UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

CARNE MECANICAMENTE SEPARADA DE FRANGO EM DIETAS PARA CÃES E GATOS: FORMAÇÃO DOS KIBBLES E AVALIAÇÃO NUTRICIONAL

Priscila Martins Ribeiro
Zootecnista

2021

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Zootecnia.

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TÍTULO DA TESE: CARNE MECANICAMENTE SEPARADA DE FRANGO EM DIETAS PARA CÃES E GATOS: FORMAÇÃO DOS KIBBLES E AVALIAÇÃO NUTRICIONAL

AUTORA: PRISCILA MARTINS RIBEIRO
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Jaboticabal, 20 de dezembro de 2021

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Priscila Martins Ribeiro, nascido em 03 de março de 1992, na cidade de Maringá – Paraná, ingressou no curso de Zootecnia da Universidade estadual de Maringá, em março de 2011. Durante a graduação foi bolsista do Programa de Educação Tutorial - PET e aluno de Iniciação Científica da Fundação Araucária, finalizando o curso em dezembro de 2016. Em março de 2016, iniciou o curso de Mestrado em Zootecnia na Universidade estadual de Maringá, sob orientação do Prof. Dr. Ricardo Souza Vasconcellos, na área de nutrição de animais de companhia. Em 2018, concluiu o curso de Mestrado e iniciou o curso de Doutorado na Faculdade de Ciências Agrárias e Veterinárias da UNESP, Câmpus de Jaboticabal, sob orientação do Prof. Dr. Aulus Cavalieri Carciofi.

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À Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Faculdade de Ciências Agrárias e veterinárias, o Programa de Pós-Graduação em

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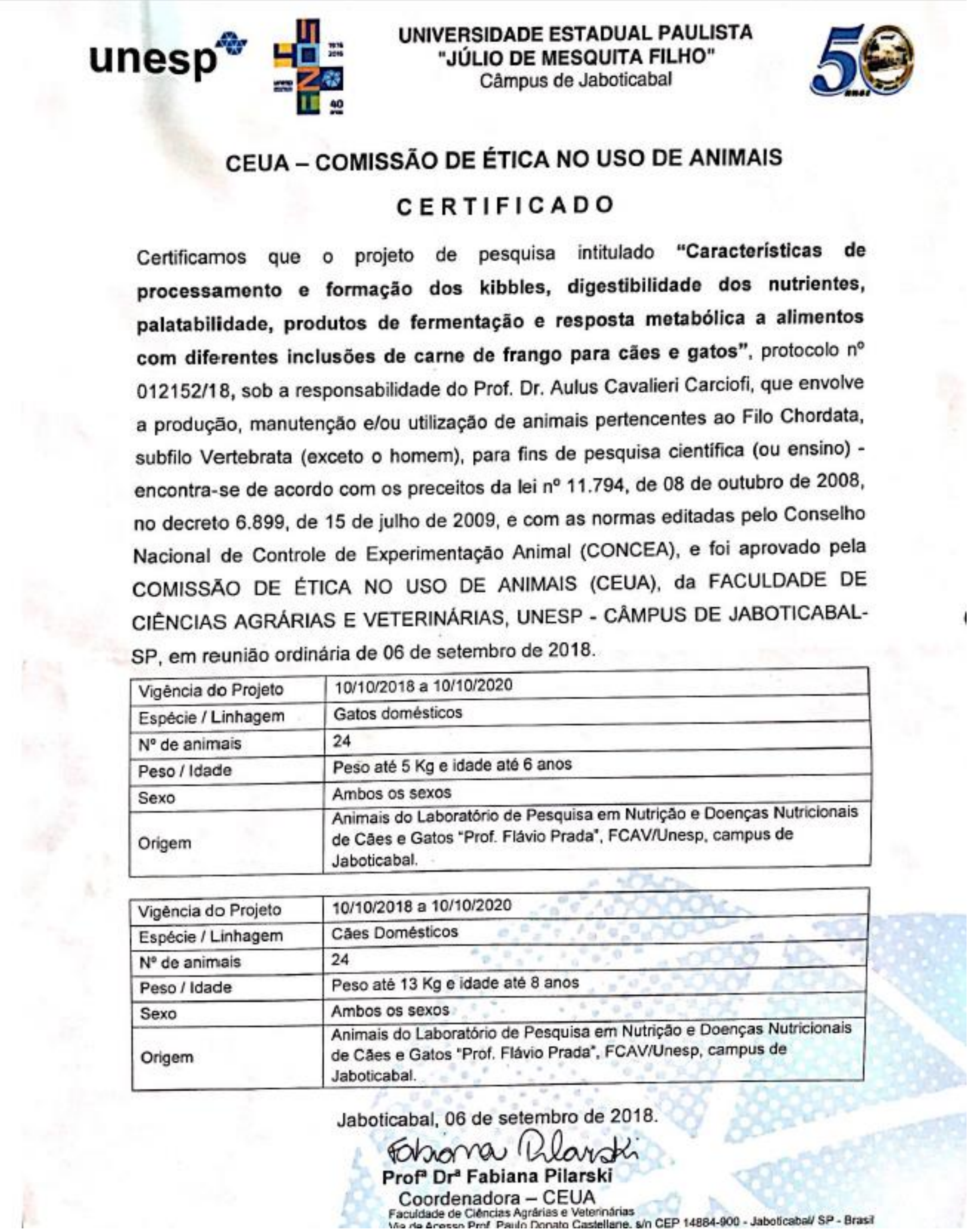

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UNIVERSIDADE ESTADUAL PAULISTA
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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Características de processamento e formação dos kibbles, digestibilidade dos nutrientes, palatabilidade, produtos de fermentação e resposta metabólica a alimentos com diferentes inclusões de carne de frango para cães e gatos"**, protocolo nº 012152/18, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 06 de setembro de 2018.

Vigência do Projeto	10/10/2018 a 10/10/2020
Espécie / Linhagem	Gatos domésticos
Nº de animais	24
Peso / Idade	Peso até 5 Kg e idade até 6 anos
Sexo	Ambos os sexos
Origem	Animais do Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof. Flávio Prada", FCAV/Unesp, campus de Jaboticabal.

Vigência do Projeto	10/10/2018 a 10/10/2020
Espécie / Linhagem	Cães Domésticos
Nº de animais	24
Peso / Idade	Peso até 13 Kg e idade até 8 anos
Sexo	Ambos os sexos
Origem	Animais do Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof. Flávio Prada", FCAV/Unesp, campus de Jaboticabal.

Jaboticabal, 06 de setembro de 2018.

Fabiana Pilarski
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CARNE MECANICAMENTE SEPARADA DE FRANGO EM DIETAS PARA CÃES E GATOS: FORMAÇÃO DOS KIBBLES E AVALIAÇÃO NUTRICIONAL

RESUMO – A utilização de ingredientes novos ou alternativos são interessantes para a indústria de alimentos para animais de estimação, especialmente ingredientes proteicos, que impactam diretamente no custo final e na saúde do animal. A presente tese está estruturada em três capítulos. O Capítulo 1 apresenta visão geral sobre proteínas, suas fontes, metabolismo e processamento, por meio de revisão bibliográfica. Os Capítulos 2 e 3 avaliaram os efeitos da inclusão de carne mecanicamente separada de frango (CMSF) em substituição à farinha de vísceras de frango (FVF) em dietas para cães e gatos, respectivamente. As analises se deram sobre: a capacidade do sistema de extrusão processar formulações com elevada umidade, formação e macroestrutura dos kibbles, digestibilidade dos nutrientes e da energia, características fecais, produtos de fermentação nas fezes, resposta pós-prandial de ureia, características de urina, balanço de nitrogênio e palatabilidade. Uma dieta livre de grãos foi formulada com FVF. Esta foi substituída pela CMSF em quatro inclusões: controle (0%), 23,1%, 40,8% e 55,2% das formulações para cães; controle (0%), 17.4%, 31.4% e 42.4% das formulações para gatos. Essa inclusão elevada de CMSF, matéria prima de elevada umidade, foi alcançada usando extrusora de rosca dupla anexada a outros equipamentos que possibilitam grandes inclusões de emulsão cárnea junto a ingredientes secos. Nós dois capítulos, maior palatabilidade foi verificada para as dietas acima do terceiro nível de inclusão de CMSF (P<0,01). Mesmo se considerando que a umidade no canhão extrusor foi superior a 43% nos tratamentos com mais alta inclusão de CMSF, foi verificada adequada formatação dos kibbles com adequado cozimento do amido (>87%). Para cães o coeficiente de digestibilidade aparente da MS aumentou de modo quadrático, enquanto o da PB e EB de modo linear com a inclusão de CMSF (P<0,05). Para gatos os coeficientes de digestibilidade dos nutrientes foram similares entre tratamentos (P>0,05). Pelo método de regressão, foi possível se extrapolar para cães inclusão de 100% de CMSF, obtendo-se para o ingrediente coeficiente de digestibilidade aparente da PB de 96% e da EB de 98%. Para cães, o escore, produção e teores de ácidos graxos de cadeia curta das fezes não diferiram (P>0,05). Amônia e isobutirico diminuíram nas fezes com a inclusão de CMSF (P<0,05), indicando menor fermentação proteica no intestino grosso. Para gatos, a concentração de ácidos graxos de cadeia ramificada (isobutirico, isovalerico e valérico) diminuiu linearmente com o aumento da inclusão de CMSF (P<0,05), indicando melhor digestibilidade ileal e menor fermentação proteica no colón para gatos. A concentração plasmática pós-prandial de ureia nos cães não diferiu entre os tratamentos (P>0,05). O volume, a densidade e o pH da urina foram também similares entre dietas para as duas espécies (P>0,05). Como conclusão, a substituição de FVF por CMSF aumentou a palatabilidade da dieta e a digestibilidade dos nutrientes, e reduziu os produtos da fermentação proteica nas fezes. O sistema de extrusão avaliado foi capaz de processar adequadamente formulações de alta umidade que apresentam a CMSF como matéria-prima principal.

Palavras-chave: proteínas, digestibilidade, ureia, palatabilidade, aminoácidos, balanço de nitrogênio.

MECHANICALLY DEBONED CHICKEN MEAT IN DIETS FOR DOGS AND CATS: KIBBLE FORMATION AND NUTRITIONAL EVALUATION

ABSTRACT – The use of new or alternative ingredients are part of the normal development of the pet food industry, especially protein ingredients, which directly impact the final cost and health of the animals. The present thesis is structured in three chapters. Chapter 1 present an overview of proteins, their sources, metabolism, and processing through a literature review. Chapters 2 and 3 evaluated the effects of including mechanically deboned chicken meat (MDCM) to replace chicken by product meal (CM) in diets for dogs and cats, respectively. The analyzes developed included: the capacity of the extrusion systems process formulations with high in-barrel moisture, kibble formation and macrostructure, nutrient and energy apparent digestibility, fecal characteristics, fermentation products formation, postprandial urea response, urine characteristics, nitrogen balance and palatability. A grain-free diet was formulated, with CM. This was replaced by MDCM in four inclusions: control (0%), 23.1%, 40.8% and 55.2% in dog diets; control (0%), 17.4%, 31.4% and 42.4% in cat diets. This high inclusion of a raw material with elevated moisture was achieved using a twin-screw extruder attached to other equipment that allowed large inclusions of meat emulsion in combination with dry ingredients. In both chapters, greater palatability was verified for diets after the third level of MDCM inclusion ($P<0.01$). Even considering that the in-barrel moisture was higher than 43% in treatments with the highest inclusion of MDCM, adequate kibbles formatting was verified with adequate starch cooking (>87%). For dogs, the coefficients of apparent digestibility of DM presented a quadratic increase, while for CP and GE a linear increase was observed with MDCM inclusion ($P<0.05$). For cats, the apparent nutrient digestibility of nutrients was similar between treatments ($P>0.05$). Using the regression method, it was possible to dogs to extrapolate the replacement of 100% of MDCM, obtaining for CP a digestibility coefficient of 96%. For dogs, the feces score, production, and contents of short-chain fatty acids did not differ ($P>0.05$). Ammonia and isobutyrate decreased in feces with the inclusion of MDCM ($P<0.05$), indicating lower protein fermentation on colon. For cats, the concentration of branched-chain fatty acids (isobutyric, isovaleric and valeric) decreased linearly with the increase on MDCM ($P<0.05$), indicating better ileal digestibility and lower protein fermentation in the colon. Postprandial plasma urea concentration did not differ between treatments in dogs ($P>0.05$). Urine volume, density and pH were also similar between diets for the two species ($P>0.05$). In conclusion, the replacement of CM by MDCM increased the palatability and digestibility of the diet and reduced the protein fermentation products in the feces. The evaluated extrusion system was able to properly process high moisture formulations that have MDCM as the main raw material.

Keywords: proteins, digestibility, urea, palatability, amino acids, nitrogen balance.

CAPÍTULO 1 – Considerações gerais

1. Introdução

O mercado pet é um setor inovador que está em constante expansão. Prova disso é que hoje, no Brasil, existem aproximadamente 55,1 milhões de cães e 24,7 milhões de gatos, que movimentam aproximadamente R$ 22,3 bilhões por ano (ABINPET, 2020). No Brasil, em 2020, foram produzidas 3,1 milhões de toneladas de rações para cães e gatos (Sindirações, 2021). Os ingredientes proteicos representam certa de 30 a 40% da composição dessas rações, ou seja, houve um consumo de mais de 1 milhão de toneladas de ingredientes proteicos. Diante dessa realidade, discussões e estudos sobre os ingredientes proteicos utilizados e sua qualidade, são questões cada vez mais pertinentes.

Fontes de proteína de origem animal são amplamente utilizadas em dietas para cães e gatos. Dentre elas, a farinha de vísceras de frango (FVF) é a mais relevante e empregada, por ser boa fonte de nutrientes, conter aminoácidos essenciais, ácidos graxos essenciais, vitaminas, minerais e apresentar boa digestibilidade para cães e gatos (Carciofi, 2008; Meeker e Meisinger, 2015). No entanto, embora o seu emprego em rações extrusadas para animais de estimação seja muito vantajoso, existe tendência crescente de inclusão e utilização de ingredientes considerados “frescos” e não processados e com qualidade de grau alimentício humano (Buff et al., 2014), dentre estes a carne mecanicamente separada de frango (CMSF).

A CMSF apresenta boas características nutricionais, no entanto, o seu uso é um desafio para o processo de extrusão, pois seus elevados teores de gordura e água reduzem o atrito na extrusora (Beaton, 2016; Baller et al, 2018), podendo limitar a transferência de energia, o cozimento e a formação dos kibbles. Além desta dificuldade técnica em se incluir esta matéria prima, estudos científicos sobre a qualidade nutricional da proteína de alimentos extrusados contendo CMSF, em comparação com formulações tradicionais à base de FVF, são escassos na literatura.

Com base no exposto, este trabalho teve como objetivos gerais avaliar formulações para cães e gatos com elevada inclusão de CMSF quanto a características dos kibbles, digestibilidade aparente dos nutrientes e da energia, características das fezes e da urina, formação de produtos de fermentação microbiana nas fezes, balanço de nitrogênio e palatabilidade. Para isso, essa tese está estruturada em três capítulos: Capítulo 1 - Considerações gerais; Capítulo 2 - Mechanically deboned chicken meat as a substitute for chicken by-product meal in extruded foods to dogs; Capítulo 3 - Mechanically deboned chicken meat as protein source for extruded diets for cats.

