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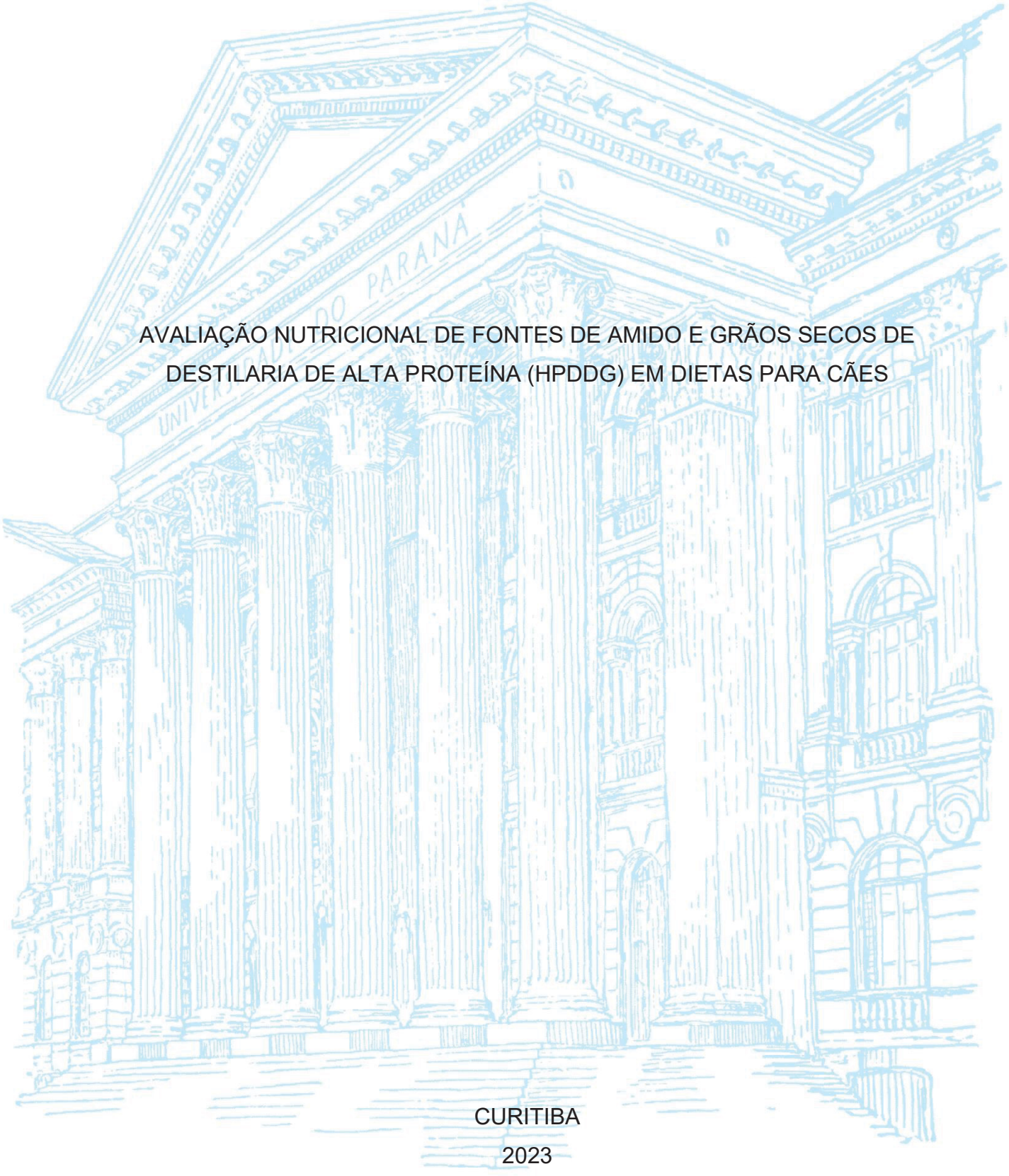
GISLAINE CRISTINA BILL KAELLE



AVALIAÇÃO NUTRICIONAL DE FONTES DE AMIDO E GRÃOS SECOS DE DESTILARIA DE ALTA PROTEÍNA (HPDDG) EM DIETAS PARA CÃES

CURITIBA

2023

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AVALIAÇÃO NUTRICIONAL DE FONTES DE AMIDO E GRÃOS SECOS DE DESTILARIA DE ALTA PROTEÍNA (HPDDG) EM DIETAS PARA CÃES

Tese apresentada ao curso de Pós-Graduação em Zootecnia, Setor de Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Zootecnia.

Orientadora: Profa. Pós-Dra. Dra. Ananda Portella Félix

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **GISLAINE CRISTINA BILL KAELLE** intitulada: **AVALIAÇÃO NUTRICIONAL DE FONTES DE AMIDO E GRÃOS SECOS DE DESTILARIA DE ALTA PROTEÍNA (HPDDG) EM DIETAS PARA CÃES**, sob orientação da Profa. Dra. ANANDA PORTELLA FÉLIX, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 28 de Março de 2023.

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Dedico

A minha família

Aos meus amigos

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A Deus, por sempre me mostrar o caminho em momentos árduos.

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Gislaine Cristina Bill Kaelle

“Sonhos grandes trazem grandes desafios (...)”

(Malala yousafzai)

RESUMO

O amido é o principal constituinte da maioria das dietas formuladas para cães. No entanto, devido às características físico-químicas das diferentes fontes de amido, aspectos envolvidos na nutrição de cães podem ser afetados. Adicionalmente, a partir do beneficiamento do milho é possível obter ingredientes com novas funcionalidades. Nesse contexto, a presente tese está estruturada em cinco capítulos. O capítulo I teve como objetivo apresentar uma visão geral sobre o tema, por meio de uma revisão de literatura. No capítulo II, objetivou-se avaliar a influência de sete fontes de amido: milho, arroz integral, sorgo, batata, batata-doce, grão-de-bico e ervilha no processo de extrusão, características do extrusado e palatabilidade das dietas. No terceiro capítulo, foram utilizados os mesmos tratamentos experimentais apresentados no segundo capítulo, com o objetivo de avaliar os coeficientes de digestibilidade aparente do trato total (CDATT) e energia metabolizável (EM) da dieta, características fecais, produtos de fermentação intestinal, microbiota e respostas glicêmicas pós-prandiais em cães. O capítulo IV avaliou os CDATT e EM do ingrediente e dos nutrientes, palatabilidade da dieta e produtos fermentativos intestinais e microbiota fecal de cães alimentados com resíduo seco de destilaria de alta proteína (HPDDG). Para isso, foram avaliados quatro níveis dietéticos: 0, 7, 14 e 21% de HPDDG. Para avaliação do CDATT e EM do HPDDG foi formulada uma dieta contendo 70% da dieta 0 e 30% de HPDDG. No capítulo V são apresentadas as considerações finais. Como resultado, no capítulo II, a inclusão de batata e batata-doce como fontes de amido resulta em maior expansão, tamanho, dureza e porosidade do extrusado e menor densidade. A inclusão de ervilha resulta em rações com extrusados menos expandidos e com menor porosidade. Em adição, o teor de umidade e a textura da ração afetam a palatabilidade da dieta em cães. No capítulo III, dietas a base de cereais, batata e leguminosas têm digestibilidade semelhante. Dietas contendo batata-doce demonstraram menor digestibilidade e, semelhante às leguminosas, resultam em maior volume e umidade fecal. Por sua vez, a inclusão de leguminosas e batata-doce resulta em pH fecal mais baixo, maior concentração de AGCC fecal, além de maior abundância de *Allobaculum*, *Bacteroides plebeius*, *Blautia* e *Turicibacter* (dietas com grão de bico e ervilha) e *Faecalibacterium* e *Blautia* (dieta com batata-doce). Os amidos de milho e batata resultam em maior incremento glicêmico pós-prandial. Sorgo, leguminosas, batata-doce e arroz integral resultam em menor incremento glicêmico pós-prandial. No capítulo IV, a inclusão de 21% de HPDDG não altera o CDATT das frações nutritivas e a EM da dieta ou as características fecais dos cães. Por sua vez, reduz *Streptococcus* e *Megamonas* e aumenta *Blautia* e *Clostridiales* nas fezes. Além disso, a inclusão de 21% HPDDG melhora a palatabilidade da dieta. Em conclusão, dietas contendo batata-doce e leguminosas melhoram indicadores de funcionalidade gastrointestinal e auxiliam no controle glicêmico pós-prandial em cães. Em adição, a fração fibrosa do HPDDG avaliado não afeta a utilização de nutrientes na dieta, mas pode modular a microbiota intestinal de cães, além de contribuir para a palatabilidade da dieta para cães.

