12275. doi: 10.1111/jfbc.12275

Mas-Capdevila, A. et al. 2019. Long-term administration of protein hydrolysate from chicken feet induces antihypertensive effect and confers vasoprotective pattern in diet-induced hypertensive rats. Journal of Functional Foods 55: 28-35. doi: 10.1016/j.jff.2019.02.006

Mas-Capdevila, A., Z. Pons, A. Aleixandre, F. Bravo, and B. Muguerza. 2018. Dose-related antihypertensive properties and the corresponding mechanisms of a chicken foot hydrolysate in hypertensive rats. Nutrients 10: 1295. doi: 10.3390/nu10091295

Matsuoka, R., H. Kurihara, N. Nishijima, Y. Oda, and A. Handa. 2019. Egg white hydrolysate retains the nutritional value of proteins and is quickly absorbed in rats. The Scientific World Journal 2019: 5475302. doi: 10.1155/2019/5475302

Meeker, D., and C. Hamilton. 2006. An overview of the rendering industry. Essential Rendering: 1-16.

Meeker, D., and J. Meisinger. 2015. COMPANION ANIMALS SYMPOSIUM: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. Journal of Animal Science 93: 835-847. doi: 10.2527/jas.2014-8524

Mentz, R. J. et al. 2013. The past, present and future of renin–angiotensin aldosterone system inhibition. International Journal of Cardiology 167: 1677-1687. doi: 10.1016/j.ijcard.2012.10.007

Mine, Y., E. Li-Chan, and B. Jiang. 2010. Biologically active food proteins and peptides in health: an overview. Bioactive proteins and peptides as functional foods and nutraceuticals. Hoboken: Wiley-Blackwell: 5-11.

Neves, A. C., P. A. Harnedy, M. B. O'Keeffe, and R. J. Fitz Gerald. 2017. Bioactive peptides from Atlantic salmon (Salmo salar) with angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory, and antioxidant activities. Food Chemistry 218: 396-405. doi: 10.1016/j.foodchem.2016.09.053

Nutrient requirements of dogs and cats (NRC). 2006. In: N. R. Council (ed.). p 398. The National Academy, Whashington, DC.

Onuh, J. O., A. T. Girgih, S. A. Malomo, R. E. Aluko, and M. Aliani. 2015. Kinetics of in vitro renin and angiotensin converting enzyme inhibition by chicken skin protein hydrolysates and their blood pressure lowering effects in spontaneously hypertensive rats. Journal of Functional Foods 14: 133-143. doi: 10.1016/j.jff.2015.01.031

Resende, M. M. d., and J. G. Mill. 2002. Alternate angiotensin II-forming pathways and their importance in physiological or physiopathological conditions. Arquivos Brasileiros de Cardiologia 78: 432-438. doi: 10.1590/s0066-782x2002000400012

Rishniw, M. 2012. Cardiovascular diseases: feline hypertension and heart disease. In: S. E. Little (ed.) The Cat: Clinical Medicine and Management. p 307. Elsevier Saunders.

Rouanet, J., Z. Infante, B. Caporiccio, and C. Pejoan. 1990. Nutritional value and intestinal effects of dipeptides and tripeptides. Annals of Nutrition and Metabolism 34: 175-182. doi: 10.1159/000177584

Soladoye, O. P., J. Saldo, L. Peiro, A. Rovira, and M. Mor-Mur. 2015. Antioxidant and angiotensin 1 converting enzyme inhibitory functions from chicken collagen hydrolysates. Journal of Nutrition & Food Sciences 5: 1. doi: 10.4172/2155-9600.1000369

Spiller, H. A. (2014). Angiotensin converting enzyme (ACE) inhibitors. Encyclopedia of Toxicology, 14–16. doi:10.1016/b978-0-12-386454-3.00686-2

Stabler, T. V, and Siegel, A. L. (1991). Chemiluminescence immunoassay of aldosterone in serum. Clinical Chemistry, 37: 11, 1987–1989. doi.org/10.1093/clinchem/37.11.1987

Steele, J. L., R. A. Henik, and R. L. Stepien. 2002. Effects of angiotensin-converting enzyme inhibition on plasma aldosterone concentration, plasma renin activity, and blood pressure in spontaneously hypertensive cats with chronic renal disease. Veterinary Therapeutics : Research in Applied Veterinary Medicine 3: 157-166.

The European Pet Food Industry Federation (FEDIAF). 2018. Nutritional guidelines for complete and complementary pet food for cats and dogs. Bruxelles: FEDIAF (feed industry association).

Tjernsbekk, M., A. H. Tauson, O. Kraugerud, and Ø. Ahlstrøm. 2017. Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility. Journal of Animal Physiology and Animal Nutrition 101: e323-e331. doi: 10.1111/jpn.12608

van Rooijen, C. et al. 2014. Quantitation of Maillard reaction products in commercially available pet foods. Journal of Agricultural and Food Chemistry 62: 8883-8891. doi: 10.1021/jf502064h

Verlinden, A., M. Hesta, J. M. Hermans, and G. P. Janssens. 2006. The effects of inulin supplementation of diets with or without hydrolysed protein sources on digestibility, faecal characteristics, haematology and immunoglobulins in dogs. The British Journal of Nutrition 96: 936-944. doi: 10.1017/bjn20061912

White, J. A., R. J. Hart, and J. C. Fry. 1986. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. The Journal of Automatic Chemistry 8: 170-177. doi: 10.1155/S1463924686000330

Yang, H. Y., and N. H. Neff. 1972. Distribution and properties of angiotensin converting enzyme of rat brain. Journal of Neurochemistry 19: 2443-2450. doi: 10.1111/j.1471-4159.1972.tb01298.x

Yusop, S. M. et al. 2016. Production of antihypertensive elastin peptides from waste poultry skin. International Journal of Food Engineering 2. doi: 10.18178/ijfe.2.1.21-25

Table 1. Ingredients and chemical composition of experimental diets containing conventional (CPM) or hydrolyzed (HPM) poultry byproduct meal.