2. Revisão de literatura

2.1. Proteínas e aminoácidos

As proteínas podem ser definidas, bioquimicamente, como um conjunto de aminoácidos que se unem por meio de ligações peptídicas e pontes de hidrogênio e dissulfeto, formando estruturas que lhes permite o enovelamento (Nelson & Cox, 2000). Os aminoácidos, constituintes básicos das proteínas, são nutrientes essenciais para o crescimento, manutenção e saúde de cães e gatos, pois as proteínas corporais oriundas dos mesmos têm uma ampla gama de funções vitais, como componentes de membranas celulares, proteínas plasmáticas, enzimas, hormônios, estruturas orgânicas especializadas, anticorpos, além de serem fonte de energia para o animal.

Cada fonte proteica contém mistura de aminoácidos única que é fundamental na determinação da sua estrutura tridimensional (Nelson & Cox, 2000). Existem 20 aminoácidos que constituem as proteínas corporais, dos quais cães e gatos são capazes de sintetizar 10, chamados de aminoácidos não-essenciais ou dispensáveis. Os 10 aminoácidos considerados essenciais, e que, portanto, devem ser fornecidos adequadamente pela dieta incluem arginina, histidina, isoleucina, leucina, lisina, metionina, fenilalanina, treonina, triptofano e valina (NRC, 2006). A taurina, apesar de ser aminoácido essencial para gatos não participa da estrutura das proteínas corporais (Case, 2010).

Deficiência de aminoácidos para o organismo pode ocorrer tanto pela quantidade insuficiente deste presente na dieta, quanto pela desbalanço de um ou mais aminoácidos essenciais. Isto acarreta uma série de problemas à saúde, tais como redução das taxas de crescimento, anemia, perda de peso, atrofia do músculo esquelético, problemas reprodutivos, má estruturação de tecidos como pelos e pele, imunossupressão, redução do apetite, hipoalbuminemia, ascite, dentre outros (Westerterp-Plantenga, 2009). Os principais fatores que afetam a quantidade proteica das dietas são sua composição de aminoácidos, digestibilidade e densidade energética (Carciofi, 2008).

2.2. Fontes de proteínas

As fontes de proteínas são ingredientes de importância para dietas de cães e gatos, por agregarem aminoácidos e energia (Thompson, 2008; Carciofi, 2008). São também especialmente relevantes para estes animais por determinarem em boa parte a palatabilidade e consumo das rações (Aldrich et al, 2015; Hall et al, 2018). As proteínas de origem animal desidratadas que apresentam ossos em sua composição, como a FVF, farinha de carne e ossos e a farinha de peixes conferem também minerais à formulação, suprindo as necessidades de cálcio, fósforo e magnésio dos animais. No mercado pet food, devido ao impacto da opinião do proprietário nas decisões de compra, as fontes proteicas são ainda bastante relevantes para as estratégias comerciais e sucesso de mercado dos produtos.

As fontes de proteína utilizadas para cães e gatos podem ser divididas, quanto a origem em duas categorias: derivadas de plantas e de animais. Ambas podem apresentar alta digestibilidade e serem palatáveis para cães e gatos, porém, isto depende da matéria prima e processamento utilizado (Carciofi, 2008; Callon et al., 2017). As fontes proteicas de origem animal são as matérias primas mais relevantes nas formulações de cães e gatos, porém apresentam maior variação em composição química em relação às de origem vegetal (Carciofi, 2008), sendo sua qualidade fortemente afetada pelas materiais primas empregadas, tempo do abate ao processamento, presença ou ausência de deterioração microbiológica, estabilidade oxidativa e condições de processamento incluindo temperatura, tempo e pressão, que afetam fortemente a digestibilidade dos aminoácidos (Bellaver, 2005; Ribeiro et al, 2019). As farinhas de origem animal, a depender das matérias primas empregadas na sua obtenção, podem ainda apresentar elevados teores de minerais, limitando a possibilidade de sua inclusão na fórmula (Bellaver, 2005).

Já os derivados proteicos de origem vegetal apresentam composição química mais estável e digestibilidade mais constante, e não contém teores expressivos de minerais em sua composição (Bednar et al.,2000; Seixas et al.,2003; Carciofi, 2008). Estes, entretanto, podem possuir fatores antinutricionais como inibidores de enzimas, lectinas, tanino, fitato, polissacarídeos não-amiláceos, dentre outros, que influenciam a disponibilidade de seus nutrientes e podem ter efeitos gerais em saúde (França et al., 2011; Benevides et al., 2011). O tratamento térmico, incluindo o processo de extrusão, pode reduzir ou mesmo eliminar alguns desses fatores, denominados de termolábeis, melhorando significativamente a qualidade dessas matérias-primas (Kaur et al., 2015). Dentre os fatores antinutricionais que podem ser inativados pelo processo térmico incluem-se oxilato (Chai e Liebman, 2005; Benevides et al., 2011), inibidores de proteases, lectina (Benevides et al., 2011; Bora, 2014), avidinas, hemaglutininas, inibidores de amilases, tiaminases entre outros (Bora, 2014). Já outros compostos que integram estes ingredientes vegetais, como fitatos, fibra e polissacarídeos não-amilaceos não são alterados pelo tratamento térmico devendo ser considerados no emprego destes ingredientes nas formulações. Quadro sinótico com os principais estudos disponíveis sobre ingredientes proteicos de origem vegetal e animal para cães e gatos encontra-se no Quadro 1.

As carnes frescas ou congeladas têm despertado interesse crescente para emprego em formulações para cães e gatos, impulsionadas por demanda do mercado com possível alegação de emprego de ingredientes frescos e minimamente processados, bem como por possíveis vantagens nutricionais relativas à digestibilidade e palatabilidade de rações à base de farinhas animais secas. No entanto, vários dificultadores limitam correntemente seu emprego, incluindo os custos associados ao congelamento, refrigeração e transporte de uma matéria prima com aproximadamente 70% de umidade (Aldrich, 2009). Além disso, a empresa deve possuir sistema de armazém para produtos congelados, moinhos, emulsificadores, digestores e bombas de injeção de líquidos integrados ao sistema de extrusão. Por fim, o processo de extrusão convencional não suporta mais do que 25% de carne fresca em uma fórmula, pois, a umidade da massa no canhão extrusor usualmente não deve ser superior a 30% para que não haja redução da eficiência de aplicação de energia mecânica (Baller et al, 2018).

Quadro 1. Principais estudos realizados com fontes proteicas para cães e gatos.

Fontes proteicas estudadas	**Autores**
Farinha de vísceras de Aves	Araújo et al.,2004; Beloshapka et al.,2016; Chen et al.,2017; Dust et al.,2005; Folador et al.,2006; Karthik et al.,2010; Carciofi, 2008; Kawauchi et al., 2014; Donadelli et al., 2018;
Carne mecanicamente separada de Frango	Meineri et al.2021; Tjernsbekk et al, 2017
Cordeiro	Araújo et al.,2004
Farinha de Sangue	Dust et al.,2005
Proteína hidrolisada de peixe	Dust et al.,2005
Proteína hidrolisada de salmão	Folador et al.,2006; Tjernsbekk et al, 2017
Proteína de soja bioprocessada	Beloshapka et al.,2016
Farinha de soja integral micronizada	Carciofi et al.,2009
Farelo de soja	Chen et al.,2017; Dust et al.,2005; Carciofi et al.2006; Tortola et al., 2013
Levedura integral de cana	Martins et al.,2013
Carcaça de frango	Salaun et al.,2016
Coração de frango	Salaun et al.,2016
Fígado de galinha	Salaun et al.,2016
Fígado Suíno	Hullar et al.,2001; Salaun et al.,2016; Dust et al.,2005
Proteína suína isolada	Murakami et al., 2018
Glúten de milho 60	Carciofi et al.,2009
Farinha de carne e ossos	Carciofi et al.,2006, De-Oliveira et al., 2012
Farinha de pena hidrolisada	Carciofi, 2008

Quando a umidade da massa se eleva acima do ideal, no sistema usual de extrusão monorosca de médio cisalhamento empregado pela indústria pet food, não ocorre suficiente cisalhamento, temperatura e pressão da massa em processamento. A capacidade de transporte da massa pela rosca pode ficar comprometida, havendo instabilidade no processo, comprometendo o cozimento do amido e a formação dos kibbles (Beaton, 2016; Baller et al.,2018). Ao considerar-se a composição típica destes derivados cárneos congelados, com 70% de água e 20% de proteína, a inclusão de 25% na formulação só agregaria 5% de proteína no alimento, o que não seria muito, mas já agregaria 17,5% de umidade, o que seria bem elevado em se considerando a umidade natural das demais matérias primas e a necessidade de infusão de vapor, principal fonte de energia térmica no processo de extrusão (Riaz, 2000). Assim, até o momento, o uso de ingredientes secos com proteína concentrada tem mostrado melhor custo-benefício para a indústria.

No entanto, inovações tecnológicas recentes no sistema de extrusão, com possibilidade de maior inclusão de ingredientes com elevada umidade surgiram no mercado. Estes ainda não foram avaliados para cães e gatos, mas permitem que mais de 50% da formulação (considerando a inclusão de ingredientes em base úmida) seja constituída por CMSF. Estas novas tecnologias podem abrir possibilidade de se rever estes princípios de formulação e se ampliar o emprego de ingredientes congelados com elevada umidade. Neste sentido, avaliações destas formulações em cães e gatos, quanto a palatabilidade, digestibilidade, formação e características das fezes e mesmo respostas metabólicas são importantes para a utilização mais informada e técnica destas matérias primas, a partir do conhecimento de seus reais efeitos no alimento e nos animais.

2.2.1. Farinha de vísceras de frango

A farinha de vísceras de frango (FVF) é produto resultante da cocção, prensagem e moagem dos seguintes constituintes: cabeças, pés, trato digestório (com conteúdo digestivo restante), trato respiratório, aparelhos reprodutivos, gorduras viscerais, aparas, peles, cutículas e carcaças ou parte dessa, que foram rejeitadas para o consumo humano (AAFCO, 2017). Cabeça e pés podem não são incluídos no Brasil por apresentarem mercado específico, para consumo humano. Esse processo ocorre em graxarias, que são locais onde se realiza o processamento de materiais residuais de abate (ABINPET, 2020). A composição química, conteúdo mineral, estabilidade oxidativa, digestibilidade e qualidade proteica podem variar, dependendo dos resíduos de abate empregados, do tempo de estocagem dos materiais antes do processamento e do método e tecnologia de processamento, incluindo a temperatura, a pressão e o tempo utilizados nas graxarias (Ribeiro et al, 2019).

A FVF comercializada é classificada em dois tipos básicos, a tradicional ou *standard (high ash)*, na qual existe inclusão de cabeças, pés e carcaça e a *low ash*, ou especial, que contêm menor proporção de partes ósseas e, desta forma, maior quantidade de proteína e menor quantidade de matéria mineral (ABINPET, 2020), com possível maior digestibilidade. A digestibilidade *in vivo* da proteína de dietas à base de FVF para cães e gatos varia de 76 a 88%, dependendo da qualidade desta matéria prima e da composição química da dieta utilizada (Carciofi et al 2008). Volpato (2021) realizou estudo diferenciando a composição química de farinhas de viceras *low* e *high ash*, com 200 amostras de 5 fornecedores diferentes. Verificaram que a FVF standard apresentou composição química média com 9,51% de umidade, 20,3% de matéria mineral, 62,4% de proteína bruta e 11,6% de extrato etéreo, enquanto a *low ash* 9,47% de umidade, 8,4% de matéria mineral, 72,4% de proteína bruta e 11,2% de extrato etéreo. O quadro 2 mostra os níveis de garantia no site de um dos principais fornecedores do Brasil de farinha de vísceras de frango *low* e *high ash*.

Quadro 2. Níveis de garantia - fichas técnicas do brasil food (BRF, 2021).

Item	*Low Ash*	*High Ash (Standard)*
Umidade	2 a 8%	3,5 a 8%
Proteína bruta	64 a 73%	56 a 70%
Extrato etéreo	11 a 13%	8,5 a 18%
Matéria mineral	6 a 12%	8 a 22%

2.2.2. Carne mecanicamente separada de frango

Brasil é o terceiro maior produtor de carne de frango do mundo, com uma produção total de 13.845 milhões toneladas em 2020 (ABPA, 2021). Estima-se que pelo menos 20% das carcaças processadas de frango são transformadas em carne mecanicamente separada de frango (CMSF) (Negrão et al, 2005). A CMSF é obtida em frigoríficos por processo mecânico de separação da carne dos ossos, carcaças ou partes de carcaça de aves, utilizando máquinas de separação mecânica e imediatamente resfriada ou congelada. Não é permitida a utilização de cabeças, vísceras e pés (ABINPET, 2020).

A CMSF é composta por tecido muscular, conjuntivo e gordura e sua composição depende da relação músculo e osso da matéria-prima, da idade do animal e da quantidade de pele (ABINPET, 2020). Em trabalho que avaliou a qualidade nutricional da CMSF, foi verificado alta umidade (62%), proteína (11%) e gordura

(24%) e baixa quantidade de matéria mineral (0,70%). Os aminoácidos essenciais presentes atenderam a demanda diária recomendada para seres humanos, de acordo com a FAO (Negrão et al, 2005). Trabalho que avaliou a CMSF em dietas extrusadas para cães observou 49,5g de aminoácidos não essenciais e 40,9g de aminoácidos essenciais para cada 100g de proteína bruta (Tjernsbekk et al, 2016).

Nos poucos dados sobre digestibilidade, valores entre 88,2% (Tjernsbekk et al, 2016) e 83,8% (Meineri et al.2021) foram relatados para o coeficiente de digestibilidade aparente da proteína bruta da dieta para cães, mas estes foram obtidos em dietas com inclusão limitada (<25%) de CMSF, situação na qual a influência dos demais ingredientes da fórmula é relevante. Verifica-se, assim, que apesar de amplamente consumido por humanos em todo o mundo como ingrediente em produtos cárneos, poucos estudos foram realizados sobre o valor nutricional da CMSF para cães e gatos.

2.5. Importância das proteínas no metabolismo de cães e gatos

Os cães e gatos necessitam das proteínas para o crescimento e manutenção corporal, pois estas constituem importante componente estrutural da pele e anexos, músculos, tendões, ligamentos, órgãos, células sanguíneas e imunes, da cartilagem, dentre outros (Case, 2010). As enzimas necessárias para as reações químicas são também proteínas (Laflamme, 2005), além disso, estas constituem alguns hormônios que atuam na célula como mensageiros químicos e os anticorpos que fazem parte do sistema imunológico (Case, 2010).

As proteínas do corpo estão em estado de constante mudança, com equilíbrio entre as reações de síntese e degradação estabelecendo-se fluxo de aminoácidos, visando dar suporte a síntese de novas proteínas para que não haja deficiência proteica no organismo dos cães e gatos (NRC, 2006). As proteínas que não são direcionadas para a reposição de células e tecidos são utilizadas como fonte de energia (Gonzalez et al.,2006). A falta de proteínas na dieta resulta em redução do apetite, crescimento lento ou perda de peso, desenvolvimento de pelagem áspera e sem brilho, as funções do sistema imunológico ficam comprometidas, dentre outros (Fascetti & Delaney, 2012). Além do suporte à síntese de proteínas corporais para crescimento, manutenção e reparo de tecidos, aminoácidos essenciais são empregados também como fonte de nitrogênio. O nitrogênio é importante para a síntese de aminoácidos não essenciais e outras moléculas compostas por esse elemento químico, como os ácidos nucléicos, purinas e pirimidinas (Case, 2010).