Palavras-chave: DDG. Funcionalidade intestinal. Glicose. Leguminosas. Tubérculos.

ABSTRACT

Starch is the main constituent of most diets formulated for dogs. However, due to the physicochemical characteristics of the different starch sources, aspects involved in dog nutrition can be affected. Additionally, from the processing of corn, it is possible to obtain ingredients with new functionalities. In this context, this thesis is structured into five chapters. chapter I aimed to present an overview of the subject, through a literature review. In chapter II, the objective was to evaluate the influence of seven starch sources: corn, brown rice, sorghum, potato, sweet potato, chickpea, and pea in the extrusion process, kibble characteristics and palatability of the diets. In the third chapter, the same experimental treatments presented in the second chapter were used, to evaluate the coefficients of total tract apparent digestibility (CTTAD) and metabolizable energy (ME) of the diet, fecal characteristics, intestinal fermentative products, microbiota, and postprandial glycemic responses in dogs. Chapter IV evaluated the CTTAD and ME of the ingredient and nutrients, palatability of the diet and intestinal fermentative products, and fecal microbiota of dogs fed high-protein distillery dryed grain (HPDDG). For this, four inclusion levels were evaluated: 0, 7, 14, and 21% of HPDDG. For evaluation of CTTAD and ME of HPDDG, a diet containing 70% of diet 0 and 30% of HPDDG was formulated. In chapter V the final considerations are presented. As a result, in chapter II, the inclusion of potato and sweet potato as starch sources results in greater expansion, size, hardness, and porosity and lower density of the kibbles. The inclusion of pea results in diets with less expanded and less porosity kibbles. In addition, the moisture content and texture of starch sources affect the palatability of the diet in dogs. In chapter III, diets based on cereals, potatoes, and pulses have similar digestibility. Diets containing sweet potato are less digestible and, similar to pulses, result in greater fecal volume and moisture. In turn, the inclusion of pulses and sweet potatoes results in lower fecal pH, higher fecal SCFA production, and a greater abundance of *Allobaculum, Bacteroides plebeius, Blautia,* and *Turicibacter* (diets containing chickpea and pea), and *Faecalibacterium* and *Blautia* (sweet potato diet). Corn and potato starches result in a greater postprandial glycemic increment. Sorghum, pulses, sweet potato, and brown rice result in a smaller postprandial glycemic increment. In chapter IV, the inclusion of 21% of HPDDG does not alter the CTTAD of the nutritive fractions and the ME of the diet or the fecal characteristics of the dogs. In turn, it reduces *Streptococcus* and *Megamonas* and increases *Blautia* and *Clostridiales*. Furthermore, the inclusion of 21% HPDDG improves the palatability of the diet. In conclusion, the inclusion of diets containing sweet potatoes and pulses improves indicators of gastrointestinal functionality and helps control the postprandial glycemic response in dogs. In addition, the fiber fraction of the evaluated HPDDG does not affect the use of nutrients in the diet, but it can modulate the intestinal microbiota of dogs, in addition to contributing to the palatability of the diet for dogs.

Keywords: DDG. Intestinal functionality. Glycose. Pulses. Tubers.

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LISTA DE ABREVIATURAS OU SIGLAS

AGCC - ácidos graxos de cadeia curta
AGCR - ácidos graxos de cadeia ramificada
ALD – amido lentamente digestível
AR - amido resistente
ARD - amido rapidamente digestível
CDATT - coeficientes de digestibilidade aparente do trato total
DDG – grãos secos de destilaria
DDGS - grãos secos de destilaria com solúveis
EET - energia específica total
EM - energia metabolizável
EME - energia mecânica específica
ETE - energia térmica específica
FB - fibra bruta FD - fibra dietética
FDT - fibra dietética total
FI - fibra insolúvel
FS - fibra solúvel
HPDDG – grãos secos de destilaria de alta proteína
MS – matéria seca
OTUs - unidades taxonômicas observadas
TGI - trato gastrointestinal

LISTA DE SÍMBOLOS

α – alfa

β - beta

® - marca registrada

SUMÁRIO

CAPÍTULO I – CONSIDERAÇÕES GERAIS

1 INTRODUÇÃO

O amido é o componente de maior inclusão na formulação de alimentos secos extrusados para cães, considerado essencial no processo de extrusão (CRANE et al., 2000), apresentando papel fundamental sobre a expansão e crocância dos extrusados, importantes para a aparência e palatabilidade das dietas (BALLER et al., 2018).

O amido é encontrado em diversos vegetais como carboidrato digestível de reserva, sendo abundante em grãos de cereais, leguminosas e tubérculos. No entanto, é notório que essas matérias-primas apresentam estruturas fisíco-químicas diferentes, destacando a forma cristalina de seus grânulos, relação amilose:amilopectina e concentração fibrosa, as quais podem alterar as propriedades do amido durante o processo de extrusão, resultando em extrusados com características físicas diferentes (DOMINGUES et al., 2019; PEZZALI e ALDRICH, 2019).

Além de efeitos sobre o processamento, essas características têm relação direta com a digestibilidade das dietas e sobre a onda pós-prandial de glicose sanguínea e a resposta insulínica do animal. Em princípio, os ingredientes à base de tubérculos, excepcionalmente a fécula de batata, apresentam maior digestibilidade, seguidos dos cereais, sendo as leguminosas reconhecidas como menos digestíveis (CARCIOFI et al., 2008; BAJAJ et al., 2018; DOMINGUES et al., 2019). Portanto, quanto mais rápido for o processo de digestão, mais rápida e intensa será a curva glicêmica desencadeada (JENKINS et al., 1981, APPLETON et al., 2004).

Outro ponto relevante na nutrição de animais de companhia é a funcionalidade dos ingredientes, principalmente relacionada ao seu efeito sobre a modulação da microbiota intestinal. Como hipótese, a maior concentração de fibra solúvel em ingredientes como a batata-doce e as leguminosas, pode resultar em maior modulação da microbiota intestinal, resultando em maior produção de ácidos graxos de cadeia curta (BRITO et al., 2021; SOUZA et al., 2021), os quais são essenciais para a homeostase intestinal (TRAMONTANO et al., 2018), assim como papel importante na redução da inflamação e na melhoria da barreira epitelial (HAMER et al. 2008; HUANG et al. 2015).

A partir do processamento de ingredientes primariamente destinados como fontes de amido, como o caso do milho e do sorgo, faz-se possível obter nova matéria-prima com características, propriedades e funcionalidades diferentes. Nesse contexto, temos os coprodutos de destilaria obtidos após o beneficiamento do milho para obtenção do etanol. Devido à alta concentração de proteína bruta (PB, 25-43%), lipídios (10 a 13%), vitaminas e minerais (SPIEHS et al., 2002; SALIM et al., 2010), os grãos secos de destilaria secos com (DDGS) ou sem (DDG) solúveis são interessantes como possíveis substitutos de fontes proteicas convencionais em dietas para suínos (PEDERSEN et al., 2007; AVELAR et al., 2010), frangos de corte (WU-HAAN et al., 2010; CUEVAS et al., 2012) e cães (SILVA et al., 2016; RISOLIA et al., 2019).

Esses ingredientes podem ser boas fontes de aminoácidos essenciais, como lisina (0,9-1,2 g/100g), metionina (0,6-0,82 g/100g) e triptofano (0,2 g/100g) (de GODOY et al., 2009; RHO et al., 2017). Complementarmente, além da proteína do milho, o DDGS e DDG apresentam proteína residual da levedura, que é utilizada durante o processo de fermentação, contribuindo para a concentração e perfil de aminoácidos (BELYEA et al., 2004), intensificando, também, a palatabilidade da dieta (KAELLE et al., 2022).

Ademais, a remoção dos sólidos insolúveis do líquido que continha os sólidos solúveis por meio do processo de centrifugação resulta em novo ingrediente, denominado como DDG com alto teor de proteína (HPDDG), o qual apresenta teores de PB acima de 40%, 4,63% de lipídeos e em torno de 26,4% de FDN (HUBBARD et al., 2009) e em média 19,7% de FDT (PARSONS et al., 2006). Estudos reportaram o uso de HPDDG em dietas para peixes (PRACHOM et al., 2013; GODA et al., 2020), suínos (WIDMER et al., 2008; ADEOLA e RAGLAND, 2012; SON et al., 2019; RAO et al., 2021) e gatos (LOGAN et al., 2022), não sendo encontrados até o momento estudos avaliando esse ingrediente para cães.