	Diet	
	CPM	HPM
Ingredients [%]		
Conventional poultry byproduct meal	25.75	-
Hydrolyzed poultry byproduct meal	-	24.72
Corn grain	25.40	24.40
Corn gluten meal (60% crude protein)	14.12	13.56
Broken rice	20.00	19.20
Cellulose	1.99	1.91
Soybean hulls	0.97	0.97
Liquid palatant*	2.00	1.92
Powder palatant*	1.00	0.96
Poultry fat	6.99	8.97
Potassium chloride	0.76	0.76
Sodium chloride	0.50	0.50
Taurine	0.10	0.10
Mineral and vitamin premix†	0.40	0.40
Calcium carbonate	-	1.61
Synthetic antioxidant§	0.02	0.02
Analyzed chemical composition [% dry matter]		
Dry matter [% as is]	92.9	93.3
Crude protein	35.8	36.5
Acid-hydrolyzed fat	12.6	12.5
Ash	5.9	5.6
Crude fiber	3.3	3.2
Gross energy [kcal/kg dry matter]	5,153	5,116

* Liquid and powder palatants produced by AFB International, São Paulo, Brazil. † Vaccinar Gatos Premix (Vaccinar, Minas Gerais, Brazil). Provided per kilogram of diet: vitamin A acetate, 9,000 IU; vitamin B_1 (thiamine mononitrate), 5 mg; vitamin B_{12}, 20 mcg; vitamin B_2, 4 mg; vitamin B_6 (pyridoxine hydrochloride), 4 mg; vitamin D_3, 750 IU; vitamin E (DL-alpha tocopherol), 30 IU; vitamin K_3, 0.1 mg; pantothenic acid (D-calcium pantothenate), 5 mg; folic acid, 0.8 mg; biotin, 0.07 mg; copper sulphate, 5 mg; choline, 0.24 g; iron (ferrous sulfate), 0.08 g; iodine (sodium iodide), 0.35 mg; manganese (manganous oxide), 7.5 mg; niacin, 0.06 g; selenium (sodium selenite), 0.1 mg; zinc oxide, 0.075 g; butylated hydroxytoluene (BHT), 20 mg. § BANOX® E

(Alltech®, USA). Composition: BHT, 100 g/kg; citric acid, 35 g/kg; butylated hydroxyanisole (BHA), 10 g/kg; ethoxyquin, 10 g/kg.

Table 2. Analyzed chemical and amino acid composition of commercial conventional and hydrolyzed poultry byproduct meals.

	Meal	
	Conventional	Hydrolyzed
Analyzed chemical composition [% dry matter]		
Dry matter [% as is]	93.5	93.3
Crude protein	68.9	80.3
Acid-hydrolyzed fat	13.3	5.8
Ash	10.9	5.0
Indispensable amino acids* [g/100 g crude protein]		
Arginine	7.47	6.77
Histidine	3.43	3.08
Isoleucine	4.85	4.81
Leucine	7.26	7.09
Lysine	8.55	8.64
Methionine	2.82	2.70
Phenylalanine	4.83	4.69
Taurine	0.48	0.56
Threonine	5.11	4.96
Valine	5.01	4.91
Dispensable amino acids [g/100 g crude protein]		
Alanine	5.33	5.02
Aspartic acid	9.64	9.59
Cysteine	1.67	1.66
Glutamic acid	11.7	11.2
Glycine	8.24	7.00
Hydroxyproline	1.83	1.47
Proline	5.89	5.52
Serine	4.62	4.37
Tyrosine	4.19	3.89
Total indispensable amino acids [g/100 g crude protein]	49.8	48.2
Total dispensable amino acids [g/100 g crude protein]	53.1	49.7

* Tryptophan was not analyzed.

Table 3. Intake, apparent total tract digestibility, apparent metabolizable energy, and fecal characteristics of cats fed experimental diets containing conventional (CPM) or hydrolyzed (HPM) poultry byproduct meal.

	Diet		SEM*	*p*-value
	CPM	HPM		
Intake [g/(kg $BW^{0.67}$ day)]†				
DM§	16.1	15.3	0.9	0.687
Organic matter	15.1	14.5	0.8	0.706
Crude protein	5.7	5.6	0.3	0.808
Acid-hydrolyzed fat	2.0	1.9	0.1	0.639
Ash	1.0	0.9	0.1	0.408
Gross energy [kcal/(kg $BW^{0.67}$ day)]	82.8	78.3	4.5	0.644
Apparent total tract digestibility [%]				
DM	86.9	88.2	0.7	0.340
Organic matter	88.9	90.2	0.6	0.327
Crude protein	90.2	90.7	0.7	0.768
Acid-hydrolyzed fat	92.9	93.7	0.6	0.527
Ash	54.2	55.7	2.0	0.722
Gross energy	89.6	90.3	0.6	0.575
Metabolizable energy [kcal/kg DM]	4.34	4.34	0.03	0.929
Fecal characteristics				
Fecal DM [%]	50.1	46.5	2.2	0.417
Fecal output [g DM/(kg $BW^{0.67}$ day)]	2.13	1.66	0.21	0.280
Fecal score‖	4.60	4.00	0.18	0.099

* SEM, standard error of the mean. † BW, body weight. § DM, dry matter. ‖ Fecal score ranging from 1 (watery feces) to 5 (hard, dry pellets).

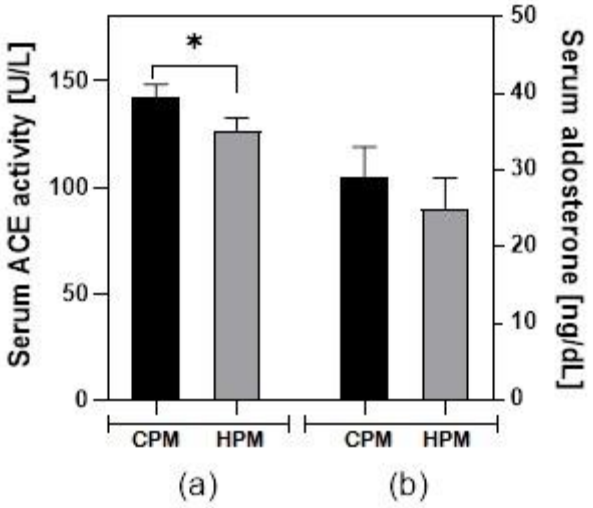

Figure 1 . Serum (a) angiotensin-converting enzyme (ACE) activity and (b) aldosterone concentration in cats fed diets containing conventional (CPM) or hydrolyzed (HPM) poultry byproduct meal.