Os gatos têm elevada necessidades de proteínas por serem animais essencialmente neogliconeogênicos, isto é, produzem parte da glicose que utilizam a partir de compostos aglicanos (não-carboidratos), utilizando para isto parte do pool de aminoácidos glicogênicos ingeridos. Esse processo ocorre principalmente no fígado, o sistema enzimático hepático do gato é especializado em remover o grupo amina dos aminoácidos e utilizar seu esqueleto carbônico para a produção de energia (Morris, 2001). Isso ocorreu devido a evolução e sua dieta em habitat natural, que era compostas por tecidos animais ricos em proteínas e pobres em carboidratos.

Além disso, outros fatores contribuem para a elevada necessidade proteica em gatos, como a necessidade de aminoácidos essenciais como a arginina e taurina. A arginina é amplamente utilizada no ciclo da ureia, responsável por transformar a amônia advinda do catabolismo das proteínas em ureia, que é menos tóxica ao organismo. Porém, os gatos não conseguem reduzir a atividade do ciclo da ureia, que se mantém constante, exigindo desta forma quantidades consideráveis de arginina para seu funcionamento (Zoran, 2002; Sturgess & Hurley, 2005).

Os gatos possuem também necessidade alimentar do aminoácido taurina (Baker, 1991), que é fundamental no metabolismo dos ácidos da bile, pois a maioria dos animais conseguem conjugar ácidos biliares em sais biliares antes de serem secretados pela bile, por meio da glicina e taurina. Entretanto, os gatos somente realizam esse processo por meio da taurina. Diferentemente da maioria dos animais, os felinos possuem baixa capacidade de sintetizar taurina devido à reduzida ação da cisteína descarboxilase e cisteína dioxigenase, duas enzimas responsáveis pela conversão de metionina e cisteína em taurina (Hora & Hagiwara, 2010; Case et. al 2011; Kirk et al., 2000). Quando essa exigência de taurina não é atendida, o animal torna-se susceptível ao desenvolvimento de doenças como degeneração da retina, infertilidade, imunossupressão, desenvolvimento fetal retardado e cardiomiopatia dilatada (Morris, 2001; Hora & Hagiwara, 2010).

Estudos sobre necessidade de proteína em cães são baseados no método de balanço nitrogenado (NRC, 2006), que se baseiam na diferença entre a ingestão e a excreção de nitrogênio pelo corpo (Kendall et al., 1982). Quando a ingestão de nitrogênio é superior ao da excreção, considera-se um balanço positivo. O balanço negativo acontece quando a excreção supera a ingestão, isto pode ocorrer por ingestão baixa de energia, por consumo de dieta desbalanceada em aminoácidos, ou ainda devido a um estado de catabolismo nos animais com doenças graves e prolongadas (Case, 2001). As pesquisas sobre o metabolismo proteico em cães e gatos têm se concentrado em estimar as necessidades mínimas de cada aminoácido (Hendriks, 2003).

2.6. Efeito da extrusão sobre a qualidade das proteínas

O processamento das fontes proteicas pode ocasionar diversas alterações nas proteínas e aminoácidos. A extrusão é tecnologia amplamente utilizada na produção de alimentos para animais de companhia (Tran et al., 2008), processo no qual um conjunto de ingredientes, previamente misturados e moídos, é cozido e formatado na presença de umidade, temperatura, pressão e cisalhamento (Riaz, 2007). Esse processo inclui as etapas de pré-condicionamento, extrusão, secagem e recobrimento (Riaz, 2000; Rokey, et al.,2010).

Durante o processo de extrusão, diversas alterações físicas e químicas ocorrem nos nutrientes, alterando sua qualidade nutricional (Singh e Smith, 1997; Rokey et al., 2010; Bordoloi & Ganguly, 2014). Diferentes alterações podem ocorrer quando os ingredientes proteicos são processados, como inativação de fatores anti-nutricionais em fontes proteicas de origem vegetal e aumento do coeficiente de digestibilidade aparente da proteína em ingredientes proteicos de origem animal e vegetal (Cavalari et al 2006). O processo de extrusão provoca o rompimento de parede celular, liberando a proteína complexada ou enclausurada, também responsável pelo baixo aproveitamento proteico do ingrediente in natura (Borges et al.2003). Outra provável vantagem do processamento é a melhora da palatabilidade do ingrediente, pois há inativação das lipoxigenases que promovem, quando presentes, a oxidação dos ácidos graxos poliinsaturados, desenvolvendo sabor e odor desagradáveis (Mustakas et al., 1969).

Outros efeitos relacionados à extrusão incluem desnaturação de enzimas, a destruição de alguns fatores tóxicos termolábeis e diminuição da contaminação bacteriana do produto (Egaña et al., 1991). A desnaturação das proteínas pode torná-las mais suscetíveis a enzimas digestivas e, portanto, melhorar a sua digestibilidade (Lankhorst et al., 2007). Durante o processo de extrusão, os aminoácidos podem sofrer alterações substanciais, por exemplo, a lisina, um aminoácido responsável pela síntese proteica é frequentemente usado para se avaliar os efeitos do processamento nas proteínas (Tran, 2008). Isto por que lisina é sensível ao calor, por conter dois grupos amino reativos, sofrendo reação de Mailard quando processado em altas temperaturas (Shibao et al 2011). Além disso, pode haver alterações como a formações de complexos proteína-lipídeo e proteína-carboidrato (Björck and Asp, 1983), fatores esses que podem danificar as estruturas dos aminoácidos e causar perda de seu valor nutricional.

Todas essas modificações são dependentes da quantidade de energia (mecânica e térmica) que é transferida para a massa durante o processo de extrusão, bem como do tempo de residência da massa e da umidade de processamento (Monti et al., 2016; Pacheco et al., 2018). A energia mecânica é aplicada através do cisalhamento ou atrito da massa no canhão extrusor, enquanto a energia térmica é adicionada a partir do vapor e da água, que podem ser acrescentados no condicionador e/ou extrusor (Riaz, 2007).

A aplicação de energia se modifica de acordo com os ingredientes contidos nas fórmulas. Ingredientes secos são mais facilmente cozidos e formatados, já que os ingredientes úmidos alteram a aplicação de energia mecânica e se tornam um desafio para o processo (Baller et al.,2018). Sendo assim, é importante se controlar e estudar o processamento de fontes proteicas, secas ou contendo alta umidade, visando manter a qualidade dos produtos finais.

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CAPÍTULO 2 – Mechanically deboned chicken meat as a substitute for chicken by-product meal in extruded foods to dogs [1]

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Mechanically deboned chicken meat as a substitute for chicken by-product meal in extruded foods to dogs

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Abbreviations: CM, chicken by-product meal; MDCM, mechanically deboned chicken meat.

Abstract

Poultry or chicken by-product meal (CM) is the most traditional protein source for extruded diets for dogs, an ingredient with low moisture after the rendering processing. New extrusion technologies have made possible elevated inclusions of high moisture raw materials, such as fresh (frozen) meat. The present study evaluated the effects of mechanically deboned chicken meat (MDCM) inclusion, replacing CM in dog diets, on kibble macrostructure, apparent digestibility of nutrients and energy, feces characteristics and fermentation products, post prandial urea response, nitrogen balance, and diet palatability. A control diet with CM as a protein source was balanced, and three levels of MDCM was included on diets with similar chemical composition, replacing 23%, 41%, and 55% of the dietary protein content. Twenty-four beagle dogs were used, distributed in a randomized block design with 6 dogs per diet. Results were submitted to analysis of variance and means compared by polynomial contrasts. Repeated measures analysis of variance was used to evaluate urea post prandial responses and the student T test for the palatability comparisons ($P<0.05$). Kibble hardness, expansion rate and piece density decreased quadratically, and specific length increased quadratically with MDCM inclusion ($P<0.01$). All diets presented elevated and similar starch gelatinization degree ($P>0.05$). The total tract apparent digestibility coefficient (TTAD) of dry matter increased quadratically and the TTAD of crude protein and gross energy increased linearly with MDCM inclusion ($P<0.05$). According to the linear effect of MDCM inclusion on crude protein TTAD, extrapolating the inclusion rate to 100%, the estimated apparent digestibility would be 0.93 ($P=0.016$; $R^2=0.63$). Feces formation was similar between dogs ($P>0.05$). A quadratic decrease was observed on ammonia and isobutiric acid fecal concentration ($P<0.05$), suggesting a reduction on protein fermentation on colon. No differences between diets were observed on amino acid intake, urea post prandial responses, and nitrogen retention of the dogs ($P>0.05$). A strong preference for diets with high MDCM inclusion (40.8% and 55.2% protein replacement) was observed, with first choices and intake ratios higher than 0.88 in comparison to control CM diet ($P<0.001$). In conclusion, the substitution of CM for MDCM increased the palatability and digestibility of the diet and reduced protein fermentation products in the feces. The evaluated extrusion system was able to properly process high moisture formulations that present MDCM as the main raw material.

Keywords: digestibility, extrusion, fermentation products, palatability, protein.

1. Introduction

Proteins are key nutrients in dog food formulation, corresponding to a relevant part of the food formulation cost (Carciofi, 2008; Meeker and Meisinger, 2015). As a characteristic of the pet food marketing, the selection of a protein source may be complex and include other factors than quality, commercial availability, and cost, encompassing aspects such as consumer opinion and beliefs (Rothgerber, 2014), marketing trends, sustainability and environmental concerns (Alexander et al., 2020; Bosch and Swanson, 2021; Acuff et al., 2021), processing issues in obtaining adequate kibbles in an efficient manner, and it influence on the overall diet palatability (Samant et al., 2021).

Rendered chicken or poultry by-products are the main driven raw material traditionally used in dog formulations worldwide (Murray et al., 2009; Farmaneshet al.,2019). Its composition includes high amounts of protein with good amino acid profile, moderate fat content, and variable amounts of ash, that may be good sources of calcium, phosphorus, and magnesium, but when too high might difficult the formulations of minerals on the diet (Dozier et al., 2003). Additionally, this raw material presents in general a good digestibility to dogs, but this is very variable according to the raw material sources and the processing conditions in the rendering industry (Johnsonet al.,1998; de-Oliveiraet al.,2011; Tortolaet al.,2013; Vanellilet al.,2021). Chicken or poultry by-product meals are generally considered raw materials that contribute to mitigate the pet food impact on the environment (Swansonet al.,2013; Meeker and Meisinger, 2015; Acuffet al.,2021), however for pet owners this raw material may be linked to non-adequate food resources and a marketing pressure toward the use of less processed frozen meat is moving pet food companies to include more raw ingredients in formulations (Buff et al., 2014), as the mechanically deboned chicken meat (MDCM) (Beaton, 2016; Meineriet al.,2021).

The MDCM is produced from edible tissue of chicken bones by deboning or separation techniques. It includes muscle and connective tissue, fat, and water with a variable chemical composition, influenced by the age of the animas, relationship among bones to muscle on raw materials, and the amount of skin. It contains bone particles and free heme groups due to the inclusion of bone marrow, and due this it is vulnerable to oxidation and spoilage (Songet al.,2014; Akramzadehet al.,2020). The last authors reported a variable chemical composition on MDCM, depending on the chicken or poultry parts used, with moisture content ranging from 60% to 76%, protein from 12% to 20%, fat from 4% to 18%, and ash from 0.6% to 2.9% (Akramzadehet al.,2020). Although be a potentially attractive raw material, few studies evaluated its use in pet food (Tjernsbekket al.,2017; Meineriet al.,2021). The issues involved with their utilization are the limited information on its effect on diet digestibility, great variation on chemical composition, potential contamination with pathogenic microorganism and presence of biogenic amines. In addition, the difficult and cost involved in transport, storage, and handle of a high moisture frozen raw material, and the limited capability of the conventional extrusion systems to incorporate high moisture ingredients currently limits their utilization do dog diets.

Usually, the available studies in pet food incorporated less than 30% of MDCM on evaluated recipes (Tjernsbekket al.,2017; Meineriet al.,2021). Considering their high moisture content (>65%), this means that probably less than 10% of the diet dry matter was constituted by MDCM, so the overall impact of the other raw materials is high and is difficult to evaluate the results. This is explained by the strong effect of the in-barrel moisture on the medium shear single screw extrusion system, typically used for pet food production (Riaz, 2007). When the mass in-barrel moisture is higher than 30 or 35% in the conventional extrusion systems, water acts as a lubricant reducing shear and consequently mechanical energy implementation (Guy, 2001). This creates a limitation of the screw capacity to convey the mass, making the mass flow instable, and reduce the shear, temperature, and pressure of the mass inside the barrel, ultimate limiting the cooking and formatting of the kibbles (Baller, et al.,2018). However, new developments in twin screw systems are capable to transfer higher amounts of energy to mass. This may compensate for the limitation in mechanical energy transference, and may promote enough total energy application, may inducing the necessary physicochemical transformation on the mass. In these systems up to 60% (on an as fed basis) of MDCM inclusion is possible, although currently no publications were found evaluating it in dogs.

Taking this in considerations, the objectives of the present study was to evaluate the effects of elevated inclusion rates of MDCM, replacing chicken by-product meal (CM) as a protein source, on kibble formation, total tract apparent digestibility of nutrients and energy, feces characteristics and fermentations products, nitrogen balance, pos-prandial urea responses, and the palatability of diets to dogs.

2. Material and Methods

All experimental procedures with animals were previously approved by the Ethics Committee on Animal Use of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil (Protocol 012152/18).

2.1. Experimental diets: formulation and processing

Four formulations with similar crude protein and crude energy content were balanced to dog maintenance (AFFCO, 2018). To this the raw materials was purchased, samples and analyzed for moisture, crude protein, and crude fat and diets formulated according to results (Table 1). A control diet with CM as a protein source was balanced, and in the three other treatments MDCM was included in amounts to replace 23%, 41%, and 55% of the dietary protein content (Table 2).

The high inclusion of meat-based high moisture raw material was achieved using a twin-screw extruder (Thermal Twin, Wenger, Sabetha, EUA). The extruder hardware configuration was kept constants for all treatments, but the software conditions varied to compensate for the elevated moisture of the MDCM, in order to obtain suitable kibbles to use. Extrusion processing parameters were recorded every second through an automated processing management system, and the average value for each treatment presented on Table 3.

Table 1. Analyzed chemical composition of the chicken by-product meal (CM) and the mechanically deboned chicken meat (MDCM) samples utilized in the experiment.

Item	CM	MDCM
g kg, on as-fed basis		
Moisture	39,0	790,5
g kg, on DM basis		
Crude protein	699,0	491,0
Crude fat	131,0	439,0
Ash	111,0	n.d.

n.d. - not determined

Table 2. Ingredient composition of the dog foods with different inclusions of mechanically deboned chicken meat (MDCM).

Ingredients	Dietary protein replacement by MDCM[1]			
	0%	23.1%	40.8%	55.2%
g/kg, on as fed basis				
MDCM	0.0	515.5	921.2	1260.1
g/kg, on DM-basis				
MDCM	0.0	108.0	193.0	264.0
Chicken by-product meal	322.2	252.0	196.7	150.5
Ground Peas	220.0	220.0	220.0	220.0
Cassava	243.5	235.5	229.7	224.9
Chicken fat	139.3	106.0	79.9	58.1
Beet Pulp	50.0	50.0	50.0	50.0
Common salt	8.0	8.0	8.0	8.0
Fish oil	5.0	5.0	5.0	5.0
Choline chloride	4.0	4.0	4.0	4.0
Potassium chloride	4.0	4.0	4.0	4.0
Vitamin premix [2]	1.8	1.8	1.8	1.8
Mold Inhibitor[3]	1.0	1.0	1.0	1.0
Mineral premix[4]	1.4	1.4	1.4	1.4
Antioxidant[5]	0.6	0.6	0.6	0.6
Dicalcium phosphate	0.0	3.4	5.6	7.5
MDCM to dry recipe ratio of inclusion (%) [6]	0	40.8	81.0	120.0

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

[2] – Addition per kilogram of product: vitamin A 18,750 IU; vitamin D 1500 IU; vitamin E, 125 IU, vitamin C 125 mg, vitamin K 0.15 mg, Thiamine 5 mg, Riboflavin 16 mg, Pantothenic acid 35, 75 mg, Niacin 62.5 mg, Pyridoxine 7.5 mg, Cobalamin 45 mcg, Folic acid 0.75 mg.