Diante do exposto, esta revisão tem como objetivo compilar informações publicadas na literatura a respeito do papel do amido em dietas para cães, incluindo aspectos relacionados à sua influência no processamento de alimentos secos extrusados e respostas glicêmicas. Além disso, objetivou-se fazer um levantamento de informações nutricionais dos coprodutos de destilaria (DDGS, DDG e HPDDG) relevantes para sua inclusão em dietas para cães.

2 REVISÃO DE LITERATURA

2.1 CARACTERÍSTICAS GERAIS E ESTRUTURAIS DO AMIDO

2.1.1 Amilose e amilopectina

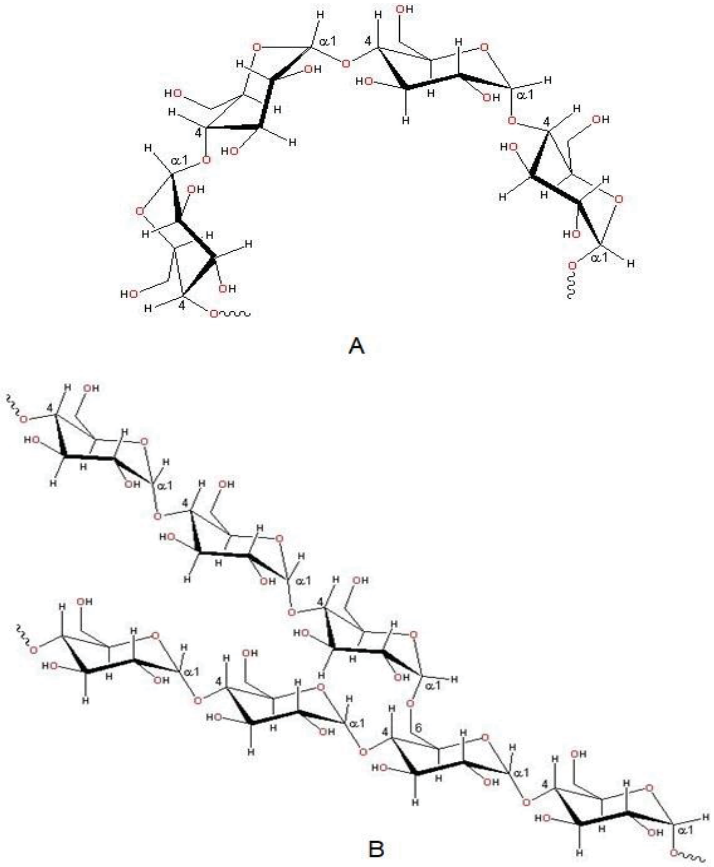
O amido encontra-se nas plantas sob a forma de grânulos. De acordo com sua origem botânica, aspectos como: a forma (redondo, oval ou poliédrico), tamanho de partícula (2 a 100 μm) e a distribuição de tamanho da partícula (unimodal, bimodal ou trimodal) dos grânulos podem variar (VANDEPUTTE e DELCOUR, 2004; TESTER et al., 2004).

Estruturalmente, o amido é um homopolissacarídeo composto por cadeias de amilose e amilopectina, as quais apresentam diferentes conformações e propriedades. A amilose é um polímero essencialmente linear (Figura 1A), formado por unidades de α-D-glicopiranose ligadas em α-(1,4), com poucas ligações α-(1,6) (entre 0,1% e 2,2%, CURÁ et al., 1995; OATES, 1997; BULEÓN et al., 1998). Essa molécula possui número médio de grau de polimerização de 500-5000 unidades de resíduos de glicose (OATES, 1997), com comprimentos médios de cadeia de 250-670 (ELIASSON, 1996; BULEÓN et al., 1998; VANDEPUTTE e DELCOUR, 2004). Seu peso molecular é da ordem de 250.000 Daltons (1500 unidades de glicose), variando entre as espécies de plantas e dentro da mesma espécie, dependendo do grau de maturação. Moléculas de amilose de cereais são geralmente menores em relação a tubérculos e leguminosas (DENARDIN e SILVA, 2009).

A amilose pode estar presente sob a forma de complexos amilose-lipídios ou de amilose livre. Esses complexos, embora detectados no amido nativo, possivelmente são formados em maior extensão durante o tratamento hidrotérmico (ELIASSON, 2004; TESTER et al., 2004; VANDEPUTTE & DELCOUR, 2004).

A amilopectina, por sua vez, é formada por cadeias de resíduos de αD-glicopiranose (entre 17 e 25 unidades) unidos em α-(1,4), sendo fortemente ramificada (Figura 1B), com 4% a 6% das ligações em α-(1,6). Seu peso molecular varia entre 50 e 500 x 10^6 Daltons (VANDEPUTTE e DELCOUR, 2004; LAJOLO e MENEZES, 2006).

FIGURA 1 - A) ESTRUTURA DA AMILOSE. B) ESTRUTURA DA AMILOPECTINA.

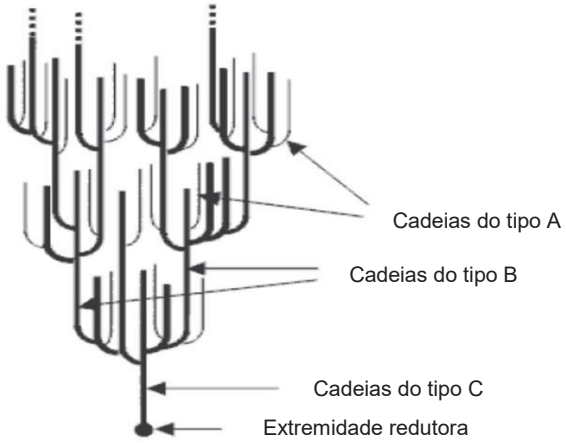


Fonte: Adaptado de LAJOLO e MENEZES (2006)

A amilopectina apresenta um grau de polimerização de 4700 a 12800 unidades de resíduos de glicose, valores médios de comprimento de cadeia de 17 a 24. As cadeias individuais podem variar entre 10 e 100 unidades de glicose (VANDEPUTTE e DELCOUR 2004).

As cadeias de amilopectina estão organizadas de maneiras diferentes, sugerindo uma classificação de cadeias A, B e C (Figura 2). O tipo A é composto por uma cadeia não-redutora de glicoses unidas por ligações α-(1,4) sem ramificações, sendo unida a uma cadeia tipo B por meio de ligações α-(1,6). As cadeias do tipo B são compostas por glicoses ligadas em α-(1,4) e α-(1,6), contendo uma ou várias cadeias tipo A e podem conter cadeias tipo B unidas por meio de um grupo hidroxila primário. A cadeia C é única em uma molécula de amilopectina, sendo composta por ligações α-(1,4) e α-(1,6), com grupamento terminal redutor (ELIASSON, 2004; VANDEPUTTE e DELCOUR, 2004; LAJOLO e MENEZES, 2006).

FIGURA 2 - CLASSIFICAÇÃO DAS CADEIAS DA AMILOPECTINA EM TIPO A, B E C.

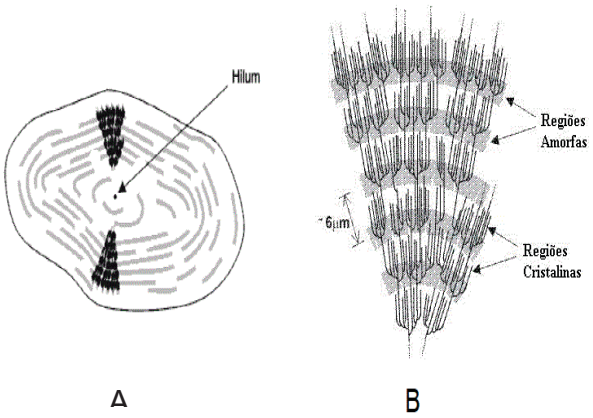


Fonte: Adaptado de PARKER & RING (2001).

2.1.2 Estrutura interna do grânulo de amido

Em geral, os grânulos de amido são formados por um hilo central envolto por anéis de crescimento, que são organizados em regiões cristalinas (Figura 3A). A fusão desses cristais e o rompimento dessa estrutura organizada formam a base para a gelatinização (ELIASSON, 2004). Essas regiões são constituídas por duplas hélices das cadeias paralelas A e B da amilopectina e regiões amorfas com pontos de ramificação das cadeias laterais da amilopectina e, possivelmente, alguma amilose (Figura 3B, OATES, 1997; ELIASSON, 2004).

FIGURA 3 - A) MODELO DA ESTRUTURA INTERNA DO GRÂNULO DE AMIDO COM A VISUALIZAÇÃO DOS ANÉIS DE CRESCIMENTO E CENTRO (HILUM). B) ESTRUTURA DA AMILOPECTINA FORMANDO AS REGIÕES AMORFAS E CRISTALINAS NO GRÂNULO DE AMIDO.