Data are presented as mean, and error bars indicate standard error (n = 8). * Cats fed the HPM diet tended to have lower serum ACE activity than cats fed the CPM diet (p = 0.094).

V. Digestibility and palatability of enzymatically hydrolyzed poultry byproduct meal and effect of hydrolysis on Maillard reaction product formation, intake, and excretion in cats

(Archives of Animal Nutrition)

ABSTRACT

Protease treatment of poultry byproduct meal results in higher levels of small peptides and free amino acids than conventional processing. Because hydrolyzed proteins are more soluble and accessible to endogenous enzymes, it is expected that they positively influence protein digestibility. Furthermore, when subjected to thermal treatment, enzymatically hydrolyzed proteins tend to form high levels of Maillard reaction products (MRPs), which might improve palatability. This study aimed to investigate the effects of enzymatic hydrolysis of poultry byproduct meal on MRP formation and metabolism in cats. The digestibility and palatability of poultry meals and diets were determined. Conventional (CPM) and enzymatically hydrolyzed poultry meals (HPM) were produced under laboratory conditions. A basal diet was formulated for adult cats, and 30% of the diet was replaced by CPM to form the conventional poultry byproduct meal diet (CD) or by HPM to form the hydrolyzed diet (HD). Eighteen cats (9 male and 9 female, 4.18 ± 0.87 kg BW) were divided into three groups, and each group was fed one of the experimental diets. In trial 1, the apparent total tract digestibility (ATTD) of diets and poultry meals was assessed in a completely randomized design. In trial 2, we determined the MRP (carboxymethyllysine, CML, and fructoselysine, FL) content of diets and measured MRP intake and excretion during three days following the digestibility trial. In trial 3, a palatability test was performed using 20 cats (11 male and 9 female, 4.52 ± 1.16 kg BW). The test lasted two days, one for adaptation and one for data collection. There were no differences in ATTD between diets (CD versus HD) or ingredients (CPM versus HPM) ($p > 0.05$). CD had higher FL and CML levels than HD.

FL and CML intakes were greater in animals fed CD ($p < 0.05$). However, there were no differences in FL or CML excretion through urine or feces between diets (CD versus HD) ($p > 0.05$). HD was the first food choice, concerning both smell and taste, for the majority of animals. The intake ratio of HD was significantly higher (test power = 0.95). Addition of CPM in extruded cat diets does not improve protein digestibility but enhances palatability. Contrary to the expected, FL and CML contents and intakes were higher in CD than in HD.

KEYWORDS: carboxymethyllysine, cat, fructoselysine, hydrolyzed protein, poultry meal

1. INTRODUCTION

Pet ownership is growing, and, with it, the pet food industry (Li et al., 2020). Poultry byproduct meal is widely used in pet foods because of its good nutritional composition and low cost compared with other conventional protein sources (Meeker & Meisinger, 2015). Currently, poultry meal is mainly produced by high-temperature treatment, after which fat and moisture are extracted. However, new processing techniques are being developed, such as enzymatic hydrolysis using proteases (Brandelli et al., 2015).

Proteases hydrolyze intact proteins into small peptides and free amino acids, which might have a positive effect on protein digestibility, as, with hydrolysis, proteins are digested and may be easily absorbed (Koopman et al., 2009). However, when in high amounts and under high-temperature conditions, peptides and amino acids readily react and complex with reduced sugars, forming Maillard reaction products (MRPs) (Van Rooijen et al., 2013). Given that poultry meal production, diet extrusion, and drying involve high-temperature treatments, we hypothesized that MRP formation is greater in hydrolyzed poultry meal and pet diets containing this ingredient.

The Maillard reaction can be divided into three stages: early, advanced, and final. In each stage, there are different pathways and reactions that lead to a variety of compounds. Fructoselysine (FL) is an Amadori product formed in early Maillard reactions, in which amino acids react with reduced sugars, decreasing amino acid availability. Amadori compounds can further react via different pathways, forming advanced MRPs, such as carboxymethyllysine (CML) and hydroxymethylfurfural. CML and hydroxymethylfurfural are the most common products, used as markers to indicate the extent of Maillard reactions in foods (Van Boekel, 2006). After these compounds are formed, the bioavailability of amino acids decreases, impairing their use by the organism (Van Rooijen et al., 2013). MRPs are associated with a variety of diseases, including diabetes mellitus, osteoarthritis, and neurodegenerative disorders. Therefore, it is important to understand MRP formation in pet food ingredients and diets, as well as intake and excretion levels, as most pets consume extruded diets throughout their life.

Although Maillard reactions can have negative impacts on diet nutritional value, they may improve pet food color and taste, positively influencing palatability (Van Boekel, 2006; Van Rooijen et al., 2013). This study aimed to investigate the effects of enzymatically hydrolyzed poultry meal on the MRP content of pet diets, MRP intake and excretion levels, and diet and ingredient digestibility and palatability in cats.

2. MATERIAL AND METHODS

Three trials were conducted to evaluate differences between diets containing conventional poultry byproduct meal (CPM) and enzymatically hydrolyzed poultry byproduct meal (HPM) in terms of digestibility (trial 1); Maillard reaction product content, intake, and excretion (trial 2); and palatability (trial 3).

2.1. Preparation of poultry byproduct meals

CPM and HPM were prepared in a pilot plant at the State University of Maringá, Brazil, as previously described (Miltenburg et al., 2020). Raw materials (viscera, offal without head or bones, and meat) were obtained from a local chicken slaughterhouse (Abatedouro Coroaves Ltd., Maringá, Brazil). Both poultry byproduct meals were prepared by similar procedures, including digestion at 105 °C for 2 h, pressing to remove excess fat, drying, and grinding. For HPM, raw materials were hydrolyzed prior to digestion using a commercial proteolytic enzyme from *Bacillus licheniformis* at 0.03% (fresh matter basis) (Protezyn APP 3000, Prozyn, São Paulo, Brazil) at 55 °C for 45 min.

Representative samples of each meal were collected and analyzed for proximate composition (dry matter, N content, acid-hydrolyzed fat, and ash) according to AOAC procedures (2006). Total amino acids, except tryptophan, were determined by high-performance liquid chromatography (Agilent 1200 Series, Santa Clara, United States of America) on a Luna C18 column (00G-4252-EQ, 100 Å, 5 µm, 250 × 4.6 mm) (White et al., 1986) with detection at 254 nm. All analyses were performed in duplicate. Total amino acids are expressed on a crude protein basis (N × 6.25).