[3]- Myco Curb, Kemin, USA: Propionic acid, sodium hydroxide, calcium hydroxide, amorphous silicon; Dioxide, Sorbic Acid, Benzoic Acid, Propylparaben, Methylparaben and BHA, vehicle q.s.p.

[4] - Addition per kilogram of product: Iron 100 mg, Copper 9.25 mg, Manganese 6, 25 mg, Zinc 150 mg, Iodine 1.87 mg, Selenium 0.13 mg.

[5]- Naturox, Kemin, USA: Silicon Dioxide, Citric Acid, Natural Mixed Tocopherols, Vegetable Oil, and Rosemary Extract.

[6] – Calculated as: (kg/h of MDCM ÷ kg/h dry recipe feed rate) * 100

The MDCM was heated, emulsified, and infused directly on extrusion preconditioner. Along the processing it was possible to see that dry recipe feed rate was reduced in approximately 25%, and MDCM increased from 0% (control diet) to a proportion of 120% in relation to the dry recipe feed rate, in an as-fed basis. Due to the high moisture content of the MDCM, however, the dry matter addition corresponded to 36% of the dry recipe feed rate. On software conditions, extruder shaft speed was reduced gradually, and water and steam infusion stopped. The system adjustment was enough to accept the increase in moisture; although the in-barrel moisture increased approximately 34%, the extruder motor load, and the specific mechanical energy application remained similar. The mass temperature at die plate was also similar, but a reduction of approximately 40% on mass pressure before the die was observed. Due this, a relevant increase on kibble bulk density was observed, of approximately 39%.

Table 3. Processing variables of extruded dog foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM [1, 2]			
	Control	23.1%	40.8%	55.2%
Dry recipe feed rate (kg/hr)	495	441	405	375
Preconditioner Information				
Steam Flow to Preconditioner (kg/hr)	28	0	0	0
Water Flow to Preconditioner (kg/hr)	100	0	0	0
MDCM Flow to Preconditioner (kg/h)	0	180	328	450
Moisture addition (kg/h)	0	126	230	315
Dry matter addition (kg/h)	0	54	98	135
Extruder Information				
Extruder shaft speed (rpm)	492	486	456	448
Extruder motor load (%)	34	46	37	35
Extruder steam (Kg/hr)	36	0	0	0
Extruder Discharge rate (kg/hr)	432	432	432	375
Extruder Discharge Density (kg/m^3)	534	570	690	742
Extruder Die Temperature (°C)	91	68	109	90
Extruder Die pressure (Kpa)	4164	6744	3720	2460
Final Product Information				
Specific mechanical energy implementation (kW-hr/Ton)	22	34	29	26
In-barrel moisture (%)	32	27	37	43

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

[2] – Diets produced in the equipment Thermal Twin, Wenger, Sabetha, KS, EUA

2.2. Particle size distribution and kibble characteristics

The mean geometric diameter (MGD) and geometric standard deviation (GSD) of the raw materials mixture (only the dry part, without the MDCM) were evaluated according to the methodology described by Zanotto & Belaver (1996), and results interpreted on the Granucalc software (Embrapa Suínos e Aves, Concordia, Brazil).

To evaluate kibble macrostructure, the hardness was analyzed in 20 kibbles using a texture analyzer (TAX/T2I, Stable Micro Systems, Godalming, UK) equipped with a load cell of 50 kgf and a cone probe. First, kibbles were stabilized at the same moisture in an oven (Quimis, Diadema, Brazil) at 35ºC for 24 h. For each treatment, the length, diameter, and mass of 20 extrudates were measured using a caliper and a scale. The data were used to obtain the radial expansion ratio, specific length, and piece density as described by Baller et al., (2018). To access the extrusion quality, the starch gelatinization degree of the diets was measured according to Hoseney (1994). The in vitro digestibility of organic matter was measured following the procedures of Hervera et al., (2007).

2.3. Digestibility protocol

Twenty-four beagle dogs aged 6.7 ± 0.8 years old and weighing 12.3 ± 1.5 kg were used. The health of the animals was previously acceded by a veterinarian, thought physical examination, hemogram, and serum biochemistry analysis. The digestibility protocol was carried out through the total collection of feces without urine collection method, considering the FEDIAF (2020) recommendations. The study followed a completely randomized design, with 3 blocks of 8 dogs, 2 dogs per diet in each block, totaling 6 dogs per food. The blocking factor was time, due the impossibility to test all the dogs in the same moment.

In each block, during the adaptation period (5 d) dogs were housed in 1.5 × 4.0 m kennels with a solarium and were released daily into a collective playground for exercise and socialization. During the feces collection period (5 d), dogs were individually housed in 1.0 × 1.0 × 1.0 m stainless steel metabolic cages. Animals were fed twice daily (09:00 and 16:00 h) in sufficient amounts to cover their metabolizable energy requirements (ME, kcal/d = 110 × Body Weight$^{0.75}$). The metabolizable energy content of the diets was estimated considering their chemical composition and the equation based on crude fiber recommended by the National Research Council (NRC, 2006). Water was offered *ad libitum*.

Fecal output was quantitatively collected and weighed at each feeding time and immediately stored at −20°C until further processing. At the end of the collection period, feces were pooled by a dog, thawed, homogenized, and dried using a forced-air oven (MA035, Marconi, Piracicaba, Brazil) at 55°C for 72 h for chemical analysis. The fecal consistency was scored on a 0 to 5 scale (Carciofi et al., 2008): 0 = watery liquid that can be poured; 1 = soft, unformed; 2 = soft, poorly formed stool that assumes the shape of its container; 3 = soft, formed, and moist stool that retains its shape; 4 = well-formed and consistent stool that does not adhere to the floor; and 5 = hard, dry pellets, which are small and hard masses.

Experimental diets and dried feces were ground in a cutting mill (MA680, Marconi, Piracicaba, Brazil) fitted with a 1.0 mm screen sieve. Samples were then analyzed according (AOAC, 1995) for dry matter (method 934.01), crude fat was determined after acid hydrolysis (method 954.02), ash content by muffle furnace incineration (method 942.05), crude fiber (method 962.09), crude protein (method 990.03) using a LECO nitrogen/protein analyzer (FP-528, LECO Corporation, Saint Joseph, USA). Organic matter (OM) was calculated as DM minus ash. Gross energy (GE) was determined in a bomb calorimeter (IKA C2000 Basic, IKA-Werke GmbH & Co., KG, Staufen, Germany). The total starch content was determined using an enzymatic method (Hendrix, 1993). All analyses were carried out in duplicate and repeated when the variation coefficient between replicates was higher than 5 %.

2.4. Fecal pH and fermentation products

Immediately after feces collection for digestibility evaluation, fresh fecal samples were taken (collected before 15 min of defecation) on three consecutive days to measure pH and fermentation products. Fecal pH was measured using a pHmeter (DM20, Digimed Analítica Ltda., São Paulo, Brazil) immediately after collection, in 2 g of fresh feces diluted in 6 ml of ultrapure water. For short-chain fatty acids (SCFA) and branched-chain fatty acid (BCFA) analyses, 10g of fresh feces were placed in a container and immediately stored at −20°C until further processing. At the end of the collection, feces were mixed in 30 ml of formic acid solution at 4.2 N, mixed and centrifuged three times (5,000 G at 4°C for 15 min). The analysis was performed by gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan) according to Erwin, Marco, & Emery (1961). The lactic acid content of feces was measured according to Pryce (1969) by mixing 3 g of fresh feces with 9 ml of ultrapure water and subsequent evaluation with a colorimetric method (Spectrophotometer Quick-Lab, Drake, Sao José do Rio Preto, Brazil). The ammonia concentration was assessed in the extracts prepared for SCFA and BCFA analysis, according to Vieira (1980) in a nitrogen distillation system (Tecnal TE-036/1, Tecnal, Piracicaba, Brazil).

2.5. Postprandial urea response

After feces collection for fermentation products evaluation, the postprandial responses of urea of the dogs were evaluated according to Tortola et al. (2013), with changes in blood collection times: 0 (zero, before meal), and 15, 30, 60, 120, 180, 240, 300, 420, and 540 minutes after the meal. On the day of the test, after trichotomy and antisepsis, the cephalic vein was cannulated with an intravenous catheter (Angiocath 20GA x 1.16in., Bekton Dickinson, USA). A blood sample was collected to determine the basal urea. The experimental diet was weighed and offered, and dogs allowed to eat for 15 minutes. Dogs that did not consume at least 90% of the stipulated amount in the period of 15 minutes were not evaluated on that day and was evaluated on the following day. Once the meal had been finished, the time counting started to conduct the blood samplings. All urea curves were started in the morning around 09:00 h.

At each sampling time, 3 mL of blood were collected, placed in a container with 0.05mL sodium fluoride (Labtest Diagnostica SA, Lagoa Santa, Brazil), centrifuged (2,000G for 5 min), and plasma separated. Immediately the catheter was washed with a saline solution to maintain patency. To evaluate the urea plasma concentrations, the Urea UV liquiform kit were used (Labtest Diagnostica SA, Lagoa Santa, Brazil), with readings in a spectrophotometer at 600nm (QUICK - Lab brand DRAKE). Urea responses were compared for average and maximum absolute values and incremental urea increase (the difference between absolute and baseline urea concentrations) and the integrated area under the post-prandial urea curve (AUC). The software GRAPHPAD PRISM (Version 4.0; GraphPad, La Jolla, CA, USA) was used for AUC computing, by the trapezoidal numerical integration method.

2.6. Urine Characteristics and Nitrogen Balance

To determine urine characteristics, for three consecutive days urine was quantitatively collected at least three times a day in plastic bottles with 100 mg of thymol as a preservative and kept under refrigeration (4ºC). Each 24 h-pooled urine sample was homogenized, and the volume, density, and pH were determined. Density was measured using a refractometer (T2-Ne Clinical; ATAGO CO., LTD.; Fujita Yorii-cho Osato gun, Saitama, Japan) and pH using a pH meter (Digimed DM20; Digicrom Analítica, São Paulo, Brazil).

The nitrogen balance was applied simultaneously to the digestibility assays. For this, during the five consecutive days of total feces collection, the urine was quantitatively collected in bottles with 2 ml of 1N sulfuric acid solution to avoid nitrogen loss. Urine was collected every 24 hours, homogenized, their volume measured and kept frozen at -20 ° C for posterior analysis. Nitrogen content was determined by the Kjedhal method (AOAC, 1995) in food, feces, and urine samples. Nitrogen balance was calculated using the digestibility trials' food ingestion and feces excretion data, as the difference between total nitrogen intake and the nitrogen excreted in feces and urine.

2.7. Food Preference Test

The first choice (product consumed first) and palatability (product consumed in greater quantity) were compared using the two-plate method (Griffin, 2003) at Panelis (Diana Petfood, Descalvado, Brasil). For this, a panel composed by at least 30 trained dogs housed individually was used for two consecutive meals. All diets were challenged against the control diet. In the morning, after a period of 12 hours, the animals received the first meal in two trays, each containing one of the experimental foods, which were available to the animals for thirty minutes. The position of the plates was alternate during the second meal, after 8 hours. The amount of offered food exceed the animal's consumption capacity, thus ensuring the occurrence of leftovers. After trays were removed, leftovers were weighed, and the consumption was calculated. To determine the palatability, the consumption rate was calculated as: Consumption rate (%) = (Food A consumption x 100) / (Food A + Food B consumption).

2.8. Statistical analysis

For extrudate macrostructure comparison, the experimental unit was one kibble with 20 repetitions per treatment. Data on digestibility, fecal and urine characteristics, and urea post prandial responses were analyzed in a completely randomized block design, with three blocks (period) and two repetitions (dogs) per block, totaling six dogs per food. The experimental unit was one dog. Urea curves were analyzed for each postprandial period using a mixed model analysis to allow repeated measures over time (diet and time). The assumptions of normality of errors and homogeneity of variances were previously evaluated. Data were submitted to analyze variance, and when differences were detected at the F test, means were compared by polynomial contrasts considering the dietary crude protein replaced by the protein of MDCM. The MIXED procedure of SAS Software was used (version 9.1, SAS Institute, Cary, NC, USA). The Student T-test was used to evaluate the palatability results, and the proportions test (Perform Exact Sign Test) to evaluate the first choice. Prior, the Shapiro-Wilk tests was used to verify error normality and if necessary, the Wilcoxon Rank Sum test was applied. Values of $P < 0.05$ was considered significant.

3. Results

3.1. Experimental diets

Diets presented similar chemical compositions, as shown on Table 4. The dry recipe flow rate and the MDCM infusion adjustments during the extrusion processing were adequate, and the diets CP was similar, with CM protein replacement by the protein of MDCM close to targeted. The particle size distribution of the dry recipe of the experimental diets was similar, with a mean geometric diameter of 280 µm and a geometric standard deviation of 1.6 µm. The amino acid composition of the two evaluated ingredients was similar, as observed by the similar amino acid profile of the four experimental diets. All evaluated nutrients were in accordance with the requirements for dog maintenance (AFFCO, 2018).

Table 4. Analyzed chemical composition (g/kg, DM basis) of the dog foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]			
	Control	23.1%	40.8%	55.2%
Moisture	73.7	63.4	88.9	95.5
Ash	71.0	71.3	72.7	71.4
Crude protein	292.5	293.8	295.2	282.0
Essential Amino Acids				
Arginine	20.4	19.1	19.9	19.2
Histidine	6.0	6.3	6.9	7.2
Isoleucine	11.0	10.7	11.4	11.2
Leucine	20.6	19.5	21.0	20.1
Lysine	18.0	17.5	18.9	18.5
Methionine	5.3	4.9	5.3	5.2
Methionine + Cystine	8.1	7.6	8.0	7.8
Phenylalanine	11.8	10.9	11.5	11.2
Phenylalanine + Tyrosine	20.3	18.9	19.8	19.6
Tryptophan	2.6	2.5	2.5	2.1
Threonine	10.6	10.1	10.6	10.6
Valina	13.5	12.9	13.4	12.9
Non-essential amino Acids				
Alanine	18.4	17.2	17.6	17.2
Aspartic Acid	24.8	23.3	24.0	24.7
Cystine	2.8	2.7	2.7	2.6
Glutamic Acid	36.4	36.2	34.2	38.0
Glycine	26.4	23.4	23.2	21.9
Proline	17.0	15.2	15.2	14.6
Serina	12.8	11.8	12.4	12.1
Tyrosine	8.5	8.0	8.3	8.4
Acid-hydrolyzed fat	205.9	212.0	210.1	200.5
Crude Fiber	38.6	31.6	27.4	30.1
Gross energy (kcal/g)	5103	5313	5341	5247

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

3.2. Kibble macrostructure

A pronounced effect on macrostructure followed the increase on MDCM addition (Table 5), with a quadratic decrease on kibble hardness, expansion rate, and piece density (P<0,01), and a quadratic increase on specific length (P<0,01). Pictures of the kibbles are shown on Figure 1. Starch gelatinization degree was high in all treatments, and did not change after MDCM inclusion, suggesting enough energy application to induce the necessary chemical transformation and starch cooking (P=0.327). The in vitro digestibility of the OM has high and similar between treatments (P=0.517).