Fonte: Adaptado de PARKER e RING (2001).

2.1.3 Classificação do amido

De acordo com a velocidade de digestão *in vitro*, o amido pode ser classificado como amido rapidamente ou lentamente digerível e como amido resistente (AR). O rapidamente digerível (ARD) é aquele que ao ser submetido à incubação com α-amilase pancreática e amiloglicosidase em uma temperatura de 37°C, converte-se completamente em glicose em até 20 minutos (Englyst et al., 1992). Este consiste principalmente de amido amorfo e disperso, sendo encontrado em grande quantidade em alimentos ricos em amido cozido por calor úmido, como a batata. O amido lentamente digerível (ALD) também é completamente digerido, entretanto em até 120 minutos nas condições de incubação anteriores. Nesta categoria incluem o amido amorfo fisicamente inacessível e o amido cru (SAJILATA et al., 2006). Essa menor velocidade de digestão acarreta benefícios fisiológicos em comparação ao ARD, ligados ao controle de diabetes e à saciedade (LEHMANN et al., 2002).

Após os 120 minutos da ingestão, a porção do amido que não é digerida pelas enzimas é denominada de AR. O AR subdivide-se em quatro frações: amido fisicamente inacessível (AR tipo 1), grânulos de amido resistente (AR tipo 2), amido retrogradado (AR tipo 3), e o amido modificado quimicamente (AR tipo 4), considerando sua resistência à digestão (RAIGOND et al., 2015; BELLO-PEREZ et al., 2018, Quadro 1). Adicionalmente, na classificação conceitua-se um quinto tipo de AR, os complexos amilose-lipídios, os quais muitos consideram um amido de digestão lenta, em vez de um verdadeiro amido resistente (DEMARTINO et al., 2020).

QUADRO 1. TIPOS DE AMIDO RESISTENTE (AR)

Tipo 1 (AR1): barreira física bloqueando o acesso ao amido, como o revestimento de uma semente em um grão inteiro. Pertence a este grupo os grãos inteiros ou parcialmente moídos de cereais, leguminosas e outros materiais contendo amido nos quais o tamanho ou a sua composição impede ou retarda a ação das enzimas digestivas.
Tipo 2 (AR2): grânulos de amido nativo que resistem à digestão devido à presença de cristalinidade tipo B (encontrados no amido retrogradado e amidos ricos em amilose) e C (amido de leguminosas).
Tipo 3 (AR3): Amido retrogradado. Amido cozido e resfriado que reestrutura novas matrizes cristalinas. Normalmente formado durante o processo de extrusão.
Tipo 4 (AR4): Amidos modificados quimicamente, muitas vezes produzidos através de alterações em sua estrutura química a de originar derivados para atender necessidades específicas da indústria de alimentos.

Fonte: Adaptado de Englyst et al. (1992) e DeMartino e Cockburn (2020)

Em relação ao seu comportamento no organismo, o AR apresenta similaridades específicas tanto das fibras de caráter insolúvel quanto solúvel (Tabela 1), uma vez que escapa da digestão no intestino delgado, chegando ao cólon e servindo como substrato para a microbiota intestinal (BELLO-PEREZ et al., 2018), resultando principalmente na produção de ácidos graxos de cadeia curta (AGCC): acetato, propionato e butirato (KNUDSEN et al., 2018), sendo o butirato o AGCC que apresenta aumento mais expressivo após o consumo de AR (DEMARTINO et al., 2020). O butirato desempenha um papel importante na saúde intestinal, incluindo: redução da inflamação, redução do risco de câncer de cólon e melhorando função de barreira intestinal (KNUDSEN et al., 2018).

TABELA 1 - CARACTERÍSTICAS DE INSOLUBILIDADE, FERMENTABILIDADE E EFEITOS SOBRE AS CARACTERÍSTICAS FECAIS E PRODUÇÃO DE ÁCIDOS GRAXOS DE CADEIA CURTA (AGCC) DO AMIDO RESISTENTE, FIBRA SOLÚVEL E INSOLÚVEL

Propriedades	Amido resistente	Fibra solúvel	Fibra insolúvel
Insolúvel em água	+	-	+
Fermentabilidade	+++	+++	-
Produção de AGCC	+++	+++	-
Aumento da produção de butirato	+++	++	-
Redução do pH fecal	+++	+++	-
Aumento da umidade fecal	++	++	+
Aumento da massa fecal	+++	+	+++
Redução do tempo de trânsito fecal	++	-	+++

Fonte: Adaptado de Pereira (2007)

Além desse efeito prebiótico, o AR melhora a resposta glicêmica, insulinêmica e o perfil lipídico e aumenta a saciedade. Além de influenciar sobre a absorção de alguns micronutrientes (REIS et al., 2017). Contudo, poucos estudos têm abordado sobre o AR e sua administração para cães (PEIXOTO et al., 2017).

2.2 INCLUSÃO DO AMIDO EM DIETAS PARA CÃES

Na maioria dos alimentos extrusados para cães e gatos, o amido constitui a maior inclusão dentro da fórmula, podendo representar de 20 a 40% (GROSS et al., 2010), dependendo do segmento e nicho de mercado ao qual ele se destina. Além da sua função nutricional como fonte de energia, o amido desempenha importante papel durante o processamento de alimentos, sobretudo no cozimento da massa e na formação dos extrusados.

2.2.1 Fontes de amido

O amido encontra-se amplamente distribuído em diversas espécies vegetais como um carboidrato de reserva. Dependendo da sua classificação botânica, essas

fontes podem ser divididas em três classes: cereais (40% a 90% na MS), leguminosas (30% a 50% na MS) e tubérculos (65% a 85% na MS) (KEMPE et al., 2004; LAJOLO e MENEZES, 2006; CARCIOFI et al., 2008; BAZOLLI et al., 2015).

Existe grande diversidade de fontes de amido que são utilizadas em alimentos para animais de companhia. Podendo ser citados como fontes de amido: milho, sorgo, arroz integral, quirera de arroz, lentilha, ervilha e as féculas de batata e mandioca (FRANÇA et al., 2011). Em adição, tem aumentado a inclusão de batata-doce em diversas fórmulas e o interesse pelo grão-de-bico como fonte de amido. Os grãos de cereais como arroz e milho são as fontes de amido mais utilizadas em alimentos para cães e gatos. O uso de leguminosas e tubérculos, por sua vez, se popularizou recentemente, nas rações denominadas *grain-free* (ALVARENGA e ALDRICH, 2020).

Adicionalmente, tem aumentado o interesse pela inclusão de ingredientes com maior teor de ALD, bem como maior proporção de fibras solúveis e insolúveis, principalmente em dietas específicas e coadjuvantes, como alimentos para perda de peso ou animais com distúrbios metabólicos, como é o caso da diabetes. Nesse contexto, a incorporação do sorgo na alimentação serve como estratégia para o controle da obesidade (AWIKA e ROONEY, 2004).

De maneira geral, os processos de extrusão e gelatinização aumentam a digestibilidade das fontes utilizadas (SPEARS E FAHEY Jr, 2004). Ao comparar a digestão do sorgo em relação ao milho, observa-se que o processo de metabolismo dos macronutrientes presentes no sorgo ocorre de forma mais lenta. Dessa forma, o processo de extrusão pode melhorar sua digestibilidade, sobretudo a digestibilidade da proteína (FAPOJUWO et al., 1987).

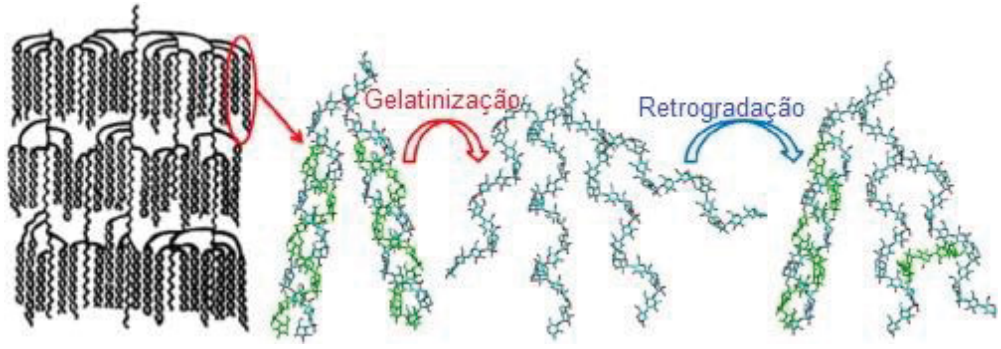
2.2.2 Processo de extrusão e influência do amido sobre as características físicas dos extrusados

A tecnologia de cozimento por extrusão é caracterizada como um processo de alta temperatura e curto tempo (DZIEZAK, 1989) em que a mistura de alimento é exposta a uma alta pressão e temperatura por um período relativamente curto (LANKHORST et al., 2007). Características benéficas da extrusão incluem: modificações de atributos texturais favorecendo a apreensão e mastigação, inativação dos fatores antinutricionais, destruição de microrganismos, aumentando a

vida de prateleira, ampliação das possibilidades de uso de matérias-primas, o aumento da digestibilidade de nutrientes e incremento na palatabilidade das dietas (LANKHORST et al., 2007).