2.2. Diets

First, a basal (BAS) diet was formulated to meet the recommendations for nutrients as defined by the European Pet Food Industry Federation (FEDIAF, 2018). Then, 30% of the BAS diet was replaced by CPM or HPM, originating conventional (CD) and hydrolyzed diets (HD), respectively (Table 4). Diets were extruded using a single-screw extruder (Imbra 120, Inbramaq, Ribeirão Preto, Brazil). Extrusion parameters were as

follows: water flow rate to preconditioner of 250–300 mL/min, preconditioner temperature of 90–95 °C, motor load of 20–30 A, and kibble density of 330–380 g/L. After extrusion, kibbles were dried in a crawler-type dryer (G–3.130, Ferraz, Ribeirão Preto, Brazil). Poultry fat and liquid palatant were sprayed onto the kibbles under continuous mixing immediately after drying, and powder palatant was applied shortly after by pulverization.

Diet samples were analyzed for proximate composition as indicated above. The metabolizable energy of BAS, CD, and HD was estimated using equations for gross energy, digestibility coefficient, and digestible energy (NRC, 2006).

2.3. Animals, housing, and feeding

All animal experimental procedures were approved by the Animal Research Ethics Committee of the State University of Maringá, Paraná, Brazil (protocol no. 1738040618). The study was conducted at the Laboratory of Nutrition and Metabolism of Domestic Cats, State University of Maringá.

All cats were mixed breed, castrated, and aged 5 to 6 years, with body condition scores ranging from 5 to 6 on a scale of 1 to 9 (Laflamme, 1997). Each animal was fed individually in a metabolic cage in sufficient amounts to supply the daily maintenance energy requirements (kcal), calculated as 75 × kg body weight $(BW)^{0.67}$ (NRC, 2006). Cats were fed two equal meals a day, except during the palatability trial (see below). Water was provided *ad libitum*. Leftover food was weighed every day, and food intake was calculated.

Cats were housed in a cattery or in metabolic cages (0.5 × 0.5 × 0.6 m) depending on the trial. The cattery (49 m^2) was environmentally enriched with shelves at various

heights, toys, and outside tree trunks. Cats had access to an outdoor area for socialization and physical activities.

2.4. Trial 1: Apparent total tract digestibility and metabolizable energy of diets and ingredients

Eighteen cats (9 male and 9 female, 4.18 ± 0.87 kg BW) were divided into three groups of six cats (3 male and 3 female), and each group was fed one of the three experimental diets. The trial lasted 10 days (5 days of adaptation and 5 days of sample collection). In the first three days of adaptation, cats were housed in the cattery and placed in metabolic cages for 120 min at predetermined feeding times (08:00 and 14:00 h). During the last two days of adaptation and five days of sampling, cats were housed full-time in individual metabolic cages.

On the first day of feces collection, all feces produced before 08:00 a.m. were removed and discarded. Feces excreted thereafter were collected for five consecutive days (120 h). Fecal samples were scored for consistency, weighed, placed in separate plastic bags for each animal, and frozen (−15 °C). After the end of the collection period, feces were thawed and dried in a forced-air oven (MA035, Marconi, Piracicaba, Brazil) at 55 °C for 72 h. Each sample was milled separately through a 1 mm Wiley mill screen (MA340, Marconi, Piracicaba, Brazil) and homogenized for subsequent laboratory analysis. Feces and diet samples were analyzed for dry matter, ash, N, acid-hydrolyzed fat, and crude fiber contents according to AOAC procedures (2006). Organic matter content was calculated on a dry matter basis by subtracting the ash content from the total dry matter. Free-nitrogen extract contents (% dry matter basis) were calculated by the difference between the sum of crude protein, acid-hydrolyzed fat, ash, and crude fiber contents and 100%. Gross energy (GE) was determined using an adiabatic

calorimetric pump (6200 Isoperibol calorimeter, Parr Instrument Company, Moline, USA).

The apparent total tract digestibility (%) of dietary nutrients was calculated as the difference between nutrient intake (g/day) and nutrient fecal excretion (g/day) divided by nutrient intake (g/day) and multiplied by 100. Metabolizable energy (ME) was determined on a dry matter basis using a correction factor for cats and the following equation: ME (kcal/kg) = [(total ration consumed × GE of ration) – (total feces produced × GE of feces) – (0.86 × digestible protein consumed)]/total ration consumed (NRC, 2006). The apparent total tract digestibility of experimental poultry byproduct meals was calculated by the difference or substitution method, considering the calculated apparent total tract digestibility values of basal and test diets and the inclusion level of each test ingredient corrected for dry matter content, using the equation described by Matterson et al. (1965).

2.5. Trial 2: MRP contents in ingredients and diets and organic balance in cats

Cats assigned to CD and HD in trial 1 were maintained on these diets for three additional days. All feces were collected during these days, weighed, placed in separate plastic bags for each animal, and frozen (−15 °C). Urine was collected during the same period in plastic bottles containing 1 mL of boric acid (50 g/L). Urinary volume was measured, and samples were pooled per cat and frozen (−15 °C). Urine and feces samples were freeze-dried before analysis.

CML and furosine (as an indirect measurement for FL) were analyzed in meals (CPM and HPM), diets (CD and HD), urine, and feces. All samples except urine were defatted by Soxhlet extraction with petroleum ether. Meals, diets, feces, and urine samples (10 mg) were hydrolyzed using 1.0 mL of 6 M HCl for 23 h at 110 °C in glass

tubes sealed under vacuum. After this period, the tubes were opened and dried with nitrogen gas. Then, CML and furosine were analyzed according to van Rooijen et al. (2016). FL contents (mg/kg) were calculated by multiplying the molar fraction of furosine by 3.1, the conversion factor for degradation of fructoselysine during acid hydrolysis

Dietary MRP intake (mg/kg $BW^{0.67}$) was calculated as food intake (g dry matter) multiplied by the diet MRP content (mg/g dry matter) divided by the metabolic weight ($BW^{0.67}$) of the animal. MRP urinary excretion (mg/kg $BW^{0.67}$) was calculated as the amount of urine excreted (g) multiplied by the MRP content of urine (mg/g dry matter) and divided by the metabolic weight ($BW^{0.67}$) of the animal. MRP fecal excretion (mg/kg $BW^{0.67}$) was calculated as the amount of feces excreted (g dry matter) multiplied by the MRP content of feces (mg/g dry matter) and divided by the metabolic weight ($BW^{0.67}$) of the animal.