Figure 1. Pictures illustrating the kibbles produced for the experiment.

[1] Control diet without MDCM addition.
[2] Replacement of 23.1% of the dietary protein by the MDCM protein.
[3] Replacement of 40.8% of the dietary protein by the MDCM protein.
[4] Replacement of 55.2% of the dietary protein by the MDCM protein.

Table 5. Kibble characteristics and macrostructure of extruded dog foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[2]	p-Value	Contrast[3]	
	Control	23.1%	40.8%	55.2%			Linear	Quadratic
Hardness (N)	117.03	53.67	107.21	65.10	4.81	0.000	0.001	0.002
Expansion rate	3.31	2.38	1.31	2.65	0.12	0.000	0.000	0.000
Piece density (g/cm3)	0.70	0.68	0.70	0.58	0.01	0.000	0.001	0.008
Specific length (cm/g)	0.91	1.30	2.30	1.39	0.08	0.000	<0.001	<0.001
Starch gelatinization degree (%)	96.24	95.22	87.23	94.46	1.82	0.327	ns	ns
In vitro digestibility of the OM (%)	83.76	85.86	86.33	85.49	0.57	0.517	ns	ns

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

[2] SEM - standard error of mean (n = 20).

[3] Linear or quadratic effect of dietary protein replacement by MDCM.

ns – not significant.

3.3. Nutrient intake and coefficient of total tract apparent digestibility

Nutrient intake and the coefficient of total tract apparent digestibility (TTAD) of nutrients and energy are reported in Table 6. All diets were well accepted by the dogs, with no episodes of refusals, vomiting, or diarrhea. The inclusion of MDCM did not change the intake of nutrients ($P>0.05$). The TTAD of DM increased quadratically ($P=0.022$), and the TTAD of crude protein and gross energy increased linearly with MDCM addition ($P<0.05$). According to the linear effect of MDCM inclusion on crude protein TTAD, extrapolating the inclusion rate to 100% (dietary protein supplied only by MDCM), the estimated CP digestibility of the diet would be 0.93 ($P=0.016$; $R^2=0.63$).

3.4. Fecal characteristics and fermentation products

Feces production, dry matter, score, and pH were similar for dogs fed the experimental diets ($P>0.05$; Table 7). On fermentation products, BCFA and lactate fecal concentration remained similar ($P>0.05$), but it was verified a quadratic decrease on isobutiric acid and ammonia concentrations, fermentation products originated from protein degradation, with lower values on the feces of dogs fed the highest inclusion of MDCM ($P<0.05$).

Table 6. Nutrient intake and total tract apparent digestibility of nutrients and energy of dogs fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[2]	p-Value	Contrast[3]	
	Control	23.1%	40.8%	55.2%			Linear	Quadratic
Nutrient intake (g/dog/day)								
Dry matter	156.23	163.28	144.86	152.85	2.93	0.116	ns	ns
Organic matter	145.14	151.64	134.33	141.94	2.73	0.110	ns	ns
Crude fat	32.16	34.61	30.44	32.06	0.62	0.085	ns	ns
Starch	58.78	61.30	57.22	59.92	1.04	0.528	ns	ns
Crude protein	45.69	47.97	42.76	43.10	0.86	0.073	ns	ns
Coefficients of total tract apparent digestibility								
Dry matter	0.847	0.845	0.836	0.864	0.36	0.033	ns	0.022
Organic matter	0.877	0.877	0.869	0.888	0.28	0.086	ns	ns
Crude fat	0.949	0.947	0.944	0.951	0.14	0.330	ns	ns
Starch	0.995	0.996	0.995	0.996	0.03	0.528	ns	ns
Crude protein	0.800	0.809	0.805	0.835	0.51	0.013	0.016	ns
Gross energy	0.879	0.885	0.880	0.899	0.28	0.022	0.020	ns

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets.

[2] SEM - standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM.

ns – not significant.

Table 7. Fecal characteristics and fermentation products of dogs fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[2]	p-Value	Contrast[3]	
	Control	23.1%	40.8%	55.2%			Linear	Quadratic
Fecal characteristics								
g/dog/day (DM basis)	23.88	25.45	24.42	20.65	0.21	0.070	ns	ns
g/dog/day (As-is basis)	65.20	74.67	69.08	60.39	0.22	0.187	ns	ns
Dry matter	36.74	34.51	35.57	34.20	0.21	0.371	ns	ns
Score	4.00	3.98	4.00	4.00	0.07	0.416	ns	ns
pH	6.41	6.17	6.14	6.23	0.21	0.113	ns	ns
Fermentation products (mMol/g of feces DM)								
Acetic acid	209.45	282.08	231.13	219.07	17.82	0.252	ns	ns
Propionic acid	126.01	163.14	140.54	151.41	7.73	0.097	ns	ns
Butiric acid	39.51	44.10	39.67	43.86	2.81	0.831	ns	ns
Total SCFA	421.70	489.32	411.33	414.34	0.21	0.266	ns	ns
Isobutiric acid	5.54	7.41	5.71	4.36	0.48	0.048	ns	0.021
Isovaleric acid	9.64	12.19	10.33	8.28	0.66	0.081	ns	ns
Valeric acid	0.73	1.22	1.22	1.20	0.11	0.256	ns	ns
Total BCFA	15.71	20.82	17.26	13.84	0.22	0.066	ns	ns
Total VFA	441.73	510.13	428.59	428.18	0.21	0.236	ns	ns
Ammonia *(mMol/g of DM)*	99.00	123.83	97.22	88.55	0.22	0.006	ns	0.006
Lactate *(mMol/g of DM)*	3.71	4.08	4.13	4.51	0.10	0.051	ns	ns

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

[2] SEM - standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM.

ns – not significant.

3.5. Postprandial urea response

To better interpret the urea post-prandial responses and nitrogen balance, the results of crude protein and essential amino acids intake are shown in Table 8. Values were similar between diets (P>0.05) and exceeded those recommended by the NRC (2006), except for Methionine plus Cysteine intake, which had a lower than recommended intake.

The postprandial urea responses are presented in Table 9. Basal, mean, maximum, and the plasma urea AUC parameters did not differ between dogs fed the experimental diets (P>0.05). These responses are illustrated on Figure 2.

3.6. Urine characteristics and nitrogen balance

Urine volume, density, and pH of dogs was similar, regardless of MDCM inclusion (P>0.05; Table 10). Urea renal excretion of dogs was also similar (P>0.05). The intake, renal excretion and retention of nitrogen was also similar between dogs (P>0.05), but nitrogen fecal excretion reduced quadratically as the MDCM inclusion increased (P=0.003), what is explained by the increase on the TTAD of crude protein after MDCM inclusion on diets.

Table 8. Intake of crude protein and essential amino acid (g/kg$^{0.75}$/day) of dogs fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				NRC requirement [3]	p- Value	Contrast[4]	
	Control	23.1%	40.8%	55.2%			Linear	Quadratic
Crude protein	6.88	6.94	6.84	6.53	3.28	0.0736	ns	ns
Essential Amino Acids								
Arginine	0.48	0.48	0.47	0.47	0.11	0.2618	ns	ns
Histidine	0.14	0.14	0.14	0.14	0.06	0.2610	ns	ns
Isoleucine	0.26	0.26	0.25	0.25	0.12	0.2686	ns	ns
Leucine	0.49	0.49	0.48	0.48	0.22	0.2525	ns	ns
Lysine	0.43	0.43	0.42	0.42	0.11	0.2537	ns	ns
Methionine	0.13	0.13	0.12	0.12	0.11	0.3103	ns	ns
Methionine + Cystine	0.19	0.19	0.19	0.19	0.21	0.3132	ns	ns
Phenylalanine	0.28	0.28	0.27	0.27	0.15	0.2344	ns	ns
Phenylalanine + Tyrosine	0.48	0.48	0.47	0.47	0.24	0.2543	ns	ns
Tryptophan	0.06	0.06	0.06	0.06	0.05	0.3408	ns	ns
Threonine	0.25	0.25	0.25	0.25	0.14	0.2328	ns	ns
Valine	0.32	0.32	0.31	0.31	0.16	0.2403	ns	ns

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

[2] SEM – standard error of the mean (n = 24).

[3] – Daily allowance for dog maintenance of the Nutrient Requirements of Dogs and Cats (NRC, 2006).

[4] – Linear or quadratic effect of dietary protein replacement by MDCM.

ns – not significant.

Table 9. Postprandial urea response of dogs fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[2]	p-Value	Contrast[3]	
	Control	23.1%	40.8%	55.2%			Lin.	Quad.
Basal urea (mg/dL)	26.64	21.50	23.70	22.04	1.19	0.481	ns	ns
Mean urea (mg/dL)	34.80	30.94	33.14	33.57	0.90	0.452	ns	ns
Maximum urea (mg/dL)	42.82	39.75	41.86	39.91	0.89	0.826	ns	ns
Time to peak (min)	340	350	330	274	15.05	0.264	ns	ns
AUC (mg/dl/h)								
0-540.’	42.82	41.16	43.46	40.30	4.30	0.521	ns	ns
0-120.’	33.09	29.40	31.97	30.67	4.29	0.540	ns	ns
120-540.’	42.82	41.16	43.46	40.30	4.30	0.570	ns	ns

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets

[2] SEM – standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM.

ns – not significant.

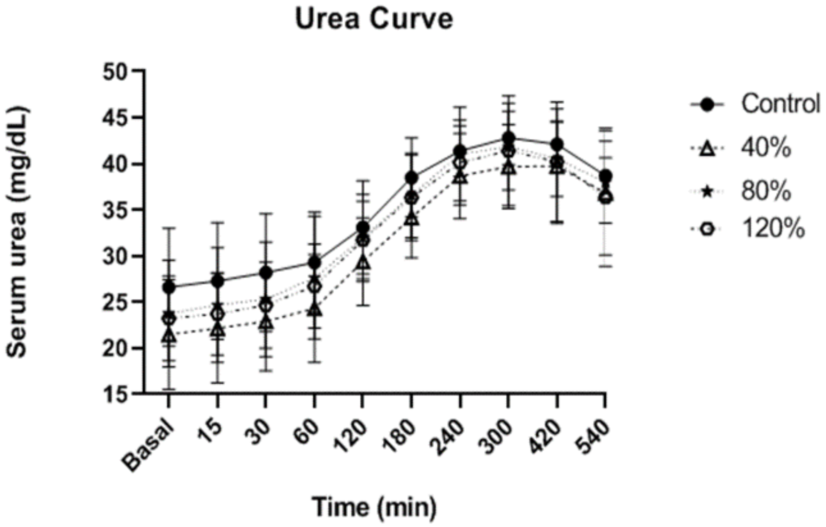

Figure 2. Postprandial urea response curves of dogs fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM). Control diet is based on chicken by-product meal, and 23.1%, 40.8%, and 55.2% corresponds to dietary protein replacement by MDCM protein, calculated considering the analyzed chemical composition of the ingredients and diets.

Table 10. Urine characteristics and nitrogen balance of dogs fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM [2]	p-Value	Contrast[3]	
	Control	23.1%	40.8%	55.2%			Linear	Quadratic
Urine characteristics								
Volume (ml/dog/day)	254.67	361.56	221.22	369.78	0.21	0.209	ns	ns
Specific density	1.03	1.02	1.03	1.02	0.22	0.236	ns	ns
pH	6.74	6.83	6.71	6.61	0.22	0.402	ns	ns
Urinary urea								
mg/kg BW/day	72.85	51.31	68.27	55.55	5.46	0.418	ns	ns
mg/g of crude protein intake	19.41	13.98	18.37	15.86	1.41	0.470	ns	ns
Nitrogen Balance (mg/kg$^{0.75}$/day)								
Intake	592.50	584.07	595.10	561.89	8.01	0.535	ns	ns
Fecal excretion	116.49	110.31	118.99	83.15	3,47	0.009	<0.001	0.003
Urinary excretion	404.81	401.26	400.24	419.83	7.65	0.842	ns	ns
Retained	71.20	72.50	75.88	58,91	7.92	0.932	ns	ns

[1] – Calculated considering the analyzed chemical composition of the ingredients and diets.

[2] SEM - standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM.

ns – not significant.

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3.7. Food preference test

Due to the impact of moisture on palatability before the palatability comparisons all diets were corrected to present the same moisture content (data not shown). Although no differences on first choice was observed when control diet and 23.1% MDCM was compared (P=0.344), a tendency of higher intake of the 23.1% MDCM food was observed (P=0.063). In higher additions, strong preference of the dogs by the diets with MDCM was observed, with first choices and intake ratios usually close do 0.9 for the diet with MDCM (P<0.001).

Table 11. Preference test of extruded dog foods with different inclusions of mechanically deboned chicken meat (MDCM).

Challenge[1]	First choice			Intake ratio[2]		
	A	B	p-Value	IR-A	IR-B	p-Value
Control vs. 23.1%	0.39	0.61	0.344	0.37	0.63	0.063
Control vs. 40.8%	0.04	0.96	<0.001	0.05	0.95	<0.001
Control vs. 55.2%	0.11	0.89	<0.001	0.12	0.88	<0.001

[1] A versus B (n=38).

[2] Intake of diet A / intake of diet A + intake of diet B

4. Discussion

New protein ingredients are used to support pet food market growth and the development of new products while maintaining animal dietary needs. In the present study, the alternative protein sources were evaluated regarding their implications for kibble formation, and intake by dogs. In general, the results suggested that MDCM induce alterations in extrusion processing and kibble formation, by your high moisture content, which should be considered to establish adequate processing conditions in future studies. In addition, the inclusion of CP from MDCM increased digestibility, palatability, and the formation of fermentation final products.

Although alternative proteins such as fresh meat are commonly included in dog formulations, the implications of including this high moisture ingredient in the extrusion process and in the formation of kibbles are little explored in the scientific literature (Wang et al, 1998). Considering that small variations in processing conditions can affect extrusion variables and product quality (Ding et al., 2005), in this study to adequately assess the effects of including MDCM on the formation and cooking of kibbles, the total steam and water in the system, by calculating the in-barrel moisture. The observed decreases in pressure have already been reported in previous studies, evaluating different in barrel moisture. These authors attributed these results to fluidizing effect of water, when mass in-barrel moisture increased to 300 g/kg to 320 g/kg, the pressure of the mass decreased, indicating lower resistance to mass flow (Baller et al.,2018). High internal quantities of in-barrel moisture turn more difficult to format the kibble, which explains the difference between treatments at kibble macrostructure. High humidity reduces friction, which can reduce starch cooking and interfere with gelatinization and viscosity formation. This may result in limited processing with relatively low expansion (Pitts et al., 2014). However, in this study, the gelatinization achieved was satisfactory, above 87%.

The high moisture content of MDCM may have influenced the chemical composition of the diets. Moisture was different between the diets because MDCM had 79% of moisture content, which makes drying and shaping the kibbles difficult. The addition of meat in the 55.2% diet was higher than the other treatments, so there was a greater addition of water by the meat, which may explain the variation in protein and fat content. Another explanation would be a failure in the MDCM's pumping system for the preconditioner. However, the variation in protein was not as significant in relation to the others. Existing studies on MDCM do not have high inclusions, which makes comparison difficult.