Durante a extrusão, a água é incorporada na estrutura dos grânulos de amido, e seus componentes mais solúveis se dissociam e difundem-se para fora do grânulo. Este processo é conhecido como gelatinização (Figura 4), tornando o amido mais acessível para as enzimas digestivas (MURRAY et al., 2001). A temperatura de ocorrência deste processo é dependente da origem botânica do amido. Tubérculos tendem a gelatinizar com bastante facilidade durante a extrusão, enquanto o amido de grãos de cereais exige temperaturas mais altas e condições de processamento mais severas (DOMINGUES et al., 2019). A fécula de batata gelatiniza em temperaturas em torno de 56ºC a 66ºC e a fécula de mandioca entre 62ºC a 66ºC (CIACCO e CRUZ,1982). Enquanto o milho, cereal comumente incluído como fonte de amido, apresenta temperatura inicial de gelatinização na faixa de 60ºC a 70ºC, e temperatura de pico endotérmica perto de 78ºC (LAI et al., 1991).

FIGURA 4 - REPRESENTAÇÃO DO PROCESSO DE GELATINIZAÇÃO E RETROGRADAÇÃO DOS GRÂNULOS DE AMIDO.



Fonte: Adaptado de Xu et al. (2013)

Ao sair pelo orifício da matriz da extrusora e entrar em contato com a atmosfera ambiente, a água presente na massa moldada se vaporiza e a estrutura plástica formada pelo amido se expande originando a estrutura celular, expansão, porosidade, dureza e forma do extrusado. Portanto, a apresentação do alimento extrusado seco está diretamente relacionada ao processo de extrusão e a gelatinização do amido (REIS et al., 2017).

Adicionalmente, características como a proporção de amilose e amilopectina favorece a obtenção de produtos com aparências diferenciadas. A inclusão de fontes em que sua composição apresenta maior no teor de amilose resulta em aumento da crocância enquanto maior teor de amilopectina melhora a expansão do produto extrusado (HUANG, 2001). O grânulo de amilose, devido a sua estrutura em hélice, forma um filamento menor, mais fino e com menor viscosidade após sua gelatinização, ou seja, um excesso de amilose em relação à amilopectina dificulta a expansão do produto. Os grânulos de amilopectina, por sua vez, por apresentarem ramificações entre suas moléculas, formam, após a gelatinização, filamentos mais longos, com maior viscosidade e aderência sendo, portanto, realmente efetivos no processo de expansão (REIS et al., 2017).

No entanto, outros fenômenos envolvendo o amido podem ocorrer durante o processo de extrusão. Como por exemplo, no interior do canhão da extrusora, o amido já gelatinizado, principalmente as cadeias de amilose, podem se associar com a gordura presente naturalmente nos ingredientes e originar complexos, denominados amido-lipídio. Estes são formados pelo encapsulamento de moléculas de triglicerídeos no interior de cadeias de amilose (GIBSON e ALAVI, 2013). A formação de complexos amilose-lipídios altera a textura e expansão dos extrusados. Quanto maior a quantidade de gordura interna na ração, menor será a eficiência de transferência de energia mecânica e da extrusão em si, reduzindo o cozimento e promovendo formação de extrusados pouco expandidos e duros (CHEFTEL, 1986).

2.2.3 Digestibilidade do amido

Apesar de sua suscetibilidade à ação enzimática, a digestibilidade do amido pode ser afetada por diferentes fatores intrínsecos ou extrínsecos à fonte (ENGLYST et al., 1992). Dentre os fatores ligados à fonte, incluem-se: inacessibilidade física do amido; resistência dos grânulos à ação enzimática, formação de amido retrogradado (LOBO e SILVA, 2003), interações entre proteína e amido, integridade de seus grânulos e pela presença de fatores antinutricionais, como o tanino (ROONEY e PFLUGFELDER, 1986). Os fatores extrínsecos, por sua vez, são: tempo de trânsito intestinal, concentração de amilase disponível para a quebra do amido e a presença de outros componentes da dieta que retardem a hidrólise enzimática (ENGLYST et al., 2003).

Em alimentos adequadamente processados, o amido é um dos componentes com uma maior digestibilidade aparente no trato digestório total para cães. Estudos mostram que a digestibilidade aparente do amido de diferentes fontes e em diferentes teores de inclusão pode variar de 95,9 a 97,0% em cães filhotes (DOMINGUES et al., 2019), enquanto em cães adultos e idosos é superior a 98,7% (CARCIOFI et al., 2008; BAZOLLI et al., 2015; MARIA et al., 2017; PACHECO et al., 2018; RIBEIRO et al., 2019). Contudo, alguns estudos demonstram que a digestibilidade do amido pode ser alterada de acordo com a fonte utilizada (TWOMEY et al., 2002; CARCIOFI et al., 2008; DOMINGUES et al., 2019). Ingredientes que apresentam menor concentração de fibra dietética total e menor relação amilose:amilopectina normalmente apresentam melhor aproveitamento pelos cães, como é o caso da fécula de batata (DOMINGUES et al., 2019).

Em estudo avaliando a digestibilidade e qualidade fecal de três dietas experimentais extrusadas, sendo a primeira com 49% de inclusão de arroz, a segunda com 51% de inclusão de milho e terceira com 46% de inclusão de sorgo para cães adultos, os autores verificaram coeficientes de digestibilidade aparente do amido de 100% para os três cereais e qualidade fecal de todos os cães considerada ideal (TWOMEY et al., 2002). Outros estudos avaliando esses mesmos cereais sugerem que diferentes proporções dos componentes de amido (amilose e amilopectina) presentes na quirera de arroz, em relação às demais fontes, bem como as reações ocorridas durante o processamento, podem contribuir para maior digestibilidade do amido total da dieta contendo arroz em relação ao milho e sorgo (KENDALL et al., 1982; NUNES, 1998; SILVA Jr et al., 2005).

Relacionando a exigência de processamento à digestibilidade do amido, os grânulos de amido do arroz e trigo apresentam digestão acima de 97% indiferente da necessidade de tratamento físico ou térmico (CARCIOFI et al., 2008). No entanto, para apresentarem boa digestão (99% e 100%) pelos cães, os amidos de milho e sorgo precisam ser moídos mais finos (CARCIOFI et al., 2008; BAZOLLI et al., 2015) e os amidos de batata e mandioca, cozidos (CARCIOFI et al., 2008). Essa afirmação foi comprovada ao comparar a digestibilidade de dois carboidratos em sua forma crua e gelatinizada (WOLTER et al., 1998). Os autores demonstraram que o processo de gelatinização não foi importante para a digestibilidade ileal do amido de trigo, resultando em 99,4% para o amido cru e 98,0% para o gelatinizado. Contudo,

para o amido de mandioca, a digestibilidade ileal aumentou de 57,6% (amido cru), para 97,4%, após o processo de gelatinização.

2.2.4 Influência do amido sobre a resposta glicêmica e insulinêmica pós-prandial de cães

O valor glicêmico fisiológico de cães e gatos adultos está entre 70 a 120 mg/dl (NRC, 2006). Estudos em cães adultos saudáveis concluíram que o amido é o principal componente responsável pela influência nos parâmetros glicêmicos e insulinêmicos (BOUCHARD e SUNVOLD, 1999; NGUYEN, et al., 1998; CARCIOFI, et al., 2008).

Tanto a fonte de amido, quanto sua forma estrutural são fatores determinantes sobre as respostas glicêmicas (ENGLYST et al., 1999). A forma estrutural do amido e sua proporção no alimento determinam a velocidade de digestão e absorção dos carboidratos simples (BEHALL et al. 1989). Dietas que apresentam alta concentração de ARD têm geralmente um índice glicêmico mais elevado. Ao ser consumido, os níveis de glicose no sangue aumentam imediatamente após a ingestão. O rápido aumento da glicose causa, consequentemente, o aumento dos níveis de insulina no sangue e essa elevação contribui para várias complicações à saúde, tais como diabetes, doenças cardiovasculares e obesidade (KITTISUBAN et al., 2014). Adicionalmente, os alimentos com elevados índices glicêmicos estão associados com a diminuição da saciedade (ZHANG e HAMAKER, 2009).