2.6. Trial 3: Preference test

Twenty cats (11 male and 9 female, 4.52 ± 1.16 kg BW) well familiar with two-bowl preference testing were used in this trial following the procedures described by Pires et al. (2020). The test lasted two days, one for adaptation and one for data collection. Two bowls, one containing 60 g of CD and the other 60 g of HD, were placed side by side in a palatability cage. Cats were placed individually inside the cages for 20 min four times a day, at 8:00, 10:00, 14:00, and 16:00 h, and allowed to eat from both bowls. The cats' first choice of diet was assessed at 8:00 h. The first bowl which the cat smelled and the first food it ate were recorded. Then, these data were used to calculate the percentage of animals that smelled and tasted HD as their first choice. The HD intake ratio was calculated as the ratio of HD intake to the total daily intake, according to the equation:

HD intake ratio = HD intake/(HD intake + CD intake). The test was repeated four times a day, and the position of bowls was changed on both days at 14:00 to reduce laterality effects.

2.7. Statistical analysis

Data from digestibility, MRP intake, and excretion assays were subjected to analysis of variance in the R environment version 3.2.4 (R Core Team, 2016), and means were compared by Tukey's test at the 5% significance level.

3. RESULTS

3.1. Chemical composition and MRP content of poultry meals

CPM and HPM had similar chemical composition (Table 5). CML content was higher in HPM; and FL content, in CPM.

3.2. Trial 1: Apparent total tract digestibility and metabolizable energy

Apparent total tract digestibility coefficients did not differ between diets (Table 6). The metabolizable energy of CD and HD was higher than that of BAS ($p < 0.001$). No differences ($p > 0.05$) in apparent total tract digestibility coefficients were observed between poultry meals (Table 7).

3.3. Trial 2: MRP content, intake, and excretion

CML and FL contents were highest in CD (Table 4). In agreement, CML and FL intakes were higher in cats fed CD (p = 0.009 and p = 0.008, respectively); however, no differences in CML or FL excretion in urine or feces were observed between diets (p > 0.05) (Table 8).

3.4. Trial 3: Preference test

Most cats had HD as their first choice of smell and taste (78.9 and 68.4%, respectively). The HD intake ratio was significantly high (0.61, test power = 0.95) (Table 9).

4. DISCUSSION

In recent years, the number of studies on MRP formation and content in pet foods as well as on MRP intake and excretion by pets has increased (Oba et al., 2019; Van Rooijen et al., 2016; Van Rooijen et al., 2013, 2014). MRPs result from the complexation of amino acids with reduced sugars, which may decrease amino acid bioavailability (mainly that of lysine) and, consequently, protein digestibility. Some studies have observed an association between MRP and diseases, such as diabetes mellitus, osteoarthritis, and atherosclerosis (Ahmed et al., 1997; Van Rooijen et al., 2013). Given that pet dogs and cats are commonly fed commercial dry food throughout their lives, it is important to understand the role of MRPs in pet food ingredients and diets as well as the intake and excretion of these compounds.

Both free amino acids and amino acids in peptides and proteins can be involved in Maillard reactions; however, free amino acids have an α-amino group and a functional side chain available for reaction, whereas peptides and proteins have only amino acid side chains (Van Rooijen et al., 2014). In this study, we hypothesized that HPM could

form a greater amount of MRPs during food processing because of its higher content of free amino acids compared with CPM. CML content was higher in HPM, but FL content was higher in CPM. FL is an Amadori compound, formed in early Maillard reactions. As the reaction progresses, Amadori compounds can react via different pathways, forming advanced MRPs (Ahmed et al., 1997). CML and hydroxymethylfurfural are advanced MRPs widely applied to indicate the extent of Maillard reactions in foods (Erbersdobler & Somoza, 2007). Therefore, the higher CML and lower FL contents in HPM compared with those in CPM could be explained by the faster conversion of FL to CML during processing, given that more free amino acids were promptly available for Maillard reactions in HPM. In addition, the raw material used to produce poultry meals contains more than 95% protein, ash, and fat and has low levels of reduced sugars. As a result, continuous FL formation in CPM might have been impaired. Van Rooijen et al. (2014) analyzed CML and FL contents of pet foods and observed a strong correlation between CML and FL content, attributed to the formation of CML from FL via an oxidative pathway.

During diet extrusion, temperature is the most important factor promoting Maillard reactions, although the nutrient profile of pet food ingredients may also affect MRP formation and content in the final diet (Van Rooijen et al., 2013). We expected HD to have a higher MRP content, as it contained hydrolyzed poultry meal. However, CD had higher levels of FL and CML. The Maillard reaction is not a one-way route; different pathways lead to a variety of reactions and compounds. For instance, Amadori compounds can react through rearrangement, oxidation, condensation, and dehydration, forming a variety of advanced MRPs, such as glyoxal, methylglyoxal, reductones, dehydroreductones, and pentosidine. Such compounds may react again to form melanoidins, the final product of Maillard reactions. In the current study, we measured

one product of the early phase (FL, an Amadori compound) and one of the advanced phase (CML). Thus, it is unknown whether FL and CML in HD had already been transformed into the next products of the pathway. It is interesting to note that HD had a darker color than CD, which might be due to a higher amount of melanoidins. These MRPs are formed at the last stage of the reaction and are responsible for the characteristic brown color of heat-treated foods (Wang et al., 2011).