High values in the TTADC of nutrients with the inclusion of MDCM were expected, as is well described in published works using MDCM in formulations for dogs (Meineri et al.2021; Tjernsbekk et al, 2017). In the few studies on MDCM, Tjernsbekk et al.,(2016), evaluating MDCM and salmon protein hydrolysate in extruded dog food on effect on protein and amino acid digestibility, observed TTADC of CP of 88% when MDCM provided 25% of dietary CP, however, the authors only used extruded dog diets, but in vivo digestibility was performed using adult male mink (Neovison mink), which may explain the differences in the values found in this study. In another study using beagles, Meineri et al., (2021), evaluating MDCM, found a digestibility of 84% for DM and 83% for CP, values very close to those found in this study, which were TTADC of DM increase in quadratic way, reaching 86%, and TTADC increase a linear way, reaching 83%, but the authors do not make explicit how much the MDCM represented of the total dietary protein.

Protein fermentation is the anaerobic digestion of proteins by the microbiota that reside in the colon (Pezzali, 2016). Proteins present in the colon originate from ingested dietary protein that escaped digestion in the proximal intestine, pancreatic or intestinal secretions, or desquamated intestinal cells (Windeyet al.,2012). Protein fermentation results in the production of BCFA and SCFA, but also metabolites such as ammonia, indole, and phenols (Macfarlane, 1992). In this work we can associate the reduction of ammonia and isobutyric acid, the lower protein fermentation. Furthermore, this result matches fecal nitrogen excretion decreased with increasing MDCM (P=0.009); can be explained by the fact that protein digestibility with inclusion, in addition to the positive nitrogen balance found in this work, protein fermentation by the intestinal microbiota significantly contributes to the metabolite pool in the large intestine and may contribute to the host's amino acid balance (Diether and Willing, 2019).

There are many aspects to consider when interpreting palatability results, such as ingredients, food processing, kibble macrostructure, added palatants, food chemical composition, and how all of these relate to sensory properties such as aroma, texture, shape and taste (Aldrich and Koppel, 2015). The MDCM were adequately accepted by dogs, as they did not reduce food preferences. In addition, the diets with some level of MDCM was preferred in comparison with the Control diet, so this coproduct seems to present some organoleptic properties that improved diet acceptability by dogs. Few studies have evaluated the palatability effects of MDCM to dog diets, but in general, a high acceptance has been described (Koppel et al., 2014; Meineri et al.2021). In a study evaluating the inclusion of MDCM on the pet food flavor and texture characteristics, Koppel et al., (2014) sensory analysis with a panel identified that MDCM inclusion tended to affect bitterness, fishiness, and cohesiveness of mass of pet foods regardless of processing method. Thus, considering the importance of the palatability attributes to the commercial performance of the diets, the results obtained pointed favorably to the use of MDCM as first protein ingredient in extruded foods for dogs.

5. Conclusion

The formulation with up to 120% inclusion of MDCM (on an as fed basis, in proportion to the dry feed rate addition) resulted in the production of acceptable formatted kibbles, with adequate starch cooking, allowing to achieve 55.2% replacement of the dietary protein by the MDCM protein in diets with 29% of CP on DM basis. The palatability was very high for the elevated inclusion of MDCM. The TTAD of dry matter, crude protein and energy increased, showing that in comparison to the CM control, MDCM presents higher digestibility. This resulted in lower ammonia and isobutirate concentration on feces, products of protein fermentation on colon. Amino acid composition was similar between CM and MDCM, as observed by the similar amino acid composition of the diets, not altering nitrogen balance and urea metabolism of dogs.

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CAPÍTULO 3 – Mechanically deboned chicken meat as protein source for extruded diets for cats [2]

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Mechanically deboned chicken meat as protein source for extruded diets for cats.

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Abstract

Protein sources are key ingredients to reach the nutritional quality and marketing claims of cat foods.The present study compared the replacement of chicken by-product meal (CM) by mechanically deboned chicken meat (MDCM) as a protein source for extruded cat foods on kibble macrostructure, apparent digestibility of nutrients and energy, feces characteristics and fermentation products, nitrogen balance, and diet palatability. A control diet with CM as a protein source was balanced, and three levels of MDCM was included replacing 17%, 31%, and 42% of the dietary protein content, on diets with similar chemical composition. In addition, a reference diet was formulated, which was replaced in dry matter by 6.7%; 13.3% and 20% of MDCM, according to the replacement method by Matterson (1965), in order to determine the apparent digestibility coefficients and metabolizable energy of the MDCM with the regression method. Twenty-four mixed breed cats were used, distributed in a randomized block design with 6 cats per diet. Results were submitted to analysis of variance and means compared by polynomial contrasts and the student T test for the palatability comparisons ($P<0.05$). Kibble hardness and specific length decreased with MDCM inclusion ($P<0.05$). All diets presented elevated and similar starch gelatinization degree ($P>0.05$). No differences between diets were observed on total tract apparent digestibility, for the both tests ($P>0.05$). Feces formation was similar between cats ($P>0.05$), and a linaer decrease was observed on Isobutyric, Isovaleric, valeric, and the total concentration of branched-chain fatty, suggesting a reduction on protein fermentation on colon. No differences between diets were observed on amino acid intake, and nitrogen retention of the cats ($P>0.05$). A preference for diets with high MDCM inclusion (31% and 42% protein replacement) was observed, with first choices and intake ratios higher than 0.65 ($P<0.05$). In conclusion, the substitution of CM by MDMC increased diet palatability and reduced protein fermentation products in the feces. The present extrusion system was able to produce appropriate formulations with high inclusion of raw materials with high moisture content.

Keywords: protein, extrusion, kibble macrostructure, nitrogen balance.

1. Introduction

The pet food industry is always on the lookout for new or alternative ingredients to appeal to consumers. The humanization of dogs and cats has led owners to prefer human-quality food, mainly using raw sources (Meineri et al, 2021). Today most commonly used protein sources in pet foods are dry ingredients with concentrated protein.

The most used protein source in dog and cat food is chicken by-product meal (CM), which offers an excellent composition of amino acids, a good source of energy and minerals (Carciofi, 2008; Meeker and Meisinger, 2015), in addition to being easily stocked due to its low moisture content, and relatively cheap, being cost-effective for the companies. On the other hand, the human-grade protein source, or fresh meats, such as mechanically deboned chicken meat (MDCM), are the most preferred sources in diet formulations for companion animals, but this is not practiced due to several problems, both financial and qualitative, such as the high cost associated with freezing and refrigeration, expenses involved in transporting raw materials with large amounts of moisture (Beaton, 2016).

In addition, the conventional extrusion process, the most used process in the production of dry food for dogs and cats (Riaz, 2007; Tran, 2008) does not support more than 25% of fresh meat in a formula, as fresh meat is required for production efficiency per are more difficult to stabilize due to their high humidity inside the extruder barrel (Baller et al, 2018). However, new extrusion technologies that are not well documented are capable of adding new loads without compromising the quality of the final product.

Considering that there are few studies so far that evaluate the effect of raw ingredients included in pet foods, the objectives of the present study were: (a) to evaluate the effects of MDCM inclusion on extrusion processing, kibble macrostructure, (b) to evaluate the effects of MDCM inclusion on total tract apparent digestibility of nutrients and energy, fecal characteristics, nitrogen balance, fermentation end products, urinary characteristics, and the palatability of dog foods.

2. Materials and methods

All experimental procedures previously approved by the Ethics Committee and Animal Use of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil (Protocol 012152/18).

2.1. Experimental diets: formulation and processing

Four formulations with similar crude protein and crude energy content were balanced to cat maintenance (AFFCO, 2018). To this the raw materials was purchased, samples and analyzed for moisture, crude protein, and crude fat and diets formulated according to results (Table 1). A control diet with CM as a protein source was balanced, and in the three other treatments MDCM was included in amounts to replace was 17.4%, 31.3%, and 42,4% of the dietary protein content (Table 2). In addition, other two formulation were balanced according to the replacement method of Matterson et al (1965), a reference diet (RD) and a test diet (Table 3), which were substituted in dry matter 19.53% of MDCM.

The high inclusion of meat-based high moisture raw material was achieved using a twin-screw extruder (Thermal Twin, Wenger, Sabetha, EUA). The extruder hardware configuration was kept constants for all treatments, but the software conditions varied to compensate for the elevated moisture of the MDCM, in order to obtain suitable kibbles to use. Extrusion processing parameters were recorded every second through an automated process management system, and he average value for each treatment presented on Table 4.

Table 1. Analyzed chemical composition of the chicken by-product meal (CM) and the mechanically deboned chicken meat (MDCM) samples utilized in the experiment (%, as fed basis).

Item	CM	MDCM
Moisture	3.90	79.05
Crude protein	69.90	49.10
Crude fat	13.10	43.90
Ash	11.10	n.d.

n.d. - not determined

Table 2. Ingredients composition (%, dry matter) of the cat foods with different inclusions of MDCM.

Ingredients	Dietary protein replacement by MDCM[1]			
	0%	17.4%	31.4%	42.4%
%, on as fed basis				
MDCM	0.0	51.55	94.03	128.25
%, on DM-basis				
MDCM	0.00	10.80	19.70	26.87
Chicken by-product meal	43.13	36.09	30.30	25.63
Ground Peas	22.00	22.00	22.00	22.00
Cassava	12.47	12.02	11.42	10.93
Chicken fat	14.69	11.37	8.63	6.42
Beet pulp	5.00	5.00	5.00	5.00
Salt	0.80	0.80	0.80	0.80
Fish oil	0.50	0.50	0.50	0.50
Choline chloride	0.40	0.40	0.40	0.40
Potassium chloride	0.40	0.40	0.40	0.40
Taurine	0.20	0.20	0.20	0.20
Vitamin premix [2]	0.18	0.18	0.18	0.18
Mold inhibitor[3]	0.10	0.10	0.10	0.10
Mineral premix[4]	0.14	0.14	0.14	0.14
Antioxidant[5]	0.06	0.06	0.06	0.06
Dicalcium phosphate	0.00	0.01	0.24	0.43
MDCM to dry recipe ratio of inclusion (%) [6]	0	41.8	81.7	118.7
Mean geometric diameter (µm)	316	321	320	311
Geometric standard deviation (µm)	1.52	1.53	1.55	1.57

[1] Calculated considering the analyzed chemical composition of the ingredients and diets

[2] Addition per kilogram of product: vitamin A 18.750 IU; vitamin D 1500 IU; vitamin E. 125 IU. vitamin C 125 mg. vitamin K 0.15 mg. Thiamine 5 mg. Riboflavin 16 mg. Pantothenic acid 35. 75 mg. Niacin 62.5 mg. Pyridoxine 7.5 mg. Cobalamin 45 mcg. Folic acid 0.75 mg.

[3] Myco Curb. Kemin. USA: Propionic acid. sodium hydroxide. calcium hydroxide. amorphous silicon; Dioxide. Sorbic Acid. Benzoic Acid. Propylparaben. Methylparaben and BHA. vehicle q.s.p.

[4] Addition per kilogram of product: Iron 100 mg. Copper 9.25 mg. Manganese 6. 25 mg. Zinc 150 mg. Iodine 1.87 mg. Selenium 0.13 mg.

[5] Naturox. Kemin. USA: Silicon Dioxide. Citric Acid. Natural Mixed Tocopherols. Vegetable Oil. and Rosemary Extract.

[6] Calculated as: (kg/h of MDCM ÷ kg/h dry recipe feed rate) * 100

Along the processing it was possible to see that dry recipe feed rate was reduced in approximately 25%. and MDCM increased from 0% (control diet) to a proportion of 118% in relation to the dry recipe feed rate. in an as fed basis. Due to the high moisture content of the MDCM. however. the dry matter addition corresponded to 26% of the dry recipe feed rate.

Table 3. Ingredients composition (%. dry matter)) of the cat foods with different inclusions of MDCM by regression method.

Item	Regression method		Ratio
	RD	TD	RD/TD
Chicken meal	33.56	41.70	80.4
Ground Peas	20.46	25.42	80.4
Cassava	19.53	0.00	-
MDCM	11.18	13.89	80.4
Chicken fat	8.10	10.06	80.4
Beet pulp	4.65	5.78	80.4
Salt	0.74	0.92	80.4
Fish oil	0.46	0.58	80.4
Choline chloride	0.37	0.46	80.4
Potassium chloride	0.37	0.46	80.4
Taurine	0.19	0.23	80.4
Vitamin premix [1]	0.14	0.17	80.4
Mold inhibitor[3]	0.09	0.12	80.4
Mineral premix[2]	0.09	0.12	80.4
Antioxidant[4]	0.06	0.07	80.4
Dicalcium phosphate	0.01	0.01	80.4
Total	100.0	100.0	-

[1] Addition per kilogram of product: vitamin A 18.750 IU; vitamin D 1500 IU; vitamin E. 125 IU. vitamin C 125 mg. vitamin K 0.15 mg. Thiamine 5 mg. Riboflavin 16 mg. Pantothenic acid 35. 75 mg. Niacin 62.5 mg. Pyridoxine 7.5 mg. Cobalamin 45 mcg. Folic acid 0.75 mg.

[2] Myco Curb. Kemin. USA: Propionic acid. sodium hydroxide. calcium hydroxide. amorphous silicon; Dioxide. Sorbic Acid. Benzoic Acid. Propylparaben. Methylparaben and BHA. vehicle q.s.p.

[3] Addition per kilogram of product: Iron 100 mg. Copper 9.25 mg. Manganese 6. 25 mg. Zinc 150 mg. Iodine 1.87 mg. Selenium 0.13 mg.

[4] Naturox. Kemin. USA: Silicon Dioxide. Citric Acid. Natural Mixed Tocopherols. Vegetable Oil. and Rosemary Extract.

On software conditions. extruder shaft speed was reduced gradually. and water and steam infusion stopped. The system adjustment was enough to accept the increase in moisture; although the in-barrel moisture increased approximately 51%. the extruder motor load. and the specific mechanical energy application remained similar. The mass temperature at die plate was also similar. but a reduction of approximately 40% on mass pressure before the die was observed. Due this. a relevant increase on kibble bulk density was observed. of approximately 39%.

Table 4. Processing variables of extruded cat foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM [1. 2]			
	Control	17.4%	31.4%	42.4%
Dry recipe feed rate (kg/hr)	485	431	396	373
Preconditioner Information				
Steam Flow to Preconditioner (kg/hr)	39	43	0	0
Water Flow to Preconditioner (kg/hr)	140	39	0	0
MDCM -fresh meat (Kg/h)	0	180	324	442
Moisture addition (Kg/h)	0	126	227	309
DM addition (kg/h)	0	88	97	133
Extruder Information				
Extruder shaft speed (rpm)	422	423	455	470
Extruder motor load (%)	36	35	34	33
Extruder steam (Kg/hr)	0	0	0	0
Extruder Discharge rate (kg/hr)	472	430	393	368
Extruder Discharge Density (kg/m^3)	650	634	628	637
Extruder Die Temperature (°C)	89	90	90	90
Die pressure (Kpa)	5726	4055	2845	2289
Final Product Information				
Specific mechanical energy implemetation (kWhr/Ton)	20	20	23	23
In-barrel moisture (%)	34	39	43	51

[1] Calculated considering the analyzed chemical composition of the ingredients and diets

[2] Diets produced in the equipment Thermal Twin. Wenger. Sabetha. EUA.