Por sua vez, dietas que apresentam alto índice da fração de ALD, apresentam uma liberação lenta e prolongada de glicose no sistema gastrointestinal durante o processo digestivo, e este apresenta uma baixa resposta glicêmica. Em humanos, as características nutricionais do ALD incluem saciedade, o aumento da tolerância à glicose e redução do nível de lipídios no sangue em indivíduos saudáveis e com hiperlipidemia (JENKINS et al., 2002).

Em relação à fonte, sabe-se que diferentes fontes de amido resultam em respostas glicêmicas e insulínicas distintas em cães sadios (CARCIOFI et al., 2008). Esses mesmos autores, ao avaliaram seis fontes de amido (milho, sorgo, arroz, mandioca, lentilha e ervilha) em cães, encontraram valores de mínimo e máximo incremento de glicose plasmática menor em animais consumindo ervilha e sorgo, e

maiores para as dietas com milho, arroz e mandioca. Em outro estudo avaliando dietas a base de milho, trigo, arroz, cevada e sorgo, observou-se que o arroz foi o ingrediente que proporcionou maior pico glicêmico, identificado pela maior área abaixo da curva (BOUCHARD e SUNVOLD, 1998).

Deve-se considerar que, além da fonte utilizada e suas características de estrutura e composição, outros fatores também influenciam a onda pós-prandial de glicose e insulina, como a fibra dietética e o próprio processamento do alimento (CARCIOFI et al., 2008). As fibras dietéticas são capazes de modular e regular as repostas glicêmicas pós-prandiais. Os efeitos atribuídos ao consumo deste componente incluem retardo do esvaziamento gástrico, absorção gradativa de carboidratos no intestino, aumento da sensibilidade à insulina no fígado e em outros tecidos e alteração dos hormônios que controlam o metabolismo dos nutrientes (GRAHAM et al., 2002). Estudos têm demonstrado que em altas concentrações a fibra dietética pode ser ferramenta interessante no manejo alimentar de cães com *diabetes mellitus* (NELSON et al., 1998; KIMMEL et al., 2000; GRAHAM et al., 2002).

Em relação ao processamento do alimento comercial extrusado, parâmetros envolvidos ao processo de extrusão, como tamanho de partícula da matéria-prima e aspectos relacionados às configurações da extrusora (temperatura, força de cisalhamento, tempo de retenção e pressão), podem trazer benefícios sobre o controle da glicemia pela formação do amido resistente (ROBERTI-FILHO, 2013).

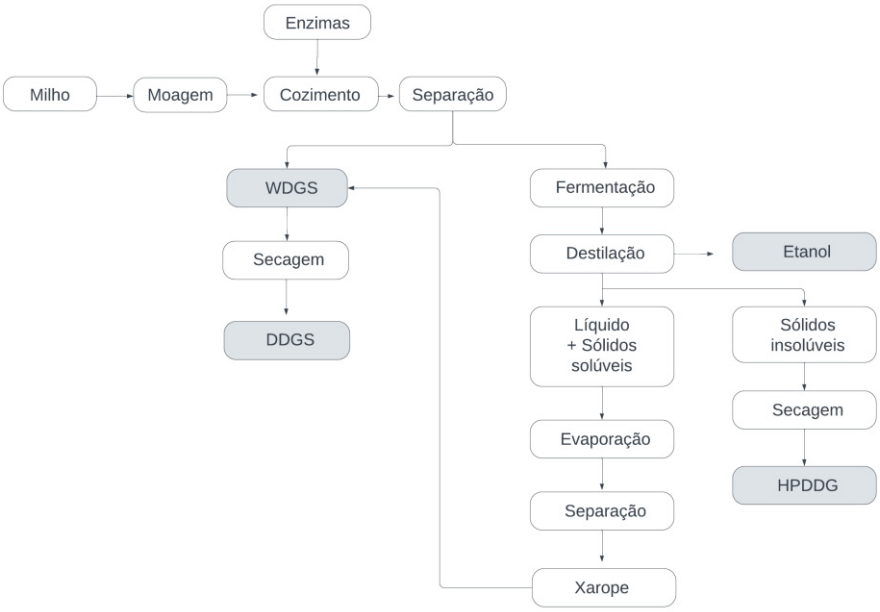
Com base no exposto, indica-se a utilização de dietas que minimizem e estabeleçam uma onda glicêmica pós-prandial que favoreça aos animais o restabelecimento mais rápido e fisiológico da glicose sanguínea (BOUCHARD e SUNVOLD, 1999; BRAND-MILLER, 1994; BAZOLLI, 2015).

2.3 COPRODUTOS OBTIDOS A PARTIR DA FERMENTAÇÃO E DESTILAÇÃO DO MILHO

A partir da fermentação alcoólica do milho é possível obter diferentes coprodutos de interesse para a nutrição animal (STEIN e SHURSON, 2009). Os mais reconhecidos e comumente utilizados pelos formuladores de dietas são os grãos úmidos (WDGS) e os grãos secos de destilaria com (DDGS) e sem solúveis (DDG). Adicionalmente, com o avanço das tecnologias de processamento e a necessidade de obtenção de coprodutos de maior potencial em termos de

composição e funcionalidade, desenvolveu-se uma nova matéria-prima, denomiada como DDG com alto teor de proteína (HPDDG). Esse ingrediente é obtido após a destilação e separação por centrifugação do resíduo contendo os sólidos insolúveis dos sólidos solúveis junto ao líquido fermentado (Figura 5).

FIGURA 5 - PROCESSO DE EXTRAÇÃO DE OBTENÇÃO DOS COPRODUTOS WDGS, DDGS e HPDDG



Fonte: Adaptado de FS Fueling Sustainability (2023)

Como potencial limitante ao uso desses ingredientes, assim como demais ingredientes originados a partir do processamento do milho, na nutrição de animais de companhia tem-se sua possível contaminação com micotoxinas, principalmente tricotecenos, aflatoxina, fumonisina e zearalenona (WU E MUNKVOLD, 2008; ZHANG e CAUPERT, 2012) sendo que as principais relatadas são a DON e FBs (ZHANG et al., 2009; KHATIBI et al., 2014).

Em cães e gatos, os efeitos das micotoxinas são severos, como a perda de nutrientes, alteração das propriedades organolépticas e redução do “tempo de prateleira” do produto no mercado (CAMPOS, 2006). Os cães são animais particularmente sensíveis aos efeitos hepatotóxicos agudos e a exposição regular a aflatoxinas pode causar dano crônico no fígado desses animais (MAIA e SIQUEIRA, 2007). Para isso, é importante a adoção de medidas capazes de reduzir o risco da

contaminação nos grãos e nos coprodutos. Sendo exemplo destas medidas a secagem eficiente, manutenção do controle de umidade e temperatura durante a armazenagem e monitoramento de fungos e micotoxinas durante todo o processo (SILVA et al., 2016a).

2.3.1 Composição nutricional dos grãos secos de destilaria

O DDGS possui bom valor nutritivo e apresenta alta concentração de proteínas (28,7 – 32,9%) e lipídios (8,8 – 12,4%, (ROBINSON et al., 2008; PEDERSEN et al., 2014; SILVA et al., 2016b; RISOLIA et al., 2019). Em adição, o DDGS apresenta em sua composição perfil de aminoácidos essenciais para cães (Lisina = 0,61 – 1,6; Arginina = 1,01 – 1,48; Metionina = 0,54 – 0,76, SILVA et al., 2016).

Entretanto, o seu alto conteúdo de fibra dietética total (FDT) pode limitar o uso para cães. Este teor elevado de FDT (em torno de 32,3%, RISOLIA et al., 2019) promove a redução da digestibilidade dos nutrientes (SILVA et al., 2016b; RISOLIA et al., 2019). Buscando minimizar tais efeitos, o uso de enzimas exógenas, como xilanases, tem se mostrado eficiente (SILVA et al., 2016b). No entanto, diversos são os efeitos positivos na saúde de maneira geral ocasionados pela inclusão de fibras solúveis e insolúveis na dieta como o manejo adequado da obesidade (HOWARTH et al., 2001), de constipação e diarreias (BAUER e MASKELL, 1996) e da diabetes (CHANDALIA et al., 2000). Além de efeitos benéficos, sobretudo da fração solúvel da fibra, sobre a funcionalidade intestinal, por meio da seleção da microbiota intestinal não patogênica e produção de AGCC (RISOLIA et al., 2019; BRITO et al., 2021; SOUZA et al., 2021).