CML and FL intakes were significantly higher in animals fed CD (0.65 and 7.1 mg/kg $BW^{0.67}$, respectively) than in those fed HD (0.45 and 4.9 mg/kg $BW^{0.67}$, respectively). Of note, the intake values were lower than those reported by Van Rooijen et al. (2016), who observed a CML intake of 0.91–2.26 mg/kg $BW^{0.67}$ and an FL intake of 9.5–27.5 mg/kg $BW^{0.67}$ in cats fed extruded diets with different levels of MRPs. Although FL and CML intakes were higher in animals fed CD compared with HD, there were no significant differences in urinary or fecal excretion levels. These results differ from those of Van Rooijen et al. (2016), who found a positive correlation between daily dietary intakes of FL and CML and urinary excretion, indicating that increased intake results in increased excretion. However, the models used had very low R^2 values, and, according to the authors, more data would be necessary to confirm the results. Delgado-Andrade et al. (2012) studied CML urinary excretion in young human volunteers fed high- and low-MRP diets and observed that, although urinary output was 25% higher in adolescents who consumed the high-MRP diet, there were no significant differences in the results. When expressed as percentage of ingested CML, urinary excretion was higher in participants fed the low-MRP diet. These findings are in line with ours, as we observed a 28% higher urinary output in animals fed CD (without statistical significance); when expressed as a percentage of ingested CML, CML excretion was 6% higher in cats fed HD. According to Delgado-Andrade et al. (2012), these

observations could indicate that the urinary elimination rate of CML was limited or saturated. CML fecal excretion, however, was also greater and proportional to CML dietary intake in the study of Delgado-Andrade et al. (2012), differing from our results. According to Somoza (2006), it is well established that MRPs can affect the action of enzymes on proteins, resulting from steric impediments or protease inhibition. Therefore, we believe that the ability to digest and absorb MRPs in HD was lower than that for MRPs in CD, probably affected by reactions with hydrolyzed poultry meal.

The total apparent balance of MRPs was positive; therefore, we assumed that these compounds can be either transformed to different products or retained in the body. As discussed by Delgado-Andrade et al. (2012), the metabolic pathway that MRPs follow once they enter the organism remains unknown. The compounds can become part of an endogenous circulating MRP/advanced glycation end-product pool, a portion can be deposited in organs (as in the liver and muscles), or MRPs can be eliminated through urine. Hultsch et al. (2006) reported that a large accumulation (34%) of intravenously administered [18^{F}]fluorobenzoylated FL was observed in the kidneys 60 min post injection and a low accumulation (less than 10%) was identified in the stomach, lungs, liver, and intestine. Furthermore, following digestion, some degraded compounds, such as CML, may be excreted in feces as different products. This possibility is supported by the fact that MRPs, especially Amadori compounds, are degraded by the colonic microbiota and can be either absorbed in the intestine or transformed and excreted in feces (Somoza, 2005).

Hydrolyzed proteins have a lower molecular weight, as well as smaller peptide chains and free amino acids, than intact proteins (Cave, 2006). In view of this, we hypothesized that HPM and HD would have higher protein digestibility. However, no significant differences in the apparent total tract digestibility of diets (CD versus HD) or

ingredients (CPM and HPM) were observed. This result is similar to that obtained in a previous study carried out by our research group (Miltenburg et al., 2020), in which no differences in protein digestibility were observed between cat diets containing a commercial hydrolyzed poultry meal or a conventional poultry meal. As discussed in our previous study, it seems that endogenous enzymes have the capability of hydrolyzing intact proteins in poultry meals to the same extent as proteases in enzymatically prehydrolyzed poultry meal, explaining these results.

Palatability is a key driver of food acceptance and preference by cats. Enzymatically hydrolyzed proteins from a variety of offal are widely used by pet food manufacturers to produce ingredients/palatant containing MRPs, as they contribute to the desired flavor and color of pet food (Van Boekel, 2006). Therefore, we hypothesized that HD could have higher palatability than CD, which was confirmed in this study. Although the total amount of MRPs was higher in CD, cats' first choice percentages and intake ratio were significantly higher for HD. As previously mentioned, we evaluated only two MRPs; there are probably other compounds not measured in this study that are key to enhancing palatability. For instance, according to Van Boekel (2006), Strecker degradation (an advanced Maillard reaction) is one of the most important reaction steps for flavor formation. In Strecker degradation, amino acids are degraded by dicarbonyls formed in Maillard reactions, leading to amino acid deamination and decarboxylation as well as aldehyde and aminoketone formation.

5. CONCLUSIONS

Addition of HPM to extruded cat diets did not increase FL or CML intake, nor did it improve the total tract apparent digestibility of proteins. However, HPM enhanced diet palatability.

Acknowledgments

The authors thank Danês Alimentos, Kemin Industries Inc., and Prozyn BioSolutions for providing the ingredients and processing know-how.

Author contributions

TZM and RSV designed the study. TZM, IOM, and MU carried out the research. TZM, IOM, and MU contributed to data analysis. RSV provided access to the Pet Nutrition Laboratories. TZM and RSV contributed to data collection and statistical analyses. TZM and RSV wrote the paper. All authors consider themselves equally responsible for the contents of the study.

Disclosure statement

The authors state that there is no conflict of interest.

Funding

This study was supported by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES, grant no. 88882.181511/2018-01) and Premier Pet, Dourado, Brazil.

6. REFERENCES

Ahmed, M. U., Brinkmann Frye, E., Degenhardt, T. P., Thorpe, S. R., & Baynes, J. W. (1997). Nε-(Carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. *Biochemical Journal*, *324*(2), 565–570. https://doi.org/10.1042/bj3240565

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 17th ed. Gaithersburg, MD: Association of Official Analytical Chemists.

Brandelli, A., Sala, L., & Kalil, S. J. (2015). Microbial enzymes for bioconversion of poultry waste into added-value products. *Food Research International*, *73*, 3–12. https://doi.org/10.1016/j.foodres.2015.01.015

Cave, N. J. (2006). Hydrolyzed Protein Diets for Dogs and Cats. *Veterinary Clinics of North America - Small Animal Practice*, *36*(6), 1251–1268. https://doi.org/10.1016/j.cvsm.2006.08.008

Delgado-Andrade, C., Tessier, F. É. J., Niquet-Leridon, C., Seiquer, I., & Navarro, M. P. (2012). Study of the urinary and faecal excretion of Ne-carboxymethyllysine in young human volunteers. *Amino Acids*, *43*(2), 595–602. https://doi.org/10.1007/s00726-011-1107-8

Erbersdobler, H. F., & Somoza, V. (2007). Forty years of furosine - Forty years of using Maillard reaction products as indicators of the nutritional quality of foods. *Molecular Nutrition and Food Research*, *51*(4), 423–430. https://doi.org/10.1002/mnfr.200600154

Hultsch, C., Hellwig, M., Pawelke, B., Bergmann, R., Rode, K., Pietzsch, J., Krause, R., & Henle, T. (2006). Biodistribution and catabolism of 18F-labeled N-ε-fructoselysine as a model of Amadori products. *Nuclear Medicine and Biology*, *33*, 865–873. https://doi.org/10.1016/j.nucmedbio.2006.07.007

Koopman, R., Crombach, N., Gijsen, A. P., Walrand, S., Fauquant, J., Kies, A. K., Lemosquet, S., Saris, W. H. M., Boirie, Y., & Van Loon, L. J. C. (2009). Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *American Journal of Clinical Nutrition*, *90*(1), 106–115. https://doi.org/10.3945/ajcn.2009.27474

Laflamme, D. P. (1997). Development and validation of a body condition score system for cats: a clinical tool. *Feline Practice (Santa Barbara, Calif. : 1990) (USA)*, *v. 25*.