2.2. Regression Method

The RD. and the diet with 19.53% of MDCM were used for mixtures. giving rise to two intermediate diets. in which the MDCM was replaced in the dry matter by 6.7% and 13.3%. Two methods were used to determine the apparent digestibility (%) and metabolizable energy coefficients of diets. the method of Adeola and Ileleji (2009) and Matterson et al..(1965).

The equation suggested by Matterson et al.. (1965) is:

$$\text{Nutrient DC}(\%) = \text{Nutrient intake RD}(\text{g}) + \frac{\text{Nutrient intake RD} - \text{Nutrient intake TD}}{\text{Replacement x 100}}$$

Where: DC- Digestibility coefficient; RD – Reference diet; TD – Test Diet.

The second method consists of using an equation obtained by regression of the digestibility of the components that were substituted at different levels of proportion and extrapolating to 100% of substitution. in order to estimate the digestibility of the test food used in the diet (MDCM).

For these twenty-four castrated cats (4.14 ± 1.16 kg) were used. distributed among treatments with six experimental units each. To determine the apparent digestibility coefficient and apparent metabolizable energy of the diets. the cats were kept in individual metabolic cages and the urine collection method was followed. according to AAFCO (2011). The amount of food offered was calculated according to the energy value of the food. and the energy requirement estimated according to NRC (2006). Diets. feces. and urine were sampled and analyzed by the AOAC (2005) methods for the determination of moisture (DM). crude protein (CP). ash (OM) and gross energy (GE) determined by adiabatic calorimeter (PARR Instrument-1281. Moline. USA).

2.3. Particle size distribution and kibble characteristics

The mean geometric diameter (MGD) and geometric standard deviation (GSD) of the raw materials mixture after grinding were performed according to the methodology described by Zanotto & Bellaver (1996) using 100 g of sample in an analytical sieve shaker with a horizontal circular motion and a vertical tapping motion (ABRT 820. Bronzinox. Sao Paulo. Brazil). An agitation time of 10 min and 12 screen sieves sizes were used: 0.841 mm. 0.595 mm. 0.500 mm.

0.420 mm. 0.354 mm. 0.297 mm. 0.250 mm. 0.210 mm. 0.177 mm. 0.149 mm. 0.105 mm. 0.74 mm. 0.53 mm plus plate. The MGD and GSD were calculated using Granucalc software (Embrapa Suínos e Aves. Concordia. Brazil).

Samples collected from the dryer were used to evaluate kibble macrostructure. The hardness was analyzed in 20 kibbles using a texture analyzer (TAX/T2I. Stable Micro Systems. Godalming. UK) equipped with a load cell of 50 kgf and a cone probe. First. kibbles were stabilized at the same moisture in an oven (Quimis. Diadema. Brazil) at 35ºC for 24 h. For each treatment. the length. diameter. and mass of 20 extrudates were measured using a caliper. and the data were used to obtain the radial expansion ratio. specific length. and piece density as described by Karkle et al..(2012). In addition. starch gelatinization (Sá et al.. 2013) and the in vitro digestibility of organic matter (Hervera et al.. 2007) were measured.

2.4. Digestibility protocol

A total of 24 healthy adult non-breed cats with a mean of 3.35 ± 1.71 years and 4.25 ± 0.79 kg were used. The digestibility protocol was carried out through of total collection of feces without urine collection method considering the FEDIAF (2020) recommendations. The health of the cats was confirmed prior to the start of the study. Cats were submitted to a 5-d diet adaptation phase. after which a 7-d total feces collection was conducted. During the adaptation period. cats were restrained to individual metabolic cages (0.9 x 0.8 x 0.9 m) from 04h00 p.m. to 10h00 a.m.. were presented with their experimental foods. and were allowed to roam free in a collective cattery of 50 m^2 to exercise and socialize from 10h00 a.m. to 04h00 p.m. the cats. where water was available but not food. During the fecal collection period. cats were permanently restricted to their metabolic cages. Fecal output was quantitatively collected and weighed at each feeding time. and immediately stored at −20°C until further processing. At the end of the collection period. feces were pooled by a cat. thawed. homogenized. and dried using a forced-air oven (MA035. Marconi. Piracicaba. Brazil) at 55°C for 72 h for chemical analysis. The fecal consistency was scored on a 0 to 5 scale (Carciofi et al.. 2008): 0 = watery liquid that can be poured; 1 = soft. unformed; 2 = soft. poorly formed stool that assumes the shape of its container; 3 = soft. formed. and moist stool that retains its shape; 4 = well-formed and consistent stool that does not adhere to the floor; and 5 = hard. dry pellets. which are small and hard masses.

2.5. Chemical analyses

Experimental ingredients. diets. and dried feces were ground in a cutting mill (MA680. Marconi. Piracicaba. Brazil) fitted with a 1.0 mm screen sieve and analyzed according to official methods (AOAC. 1995) for dry matter (method 934.01); crude fat was determined by acid hydrolysis (method 954.02); ash content. by muffle furnace incineration (method 942.05); crude fiber (method 962.09); crude protein (method 990.03) using a LECO nitrogen/protein analyzer (FP-528. LECO Corporation. Saint Joseph. USA); and total and insoluble dietary fiber by the enzymatic-gravimetric method (method 991.43). Soluble dietary fiber was calculated as total fiber minus insoluble fiber. Organic matter (OM) was calculated as DM minus ash. Gross energy (GE) was determined in a bomb calorimeter (IKA C2000 Basic. IKA-Werke GmbH & Co.. KG. Staufen. Germany). The total starch content was determined using an enzymatic method (Hendrix. 1993). All analyses were carried out in duplicate and repeated when the coefficient of variation was higher than 5 %.

2.6. Fecal pH and fermentation end-products

Fresh fecal samples were taken before 15 min of defecation on three consecutive days to pH. short-chain fatty acids (SMDCM). branched-chain fatty acids (BMDCM). lactate. and ammonia determination. Fecal pH was measured using a pHmeter (DM20. Digimed Analítica Ltda.. São Paulo. Brazil) immediately after collection in 2 g of fresh feces diluted in 6 ml of ultrapure water. For SMDCM and BMDCM analyses. 10 g of fresh feces were placed in collector containers and immediately stored at −20°C until further processing. At the end of the collection. each sample taking was mixed in 30 ml of formic acid solution at 4.2 N. pooled by a cat. and the supernatant was centrifuged three times (5.000 G at 4°C for 15 min). The analysis was performed by gas chromatography (GC-2014. Shimadzu Corporation. Kyoto. Japan) according to Erwin. Marco. & Emery (1961). Lactic acid was measured according to Pryce (1969) by mixing 3 g of fresh feces with 9 ml of ultrapure water and subsequent evaluation with a colorimetric method (Spectrophotometer Quick-Lab. Drake. Sao José do Rio Preto. Brazil). The ammonia concentration was assessed in the extracts prepared for SMDCM and BMDCM analysis according to Vieira (1980). For this. 2 ml of extracts were thawed at room temperature. diluted in 13 ml of ultrapure water. and in 5 ml of potassium hydroxide solution at 2 N. The solution was distilled in a nitrogen system (Tecnal TE-036/1. Technal. Piracicaba. Brazil) and recovered in a beaker containing 10 ml boric acid solution. Ammonia was titrated using hydrochloric acid at 0.005 N and then calculated.

2.7. Urinary Characteristics and Nitrogen Balance

To determine urine characteristics. for three consecutive days. urine was quantitatively collected at least three times a day in plastic bottles with 100 mg of thymol as a preservative and kept under refrigeration (4ºC). Each 24 h-pooled urine sample was homogenized. and the volume. density. and pH were determined. Density was measured using a refractometer (T2-Ne Clinical; ATAGO CO.. LTD.; Fujita Yorii-cho Osato gun. Saitama. Japan) and pH using a pH meter (Digimed DM20; Digicrom Analítica. São Paulo. Brazil).

The nitrogen balance was applied simultaneously to the digestibility assays. For five consecutive days. the urine collector received 1 ml of the 1N sulfuric acid solution to avoid nitrogen loss. Urines collected every 24 hours were homogenized. and their volume measured as aliquots frozen at -20 ° C for posterior analysis. Nitrogen content was determined by the Kjedhal method (AOAC. 1995) in food. feces. and urine samples. Nitrogen balance was calculated using the digestibility trials' ingestion and excretion data as the difference between total nitrogen intake and nitrogen excreted in feces and urine.

2.8. Palatability testing

Palatability comparisons were conducted at the Research Laboratory in Nutrition and Metabolism of Domestic Cats (Maringá. Paraná. Brazil). All test foods were compared against the control diet. totaling 3 comparisons. The first choice (product consumed and smell first) and palatability (product consumed in greater quantity) were compared using the two-plate method (Griffin. 2003). For this. 20 cats trained panel animals. were housed individually. and tested on two consecutive days. In the morning. after a fasting period of 12 hours. the animals received the first meal in two trays. each containing one of the experimental feeds. which were available to the animals for thirty minutes. The position of the dishes was alternate during the second meal. The amount of food to be offered was calculated to exceed the consumption capacity of the animal. thus ensuring the occurrence of leftovers. After 30 minutes. trays were removed. leftovers were weighed. and consumption was calculated. Due to the large differences in body weight. the results were calculated as the relative consumption of each diet during the two days. To determine the palatability of foods. the consumption rate will be calculated by the formula: Relative consumption (%) = (Food consumption A x 100) / (Food consumption A + Food consumption B).

2.9. Statistical analysis

The analysis of the extrusion process followed a completely randomized design. For extrudate macrostructure comparison. the experimental unit was one kibble with 20 repetitions per treatment. Data on digestibility fecal characteristics. urine characteristics. and fermentation end-products were analyzed in a randomized complete block design. with three blocks (period) and two repetitions (cats) per block. totaling six cats per food. The experimental unit was one cat. The assumptions of normality of errors and homogeneity of variances were previously evaluated. Data were submitted to analyze variance and polynomial contrasts considering the replaced of CP by the MIXED procedure of SAS Software (version 9.1. SAS Institute. Cary. NC. USA). If differences were obtained in the F Test. the means were submitted to non-orthogonal contrast considering protein replacement in the diets. For the food preference test. the Student T-test was used. considering a 5% probability. To evaluate the first choice (AxB). we used the proportions test (Perform Exact Sign Test). In both. the Normal distribution of data was verified by the Shapiro-Wilk tests. In case of abnormal data. the Wilcoxon Rank Sum test was applied.

3. Results

3.1. Regression Method

The digestibility coefficients of the diets and the test ingredient according to the substitution method (Table 5). There was no statistical difference between the digestibility coefficients with increasing inclusion. The regression method showed better use of the ingredient by the animals. Through the regression method. regressions were generated for CP. OM. DM. GE and ME. By the regression method. it was possible to extrapolate the consumption of 100% of the ingredient. obtaining CP apparent digestibility coefficient of 96%; 98% GE. Diets have the same chemical composition.

Table 5. Total tract apparent digestibility of extruded cat foods with different inclusions of mechanically deboned chicken meat (MDCM) by the regression method.

Item	Regression method				SEM[1]	p-Value	Contrast[2]		Equation	R^2	Ingredient (100%)[3]
	RD	6.70%	13.30%	TD			Linear	Quadratic			
Dry matter	78.20	78.18	80.06	80.62	7.26	0.287	ns	ns	Y=0.1369x + 77.89	0.87	91.58
Organic matter	82.23	82.18	83.90	84.54	7.62	0.356	ns	ns	Y=0.1295x + 81.22	0.87	94.17
Crude protein	77.51	77.51	79.96	80.98	0.30	0.203	ns	ns	Y=0.1987x + 76.97	0.91	96.84
Gloss energy	83.52	83.95	85.84	86.13	0.23	0.109	ns	ns	Y= 0.1460x + 83.40	0.91	98.00

[1] SEM - standard error of mean (n = 24).

[3] Linear or quadratic effect dietary protein replacement by MDCM; ns – not significant at 5% probability.

[3] Values calculated according to Adeola and Ileleji (2009);

3.2. Experimental diets

The food's nutritional composition was formulated to be iso-nutrient (Table 6). Moisture. fat. ash. and gross energy contents were similar among diets. Crude protein levels decrease with the inclusion of MDCM. probably explained by a lower meat slurry addition by the pump system. The amino acid composition was similar and. as expected. according to requirements from FEDIAF (2020).

Table 6. Analyzed chemical composition (%. dry matter) of the cat foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]			
	Control	17.4%	31.4%	42.4%
Moisture	3.04	3.01	2.15	3.70
Ash	7.88	8.08	8.33	8.51
Crude protein	38.97	38.35	38.14	36.66
Essential Amino Acids				
Arginine	2.57	2.53	2.44	2.50
Histidine	0.77	0.81	0.80	0.83
Isoleucine	1.36	1.36	1.34	1.37
Leucine	2.45	2.45	2.41	2.46
Lysine	2.21	2.24	2.21	2.26
Methionine	0.63	0.64	0.64	0.65
Methionine + Cystine	0.98	0.99	0.98	1.01
Phenylalanine	1.43	1.42	1.39	1.41
Phenylalanine + Tyrosine	2.45	2.42	2.38	2.48
Tryptophan	0.26	0.25	0.31	0.33
Threonine	1.30	1.30	1.27	1.34
Valina	1.65	1.62	1.59	1.65
Taurina	0.44	0.45	0.43	0.49
Non-essential Amino acids				
Alanine	2.22	2.18	2.10	2.22
Aspartic Acid	2.85	2.85	2.76	2.93
Cystine	0.35	0.35	0.34	0.35
Glutamic Acid	4.51	4.46	4.40	4.67
Glycine	3.24	3.07	2.89	3.18
Proline	2.06	1.98	1.88	2.04
Serina	1.52	1.52	1.45	1.55
Tyrosine	1.02	1.00	0.99	1.07
Fat	21.79	22.75	22.44	22.69
Crude Fiber	3.0239	3.0767	3.6548	3.1458
Gross energy (kcal/g)	5157	5331	5415	5286

[1] Calculated considering the analyzed chemical composition of the ingredients and diets.

3.3. kibble macrostructure

The parameters for kibble macrostructure are presented in Table 7. The kibble hardness was significant in linear and quadratic contrast. The expansion rate showed a difference between treatments. but the data did not fit any of the tested models. The piece density increased linearly with the inclusion of MDCM. Specific length will show $p < 0.05$ in linear and quadratic contrasts. However. even with these differences found in the macrostructure of kibbles. the diets were similar in appearance (Figure 1). Starch gelatinization and in vitro digestibility has high. and no differences were found between the treatments.

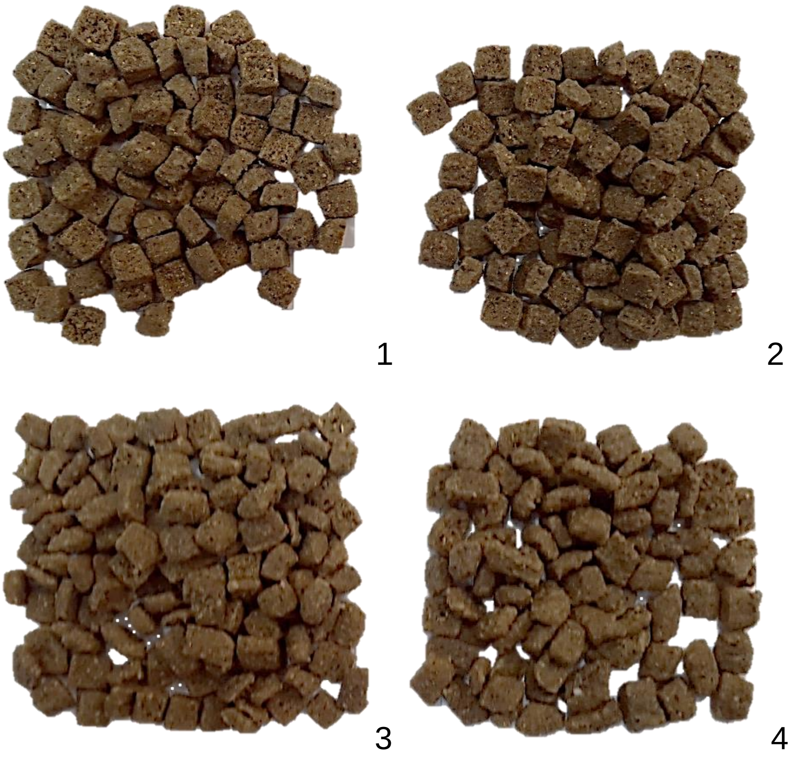

Figure 2. Pictures illustrating the kibbles produced for the experiment.