O HPDDG, por sua vez, apresenta maior proporção de PB (acima de 40% PB) e menores teores de gordura (4,63%) e FDT (em torno de 19,7%, PARSONS et al., 2006) em relação ao DDGS convencional (HUBBARD et al., 2009)

2.3.2 Uso dos grãos secos de destilaria na nutrição de cães

O DDGS é comumente utilizado na nutrição de ruminantes, no entando, estudos demonstram que este ingrediente também pode ser utilizado na alimentação

de suínos (PEDERSEN et al., 2007; AVELAR et al., 2010) e frangos de corte (WU-HAAN et al., 2010; CUEVAS et al., 2012).

Existem poucos estudos relacionados à inclusão de DDGS na nutrição de animais de companhia. Em estudo realizado utilizando baixos níveis de inclusão (0, 4, 6 e 8%) deste ingrediente para cães adultos não foram encontradas alterações na digestibilidade aparente do amido e da MS, bem como a MS das fezes (CORBIN et al., 1980). No entanto, avaliando-se altos níveis de inclusão (0, 8,9 e 15,7%) observou-se redução da digestibilidade da MS somente na inclusão mais alta de DDGS. Neste nível (15,7%) também foi observado o aumento da matéria seca fecal (CORBIN et al., 1980).

Outro estudo avaliando o uso de DDGS em níveis crescentes de inclusão (6%, 12% e 18%) em dietas para cães, com e sem a adição da enzima xilanase, encontrou relação inversa entre a inclusão do DDGS com a digestibilidade dos macronutrientes da dieta. Com a adição da enzima, observou-se melhora na digestibilidade a partir de 12% de inclusão do DDGS associada ao uso da xilanase em relação às que não receberam a suplementação enzimática. Em relação às fezes, não houve alteração de escore, porém o pH foi reduzido de forma inversa à inclusão do ingrediente. Os animais apresentaram boa aceitabilidade da dieta contendo o DDGS, sendo que a dieta com 18% de inclusão foi a mais consumida pelos cães (SILVA et al., 2016b).

Em estudo mais recente, avaliando dietas contendo 0% DDGS (sem enzimas e com inclusão de xilanase e protease) e 20% de DDGS (sem enzima, com xilanase, com protease e com as enzimas associadas), foi encontrado como resultado que a adição do DDGS, independentemente das enzimas, proporciona redução na digestibilidade dos nutrientes da dieta. No entanto, a fermentação dos compostos fibrosos presentes nesse ingrediente resultou no aumento da produção total de AGCC com a adição do DDGS, em especial os ácidos acético e propiônico (RISOLIA et al., 2019).

Em relação ao HPDDG, estudos evidenciam seu potencial uso em dietas para peixes (PRACHOM et al., 2013; GODA et al., 2020) e suínos (WIDMER et al., 2008; ADEOLA e RAGLAND, 2012; SON et al., 2019; RAO et al., 2021), não sendo encontrados estudos em cães. Recentemente, um estudo envolvendo o uso de HPDDG em gatos encontrou resultados indicando capacidade para substituição ao farelo de soja (KILBURN-KAPPELER et al., 2022). No entanto, os autores

salientaram a necessidade de uma inclusão máxima de 10%, uma vez que níveis de 15% de inclusão resultaram em maior produção fecal e redução na digestibilidade dos macronutrientes da dieta.

3 CONSIDERAÇÕES FINAIS

Com base nas informações expostas, é notável a importância da inclusão do amido para produção das dietas extrusadas e sobre a qualidade final dos extrusados formados. No entanto, as fontes utilizadas não são amidos purificados, contendo diferentes componentes, como a presença das fibras dietéticas (de caráter solúvel e insolúvel) e macromoléculas ligadas aos grânulos de amido, como proteínas e lipídeos. Essas diferentes composições, por sua vez, podem interferir sobre a digestibilidade da dieta. Contudo, a fração fibrosa tem potencial para promover e manter a funcionalidade intestinal dos cães.

A grande variabilidade em termos de perfil nutricional dos ingredientes é evidenciada nos estudos avaliando o DDGS em animais de produção e de companhia. No entanto, com o avanço nas tecnologias de processamento, é possível obter ingredientes de melhor qualidade. O HPDDG possui grande potencial de utilização em dietas para animais de companhia. Além de suas características nutricionais, apresenta propriedades funcionais capazes de modular a microbiota intestinal.

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CHAPTER II - STARCH SOURCES AND THEIR INFLUENCE ON EXTRUSION PARAMETERS, PHYSICAL CHARACTERISTICS OF KIBBLES, AND PALATABILITY OF DIETS FOR DOGS

This chapter is written in accordance with the guidelines for authors in Animal Feed Science and Technology.

Starch sources and their influence on extrusion parameters, physical characteristics of kibbles, and palatability of diets for dogs

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ABSTRACT

The study aimed to evaluate the effects of different starch sources on the extrusion process, the physical characteristics of kibbles, and diet palatability for dogs. Seven diets with corn, brown rice, sorghum, potato starch, sweet potato flour, chickpea, and pea flour were evaluated. The conditioning temperature and the volume of water added to the conditioner during the processing of the diets were measured, and the extruder variables evaluated were knife speed, feed rate, screw speed, amperage, and productivity. For the physical characteristics of kibbles, the density, size, expansion index, and hardness of the experimental diets were measured. The porosity variables evaluated were the total pore area, average pore area, and the number of pores. Diet palatability was evaluated in 16 adult beagle dogs in a completely randomized design. Six paired tests were performed, with all diets compared to the corn diet, with two consecutive days per test, totaling 32 repetitions. Diets with tubers had lower kibble density and higher size, expansion index, and hardness than the diets with cereals and pulses ($P<0.001$). Besides, diets with tubers had a higher number of pores than pulses ($P<0.001$). The inclusion of pea results in kibbles less expanded and with fewer pores ($P<0.001$). Dogs preferred all diets compared with the corn-based diet ($P<0.001$), except for the diet containing sweet potato ($P=0.102$). The inclusion of tubers as starch sources results in higher kibble expansion, size, hardness, and porosity and in lower density. The inclusion of pea results in kibbles less expanded and with a lower number of pores. The diet's moisture content and texture affects diet palatability in dogs.

Keywords: amylose; amylopectin; dietary fiber; pulses; tubers.

Abbreviations: BCS, body condition score; DM, dry matter; EI, expansion index; SEM, standard error of the mean; SME, specific mechanical energy; TDF, total dietary fiber.

1. Introduction

The extrusion process aims to cook, shape, and texturize a homogeneous mass of ingredients by combining moisture, pressure, temperature, and mechanical friction in a short time (Riaz, 2007), being the main process used to produce pet food. Among the ingredients included in the dough, starch is the main responsible for the rheological properties that characterize most of the extruded products. In general, the starch fraction works as a thermoplastic polymer during extrusion. When leaving the extruder, the pressure and temperature differential induce the water vaporization that deforms the gelatinized starch. When water, energy, and time are sufficient in the processing, starch granules lose their crystallinity, swell, and disrupt, forming an amorphous mass that binds all food components forming a continuous structure (Ding et al., 2005). This results in the formation of a cellular structure in the kibbles, responsible for their expansion and crispness formation, which are important for the appearance and palatability of the diets (Baller et al., 2018).

Starch is found in several plant species as a reserve digestible carbohydrate, being abundant in cereal grains (580 g/kg to 830 g/kg in dry matter, DM; Carciofi et al., 2008; Bazolli et al., 2015), pulses (270 g/kg to 570 g/kg in DM; Carciofi et al., 2008; He and Wei, 2017), and tubers (950 g/kg to 970 g/kg in DM, Carciofi et al., 2008; Domingues et al., 2019). Although corn and broken rice are widely used in extruded dog foods, the interest in different starch sources has increased, due to commercial trends, exploring the use of whole grains (brown rice and sorghum), pulses (lentil, chickpea, and pea), and tubers (potato and cassava).

However, it is known that the composition of these raw materials, mainly the differences in the crystalline form of their granules, amylose:amylopectin ratio, and fibrous concentration can influence starch gelatinization, viscosity, and shear inside the extruder barrel (Eliasson, 2004). For example, pulses, such as pea and chickpea, present higher amylose (240-490 g/kg) and fiber concentration (around 170 g/kg) than cereals (around 200-300 g/kg amylose and 80 g/kg fiber in corn) (Pérez and Bertoft, 2010; Hoover et al., 2010), which may impact the rheological properties of starch during the extrusion process, resulting in kibbles with different characteristics (Domingues et al., 2019; Pezzali and Aldrich, 2019). The differences in kibble texture due to different behaviors of starch sources during the extrusion process may affect diet palatability in dogs (Koppel et al., 2015; Domingues et al., 2019).