Li, H., Wyant, R., Aldrich, G., & Koppel, K. (2020). Preference ranking procedure: Method validation with dogs. *Animals*, *10*(4), 1–10. https://doi.org/10.3390/ani10040710

Meeker, D. L., & Meisinger, J. L. (2015). Companion animals symposium: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. *Journal of Animal Science*, *93*(3), 835–847. https://doi.org/10.2527/jas.2014-8524

Miltenburg, T., Uana da Silva, M., Bosch, G., & Vasconcellos, R. S. (2020). Effects of enzymatically hydrolysed poultry byproduct meal in extruded diets on serum

angiotensin-converting enzyme activity and aldosterone in cats. *Archives of Animal Nutrition*, *00*, 1–14. https://doi.org/10.1080/1745039X.2020.1849899

Nutrient requirements of dogs and cats (NRC). 2006. In: N. R. Council (ed.). p 398. The National Academy, Whashington, DC.

Oba, P. M., Utterback, P. L., Parsons, C. M., De Godoy, M. R. C., Swanson, K. S., & ‡2, †. (n.d.). *Chemical composition, true nutrient digestibility, and true metabolizable energy of chicken-based ingredients differing by processing method using the precision-fed cecectomized rooster assay 1*. https://doi.org/10.1093/jas/sky461/5238082

Pires, K. A., Miltenburg, T. Z., Miranda, P. D., Abade, C. C., Janeiro, V., Menolli, A. L. A., Mizubuti, I. Y., Ribeiro, L. B., & Vasconcellos, R. S. (2020). Factors affecting the results of food preference tests in cats. *Research in Veterinary Science*, *130*, 247–254. https://doi.org/10.1016/j.rvsc.2020.03.023

Somoza, V. (2005). Five years of research on health risks and benefits of Maillard reaction products: An update. *Molecular Nutrition and Food Research*, *49*(7), 663–672. https://doi.org/10.1002/mnfr.200500034

Somoza, V., Wenzel, E., Weiß, C., Clawin-Rädecker, I., Grübel, N., & Erbersdobler, H. F. (2006). Dose-dependent utilisation of casein-linked lysinoalanine, N(epsilon)-fructoselysine and N(epsilon)-carboxymethyllysine in rats. *Molecular Nutrition and Food Research*, *50*(9), 833–841. https://doi.org/10.1002/mnfr.200600021

The European Pet Food Industry Federation (FEDIAF). 2018. Nutritional guidelines for complete and complementary pet food for cats and dogs. Bruxelles: FEDIAF (feed industry association).

Van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, *24*(2), 230–233. https://doi.org/10.1016/j.biotechadv.2005.11.004

van Rooijen, C., Bosch, G., Butré, C. I., van der Poel, A. F. B., Wierenga, P. A., Alexander, L., & Hendriks, W. H. (2016). Urinary excretion of dietary maillard reaction products in healthy adult female cats. *Journal of Animal Science*, *94*(1), 185–195. https://doi.org/10.2527/jas.2015-9550

Van Rooijen, Charlotte, Bosch, G., Van Der Poel, A. F. B., Wierenga, P. A., Alexander, L., & Hendriks, W. H. (2013). The Maillard reaction and pet food processing: Effects on nutritive value and pet health. *Nutrition Research Reviews*, *26*(2), 130–148. https://doi.org/10.1017/S0954422413000103

Van Rooijen, Charlotte, Bosch, G., Van Der Poel, A. F. B., Wierenga, P. A., Alexander, L., & Hendriks, W. H. (2014). Quantitation of Maillard reaction products in commercially available pet foods. *Journal of Agricultural and Food Chemistry*, *62*(35), 8883–8891. https://doi.org/10.1021/jf502064h

Wang, H. Y., Qian, H., & Yao, W. R. (2011). Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry*, *128*(3), 573–584. https://doi.org/10.1016/j.foodchem.2011.03.075

White, J. A., R. J. Hart, and J. C. Fry. 1986. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. The Journal of Automatic Chemistry 8: 170-177. doi: 10.1155/S1463924686000330

Table 4. Ingredients, analyzed chemical composition, and Maillard reaction products of a basal diet (BAS) and diets based on conventional poultry byproduct meal (CD) and hydrolyzed poultry byproduct meal (HD).

	Diet		
	BAS	CD	HD
Ingredients [%]			
Conventional poultry byproduct meal	-	30.0	-
Hydrolyzed poultry byproduct meal	-	-	30.0
Salmon meal	13.00	9.10	9.10
Corn grain	22.29	15.60	15.60
Corn gluten meal (60% crude protein)	18.94	13.26	13.26
Broken rice	20.00	14.00	14.00
Cellulose	2.00	1.40	1.40
Soybean meal	10.00	7.00	7.00
Liquid palatant	2.00	1.40	1.40
Powder palatant	1.00	0.70	0.70
Poultry fat	8.97	6.28	6.28
Potassium chloride	0.38	0.27	0.27
Sodium chloride	0.50	0.35	0.35
Taurine	0.10	0.07	0.07
Mineral and vitamin premix[*]	0.40	0.28	0.28
Limestone	0.40	0.28	0.28
Synthetic antioxidant[†]	0.02	0.01	0.01
Analyzed chemical composition [% DM][‡]			
Dry matter [% as is]	92.9	92.9	92.6
Crude protein	32.3	42.6	42.9
Crude fat (by acid hydrolysis)	13.2	13.9	14.0
Ash	6.02	5.99	5.99
Gross energy [kcal/kg DM]	5,017	5,405	5,360
Analyzed Maillard reaction products [mg/kg DM]			
Carboxymethyllysine	-	36.8	29.8
Fructoselysine	-	404.6	324.7

[*]Provided per kilogram of diet: vitamin A, 9,000 IU; vitamin B_1, 5 mg; vitamin B_{12}, 20 mcg; vitamin B_2, 4 mg; vitamin B_6, 4 mg; vitamin D_3, 750 IU; vitamin E, 30 IU; vitamin K_3, 0.1 mg; pantothenic acid, 5 mg; folic acid, 0.8 mg; biotin, 0.07 mg; copper sulphate, 5 mg; choline, 0.24 g; iron, 0.08 g; iodine, 0.35 mg; manganese, 7.5 mg; niacin, 0.06 g; selenium, 0.1 mg; zinc, 0.075 g; and BHT, 20 mg. [†]Synthetic antioxidant composition: BHT, 100 g/kg; citric acid, 35 g/kg; BHA, 10 g/kg; ethoxyquin, 10 g/kg. [‡]DM, dry matter basis.