[1] Control diet without MDCM addition.

[2] Replacement of 17.4% of the dietary protein by the MDCM protein.

[3] Replacement of 31.4% of the dietary protein by the MDCM protein.

[4] Replacement of 42.4% of the dietary protein by the MDCM protein.

Table 7. Kibble characteristics and macrostructure of extruded cat foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[2]	p-Value	Contrast[3]	
	Control	17.4%	31.4%	42.4%			Linear	Quadratic
Hardness (N)	31.62	43.94	26.49	16.86	1.91	0.000	0.000	0.000
Expansion rate	0.53	0.61	0.51	0.58	0.01	0.000	ns	ns
Piece density (g/cm3)	0.87	1.00	0.97	1.19	0.03	0.001	0.001	ns
Specific length (cm/g)	4.22	3.43	4.44	3.04	0.12	0.000	0.000	0.008
Starch gelatinization (%)	94.21	94.18	87.30	96.46	1.77	0.333	ns	ns
In vitro digestibility of the OM (%)	80.07	80.65	81.97	80.79	0.38	0.406	ns	ns

[1] Calculated considering the analyzed chemical composition of the ingredients and diets.

[2] SEM - standard error of mean (n = 20).

[3] Linear or quadratic effect dietary protein replacement by MDCM; ns – not significant at 5% probability.

3.4. Nutrient intake and digestibility

Cats readily consumed the diets. without episodes of refusal. diarrhea. or vomiting. Nutrient intake was similar for all treatments (P>0.05). as well as the apparent digestibility of nutrients and energy (P>0.05). as 6 observed in Table 8. The results for crude protein and essential amino acid intake were present in table 9. All values exceeded those recommended by the NRC (2006) and FEDIAF (2020).

Table 8. Nutrient intake and total tract apparent digestibility of extruded cat foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[2]	p-Value	Contrast[3]	
	Control	17.4%	31.4%	42.4%			Linear	Quad.
Nutrient intake (g/cat/d)								
Dry matter	58.14	51.34	59.45	52.51	0.21	0.715	ns	ns
Organic matter	53.56	47.19	54.50	48.04	2.05	0.698	ns	ns
Crude fat	12.91	12.06	11.16	11.55	0.49	0.626	ns	ns
Starch	12.67	11.68	12.55	11.91	0.49	0.885	ns	ns
Crude protein	22.61	19.69	21.39	19.24	0.85	0.501	ns	ns
Total tract apparent digestibility (%)								
Dry matter	79.05	79.29	76.47	79.32	0.65	0.335	ns	ns
Organic matter	82.80	83.12	80.48	83.17	0.55	0.249	ns	ns
Crude fat	90.97	92.16	91.07	91.62	0.61	0.499	ns	ns
Starch	98.96	98.99	98.92	98.09	0.11	0.971	ns	ns
Crude protein	77.47	78.72	75.98	79.33	0.68	0.318	ns	ns
Gross energy	83.03	84.07	81.85	83.92	0.52	0.376	ns	ns

[1] Calculated considering the analyzed chemical composition of the ingredients and diets.

[2] SEM - standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM; ns – not significant at 5% probability.

Table 9. Intake of crude protein and essential amino acid ($g/kg^{0.67}/day$) of cats fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]						Contrast[4]	
	Control	17.4%	31.4%	42.4%	NRC requirement [3]	p- Value	Linear	Quadratic
Crude protein	8.58	7.40	8.58	7.41	4.96	0.5038	ns	ns
Essential Amino Acids								
Arginine	0.57	0.49	0.55	0.51	0.19	0.6523	ns	ns
Histidine	0.17	0.16	0.18	0.17	0.06	0.8734	ns	ns
Isoleucine	0.30	0.26	0.30	0.28	0.11	0.7316	ns	ns
Leucine	0.54	0.47	0.54	0.50	0.25	0.7244	ns	ns
Lysine	0.49	0.43	0.50	0.46	0.08	0.7778	ns	ns
Methionine	0.14	0.12	0.14	0.13	0.04	0.7400	ns	ns
Methionine + Cystine	0.22	0.19	0.22	0.20	0.08	0.7642	ns	ns
Phenylalanine	0.32	0.27	0.31	0.29	0.10	0.6834	ns	ns
Phenylalanine + Tyrosine	0.54	0.47	0.54	0.50	0.38	0.7119	ns	ns
Tryptophan	0.06	0.05	0.07	0.07	0.03	0.0708	ns	ns
Threonine	0.29	0.25	0.29	0.27	0.13	0.7772	ns	ns
Valina	0.36	0.31	0.36	0.33	0.13	0.6212	ns	ns
Taurina	0.10	0.09	0.10	0.10	0.01	0.8496	ns	ns

[1] Calculated considering the analyzed chemical composition of the ingredients and diets

[2] SEM – standard error of the mean (n = 24).

[3] Daily allowance for dog maintenance of the Nutrient Requirements of Dogs and Cats (NRC. 2006).

[4] Linear or quadratic effect of dietary protein replacement by MDCM; ns – not significant at 5% probability.

3.5. Fecal characteristics and fermentation products

Feces production. dry matter of feces. and pH were similar for all the experimental treatments ($P > 0.05$; Table 10). The feces score did not change and remained close to an adequate score for cats. In the final fermentation products. we observed that Isobutyric. Isovaleric. valeric. and the total concentration of branched-chain fatty acids decreases linearly. which may suggest a better use of the protein in the animal's ileum. reducing the fermentation in the colon. In this way. deboned chicken meat can have higher true digestibility. Lactate and ammonia were similar for all treatments ($P>0.05$).

Table 10. Fecal characteristics and fermentation products of cats fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Dietary protein replacement by MDCM[1]				SEM[1]	p-Value	Contrast[2]	
	Control	17.4%	31.4%	42.4%			Linear	Quadratic
Fecal characteristics								
g/cat/day (DM basis)	12.24	10.54	13.10	10.89	0.57	0.424	ns	ns
g/cat/day (As-is basis)	36.97	31.38	38.86	33.2	2.12	0.621	ns	ns
Dry matter	33.59	35.40	30.50	35.04	0.97	0.290	ns	ns
Score	3.50	3.33	3.30	3.54	0.11	0.830	ns	ns
pH	5.83	5.85	5.76	5.87	0.04	0.543	ns	ns
Fermentation products (mMol/g of DM)								
Acetic acid	251.50	230.73	314.84	278.80	18.26	0.430	ns	ns
Propionic acid	150.39	136.04	185.46	150.74	7.74	0.136	ns	ns
Butiric acid	59.62	50.25	54.69	50.91	2.63	0.576	ns	ns
Total SMDCM	461.90	407.95	564.62	473.56	27.64	0.265	ns	ns
Isobutiric acid	7.97	5.26	5.82	5.50	0.39	0.029	0.016	ns
Isovaleric acid	13.57	9.14	9.95	9.41	0.61	0.015	0.008	ns
Valeric acid	26.15	17.58	20.53	16.75	1.22	0.009	0.004	ns
Total BMDCM	47.71	36.29	36.32	36.55	2.01	0.058	0.033	ns
Total VFA	509.75	439.93	605.51	505.21	28.74	0.259	ns	ns
Ammonia	136.66	108.51	130.70	102.75	6.30	0.173	ns	ns
Lactate	1.79	1.77	1.78	1.69	0.05	0.910	ns	ns

[1] Calculated considering the analyzed chemical composition of the ingredients and diets.

[2] SEM - standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM; ns – not significant at 5% probability.

3.6. Urinary characteristics and nitrogen balance

Urinary characteristics. urinary urea. and nitrogen balance are shown in Table 11. Urinary volume. urinary density. and pH are considered adequate. within the normal range. and no differences are found with the inclusion of MDCM (P>0.05). Urine urea concentration did not differ between diets. which demonstrates a similarity in protein quality between Control diets and diets containing some MDCM inclusions.

Nitrogen balance was positive and the inclusion of MDCM did not significantly influence (P> 0.05). This fact indicates a correct profile of amino acids present in food in relation to the animal's needs. as previously shown (Table 6). Fecal nitrogen excretion. nitrogen intake. and urinary nitrogen excretion did not differ between treatments.

Table 11. Urine characteristics and nitrogen balance of cats fed extruded foods with different inclusions of mechanically deboned chicken meat (MDCM).

Item	Diets[1]				SEM[2]	p-Value	Contrast[3]	
	Control	17.4%	31.4%	42.4%			Linear	Quadratic
Urinary characteristics								
Volume (ml/dog/day)	51.47	53.50	55.77	48.56	2.63	0.821	ns	ns
Specific density	1.06	1.05	1.05	1.05	0.00	0.603	ns	ns
pH	6.96	6.93	6.96	6.93	0.05	0.993	ns	ns
Urinary urea (mg/dL)								
Ureia 24h	1824.57	1800.03	1747.27	1776.01	29.00	0.820	ns	ns
Ureia/kgBW	432.36	427.56	430.50	428.87	18.28	0.690	ns	ns
Ureia/gCPi	82.07	95.80	87.94	96.35	4.42	0.678	ns	ns
Nitrogen Balance								
Food N Intake. g/day	3.62	3.19	3.39	3.07	0.14	0.536	ns	ns
Faecal N excretion. g/day	0.81	0.70	0.79	0.62	0.04	0.332	ns	ns
Urine N excretion. g/day	2.25	2.23	2.36	2.13	0.08	0.818	ns	ns
N absorbed. g/day	2.81	2.49	2.59	2.44	0.11	0.669	ns	ns
N retained. g/day	0.56	0.26	0.23	0.32	0.06	0.222	ns	ns
N balance. %	56.32	26.38	22.61	31.79	6.56	0.222	ns	ns

[1] Calculated considering the analyzed chemical composition of the ingredients and diets.

[2] SEM - standard error of the mean (n = 24).

[3] Linear or quadratic effect of dietary protein replacement by MDCM; ns – not significant at 5% probability.

3.7. Palatability test

For the palatability study. all diets were corrected to achieve the same moisture content. all diets with MDCM inclusion were compared to the control diet (Table 12). The results indicated that diets containing 31.4% dietary protein from MDCM. or more were highly palatable. the same preference was observed at the first choice (intake or smell). where the higher preference was at diets with 31.4% or more substitution of MDCM.

Table 12. Food palatability test of extruded cat foods with different inclusions of mechanically deboned chicken meat (MDCM).

Challenge [1]	First choice (Smell)[2]			First choice			Intake ratio[3]		
	A	B	p-Value	A	B	p-Value	IR-A	IR-B	p-Value
Control vs. 17.4%	0.54	0.46	0.341	0.51	0.49	0.684	0.46	0.54	0.292
Control vs. 31.4%	0.33	0.67	0.040	0.23	0.77	0.000	0.35	0.65	0.000
Control vs. 42.4%	0.30	0.70	0.009	0.35	0.65	0.076	0.30	0.70	0.000

[1] A versus B.

[2] 40 observações em 20 gatos em 2 dias.

[3] Intake ratio A = ingestion diet A (g) / total intake of both diets (g) / Intake ratio B = ingestion diet B (g) / total intake of both diets (g).

4. Discussion

High quality diets for dogs and cats require highly digestible ingredients. Animal by-products have been the main contributor as a protein source in these diets. especially processed sources such as CM. Despite this. there is currently a tendency to replace these flours with fresh meat in commercial diets. such as MDCM. added directly in the extrusion process. There are few scientific publications comparing these sources that can contribute to knowledge about the advantages or disadvantages of each. There are no published data using MDCM for dogs and cats. but Murray et al..(1997) compared diets containing CM and MDCM determined higher CP digestibility coefficients in diets containing MDCM (82.8%) than CM (73.9%). In the present study. the MDCM used can be considered an ingredient of high digestibility and metabolizable energy. with 91.27%. and 5.432 kcal/kg. respectively. These results are favorable to the inclusion of MDCM in commercial diets for cats. when the objectives are the inclusion of high digestible protein and energy density.

Dry cat foods require a concentrated protein source to support the nutritional needs and product claims. Diets with human food grade ingredients are becoming more and more popular in the pet food market. they are coming with greater commercial appeals to affect the ultimate sale ((Buff et al.. 2014; Carter et al.. 2014; Tjernsbekk et al.. 2016; Meineri et al. 2019). Only a few studies with dogs (Koppel et al.. 2014; Meineri et al.2021; Tjernsbekk et al.. 2017). Moisture was different between the diets because MDCM had 79% of moisture content. which makes drying and shaping the kibbles difficult. The addition of meat in the 42.4% diet was higher than the other treatments. so there was a greater addition of water by the meat. which may explain the variation in protein and fat content. Another explanation would be a failure in the MDCM's pumping system for the preconditioner. Existing studies on MDCM do not have high inclusions. which makes comparison difficult. The diets showed good kibbles formatting and starch gelatinization (>87%). even considering that the in-barrel moisture was 43%.

The digestibility of DM. organic matter. crude protein. fat. starch and energy were similar between treatments (P>0.05). There are no studies comparing the use of MDCM for cats. but when purchased with dogs. the felines in this study had a lower coefficient of digestibility. Evaluating MDCM and isolated salmon protein. Tjernsbekk et al..(2016). observed TTADC of CP of 88% when MDCM provided 25% of dietary CP. using the adult male mink (Neovison mink). In another study using beagles. Meineri et al.. (2021). evaluating MDCM. found a digestibility of 84% for DM and 83% for CP. the protein digestibility coefficients found in this work reached a maximum of 79% when 42.4% of CP came from MDCM.

The food preference of pets is linked to the palatability of processed foods; thus. palatability is a concern (Griffin. 2003). We note that. as is already known. cats have a refined sense of smell and taste and are able to differ small changes in the formulation or process (Menolli et al.. 2019). The MDCM were suitably accepted by cats. the diets with some level of MDCM were preferred in comparison with the Control diet. only the MDCM present some organoleptic properties that improved diet acceptability by cats on intake ratio. or even smell and first choice of taste. No study was found evaluating the palatability of extruded diets containing MDCM for cats and considering the importance of the palatability attributes to the commercial performance of the diets. the results obtained favorably to the use of MDCM as protein ingredients in extruded foods for cats.

5. Conclusion

The replacement of CM by MDCM increased the palatability of diets when the dietary protein was >31.4%MDCM. with no effect on the apparent digestibility of nutrients. however. using the replacement method. MDCM contributes to improve the digestibility and energy density values of the diets. The apparent digestibility coefficient of CP and metabolizable energy of this ingredient are. respectively. 91.27% and 5.432 kcal/kg. All branched chain fatty acids decreased linearly. showing that there was a reduction in protein fermentation in the colon. The evaluated extrusion system was able to properly process formulations with high process humidity. with MDCM as the main raw material.

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