Table 5. Analyzed chemical and amino acid composition and Maillard reaction product contents of conventional and hydrolyzed poultry byproduct meals (CPM and HPM, respectively).

	Meal	
	CPM	HPM
Analyzed chemical composition [% DM]*		
Dry matter	93.8	93.7
Crude protein	64.7	65.8
Crude fat (by acid hydrolysis)	18.2	17.2
Ash	5.18	5.27
Analyzed Maillard reaction products [mg/kg DM]		
Carboxymethyllysine	49.3	62.3
Fructoselysine	324.2	292.0
Indispensable amino acids† [g/100 g crude protein]		
Arginine	6.69	6.53
Histidine	2.58	2.55
Isoleucine	4.81	4.73
Leucine	7.23	7.10
Lysine	8.36	8.22
Methionine	2.67	2.86
Phenylalanine	4.79	4.64
Taurine	0.88	0.85
Threonine	5.15	5.05
Valine	5.05	4.97
Dispensable amino acids [g/100 g crude protein]		
Alanine	4.54	4.47
Aspartic acid	9.78	9.60
Cysteine	1.47	1.87
Glutamic acid	10.88	10.67
Glycine	5.61	5.49
Hydroxyproline	0.77	0.73
Proline	4.73	4.64
Serine	4.70	4.60
Tyrosine	4.37	4.24
Total indispensable amino acids [g/100 g crude protein]	48.22	47.49
Total dispensable amino acids [g/100 g crude protein]	46.86	46.31

*DM, dry matter basis. †Tryptophan was not analyzed.

Table 6. Intake, apparent total tract digestibility, and apparent metabolizable energy of a basal diet (BAS) and diets based on conventional poultry byproduct meal (CD) and hydrolyzed poultry byproduct meal (HD) in cats.

	Diet			SEM*	*p*
	BAS	CD	HD		
Intake [g/(kg $BW^{0.67}$ day)]†					
Dry matter	18.1	18.2	18.0	0.17	0.879
Organic matter	17.0	17.1	16.9	0.16	0.879
Crude protein	5.85[b]	7.74[a]	7.70[a]	0.23	<0.001
Crude fat (by acid hydrolysis)	2.39	2.52	2.51	0.03	0.082
Ash	1.09	1.09	1.08	0.01	0.857
Gross energy [kcal/(kg $BW^{0.67}$ day)]	90.7[b]	98.3[a]	96.3[ab]	1.20	0.017
Apparent total tract digestibility [%]					
Dry matter	83.8	86.9	86.3	0.76	0.228
Organic matter	86.9	89.2	88.8	0.63	0.297
Crude protein	88.0	90.8	90.0	0.65	0.195
Crude fat (by acid hydrolysis)	94.9	94.9	95.5	0.33	0.711
Ash	35.1	49.7	46.8	3.03	0.110
Gross energy	87.5	90.0	89.5	0.61	0.235
Metabolizable energy [kcal/kg DM]‡	4,150[b]	4,530[a]	4,463[a]	0.04	<0.001

*SEM, standard error of the mean. †BW, body weight. ‡DM, dry matter basis. [a,b]Means within a row followed by different letters are significantly different by Tukey's test ($p < 0.05$).

Table 7. Apparent total tract digestibility of conventional (CPM) and hydrolyzed (HPM) poultry byproduct meals in cats.

	Meal		SEM*	*p*
	CPM	HPM		
Apparent total tract digestibility [%]				
Dry matter	93.8	92.3	2.35	0.781
Organic matter	93.7	92.7	1.94	0.777
Crude protein	95.7	95.6	1.33	0.979
Gross energy	97.6	94.7	1.71	0.416

*SEM, standard error of the mean.

Table 8. Daily intake, urinary and fecal excretion, and total apparent absorption and balance of Maillard reaction products (carboxymethyllysine and fructoselysine) in cats fed diets containing conventional poultry byproduct meal (CD) or hydrolyzed poultry byproduct (HD).

	Diet		*p*
	CD	HD	
Intake [mg/kg $BW^{0.67}$]*			
Carboxymethyllysine	0.65 [a]	0.45	0.009
Fructoselysine	7.10 [a]	4.90	0.008
Urinary excretion [mg/kg $BW^{0.67}$]			
Carboxymethyllysine	0.32	0.25	0.352
Fructoselysine	0.13	0.10	0.550
Fecal excretion [mg/kg $BW^{0.67}$]			
Carboxymethyllysine	0.15	0.15	0.869
Fructoselysine	0.49	0.42	0.622
Total apparent absorption [%]			
Carboxymethyllysine	76.5	65.8	0.084
Fructoselysine	93.1	91.0	0.309
Total apparent balance [mg/kg $BW^{0.67}$]			
Carboxymethyllysine	0.18	0.05	0.450
Fructoselysine	6.48	4.38	0.112

* BW, body weight. [a,b]Means within a row followed by different letters are significantly different by Tukey's test ($p < 0.05$).

Table 9. First choice and intake ratio (IR) of cats challenged with a diet containing hydrolyzed poultry byproduct meal (HD) versus a diet containing conventional poultry byproduct meal (CD).

Challenge	HD first choice [%]*		IR†	SD‡	Test power§
	Smell	Taste			
HD × CD	78.9	68.4	0.61	0.30	0.95

*Percentage of cats that smelled and tasted the HD diet first. †Intake ratio of HD to total diet intake (HD + CD). ‡Standard deviation. §A test power above 0.5 is considered significant (Griffin, 2003).