Modifications caused by extrusion on nutritional quality and microbiological safety of the diets are already described in the literature (Riaz, 2007; Tran et al., 2008; Gibson and Alavi, 2013). However, few published studies evaluated the effects of starch sources on kibble characteristics and diet palatability of pet food (Bazolli et al., 2015; Baller et al., 2018; Domingues et al., 2019; Pezzali and Aldrich, 2019). As a differential, the present study provides an evaluation of alternative starch sources to corn in extruded dog food. Thus, the objective of this study was to evaluate the influence of different starch sources on extrusion parameters and physical characteristics of kibbles. In addition, the objective was to evaluate the palatability of the diets for dogs.

2. Material e methods

All animal care and experimental procedures were approved by the Animal Use Ethics Committee of the Agricultural Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol n. 001/2020.

2.1 Experimental diets

The diets were formulated to meet the nutritional needs of adult dogs according to the European Pet Food Industry Federation (FEDIAF, 2019). The ingredients were ground in 0.8 mm sieves and extruded in a twin-screw extruder (Ferraz, E-96; Ribeirão Preto, SP, Brazil). After the extrusion process, the diets were dried in a horizontal dryer for 25 minutes and coated with poultry fat and palatability enhancer.

Seven diets containing different starch sources were evaluated: corn, brown rice, sorghum, potato, sweet potato, chickpea, and pea. The diets were formulated to have similar starch (300 g/kg starch), protein, and fat concentrations. The ingredients of the evaluated diets are presented in Table 1.

2.2 Chemical analysis

Diets and starch sources were analyzed for dry matter at 105 ºC (DM105), ether extract in acid hydrolysis (EEAH, method 954.02), ash (method 942.05), calcium (method 927.02), phosphorus (method 984.27), nitrogen (N, method 954.01) and then crude protein (CP) was calculated as N × 6.25 according to the Association of Official Analytical Chemists (AOAC, 1995). The total dietary fiber, insoluble fiber, and soluble fiber of the diet were analyzed according to Prosky et al. (1988). Gross energy (GE) was determined by an isoperibol calorimeter (Parr Instrument Co., model 1261, Moline, IL, USA).

The chemical composition analyzed of the diets is shown in Table 2.

2.3 Extrusion parameters and physical characteristics of kibbles

The conditioning temperature and the volume of water added to the conditioner during the processing of the diets were measured. The temperature was measured with a digital infra-red thermometer (LaserGrip Model GM400, São Paulo,

SP, Brazil). The following extruder variables were measured: knife speed (Hz), feed rate (Hz), screw speed (Hz), and amperage (A). The productivity was kept constant at approximately 1070 kg/h.

The density, size, expansion index (EI), and hardness of the experimental diets were measured in 20 samples per treatment. Density was expressed as the ratio of diet weight (grams) to volume (liters). Kibble size was determined using a digital caliper (MTX-316119). The EI was calculated as the ratio between the radial size of the kibble and the diameter of the extruder die.

For hardness analysis, 20 kibbles from each diet were selected. These samples were analyzed using a durometer (Ethik Technology; 298 DGP II hardness tester, Brazil), which measures the diametrically applied force required to break a kibble. The force was measured in Newton (N) and converted and expressed in kgf/cm2.

Diets were examined under scanning electron microscopy at 10x magnification to determine kibble porosity (3 replicates per treatment). Each kibble was cut longitudinally to allow better visualization of the pores. Kibble pore area (mm^2) was measured using the ImageJ® software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The variables evaluated were: total pore area, average pore area, and a number of pores.

2.4 Palatability assay

For the palatability test, 16 adult Beagle dogs (eight males and eight females), with 4 years of age, mean weight of 10.4 ± 1.76 kg were used. The body condition score (BCS) of the dogs was analyzed at the beginning and the end of the experiment, on a scale of one to nine, according to Laflamme (1997). Dogs had a mean BCS of 5.6 ± 0.6.

Diets containing sorghum, brown rice, potato, sweet potato, chickpea, and pea were compared one by one to the diet containing corn, totaling 6 comparisons. Each test was performed for two days. The two foods were offered simultaneously to the dogs once a day (08:00h). The amount provided was 30% higher than the National Research Council recommendations (National Research Council Committee on Dog & Cat Nutrition [NRC], 2006) for the maintenance of adult dogs. Once one of the diets was completely consumed, both bowls were removed and the remaining amount was quantified. The relative position of the feeders was alternated on the second day of the experiment so that the animal was not conditioned to the feeding site.

The palatability test was determined using the intake ratio and the first choice among the diets offered to the dogs. The first choice was defined by looking at the first bowl that the animal was directed to. To determine the intake ratio, the remaining fraction was quantified for later calculation.

2.5 Calculations and statistical analysis

The energy consumption (kW/h) and the specific mechanical energy (SME) transferred to the mass (kW/h/ton) were calculated according to Riaz (2000), with the following equations:

√Electric phase of the system • feeding rate • amperage • motor $\cos_{\gamma}$ / 1000. In which, motor $\cos_{\gamma} = 0.86$

SME (kW/h/ton) = (energy consumption) • 1000 / extruder output (kg/h)

The density, size, and EI data of the kibbles were analyzed for normality by the Shapiro-Wilk test ($P<0.05$) and submitted to regression analysis, considering 20 repetitions per treatment with a significance level of $P<0.05$. Porosity data were

submitted to regression analysis, considering 3 replications per treatment with a significance level of $P<0.05$. The variables measured for extrusion parameters were presented descriptively.

Palatability data were analyzed in a completely randomized design. Data were first submitted to the Kruskal-Wallis test, which revealed no influence ($P>0.05$) of gender (male and female), or test day (day one and day two) on the results. The results of the intake ratio were compared by Student t-test at 5% significance and the first choice by the chi-square test, totaling 32 repetitions per test (16 dogs x 2 days of evaluation). Data were analyzed using the SAS statistical package (version 8, SAS Institute Inc., Cary, NC, United States of America). The intake ratio was estimated according to the calculation:

Intake ratio = g consumed from diet A or B / total g consumed (A + B)

3. Results

3.1 Extrusion parameters and physical characteristics of kibbles

The results of the parameters evaluated during the extrusion process are presented in Table 3. Kibbles from diets containing potato starch had greater size and EI and lower density when compared to kibbles from diets based on cereals (corn, brown rice, and sorghum), pulses (chickpea and pea), and sweet potato ($P<0.001$), except for sorghum for size and brown rice for density. On the other hand, kibbles from diets containing pea had EI similar to the diets with corn and brown rice and lower than the other diets ($P<0.001$).

Kibbles from the chickpea-based diet showed a density similar to brown rice and higher than the other starch sources ($P<0.001$). Furthermore, kibbles from the

chickpea diet had a similar size to diets containing corn and brown rice and were lower when compared to the other diets ($P<0.001$).

Hardness was higher in kibbles containing potato in relation to the other diets and lower for chickpea-based kibbles ($P<0.001$). The other diets showed similar hardness ($P<0.001$).

Total pore area was greater in tuber-based diet kibbles compared to pea-based diet kibbles ($P=0.003$). A greater average pore area was found in kibbles of the diet containing potato in relation to corn, brown rice, and pulses ($P<0.001$). The pulses-based and brown rice-based diet presented a lower average pore area compared to sorghum and tubers ($P<0.001$). Diets containing tubers had a higher number of pores, while diets containing pulses had a lower number of pores ($P<0.001$). The number of pores in the diets containing cereals was similar to potato and pulses ($P<0.001$).

The results of the physical characteristics of kibbles are presented in Table 4 and the porosity is illustrated in Figure 1.

3.2 Palatability

There was a difference in the first choice only between corn vs. chickpea, with dogs preferring the chickpea diet ($P<0.05$). All comparisons resulted in a lower intake ratio to the corn diet in comparison to the other starch sources ($P<0.001$), except for the sweet potato diet, which did not differ from corn ($P>0.05$, Table 5).

4. Discussion

As expected, the physical characteristics of kibbles, mainly density, expansion, hardness, and porosity are highly influenced by the starch source used.

Besides, the other ingredients from the formulation and processing conditions, such as the mechanical and thermal energy transferred to the dough, residence time in the conditioner and extruder barrel, and moisture can also influence kibble characteristics (Riaz, 2007).

Starch composition significantly influences starch behavior during food extrusion. Tuber starches, such as potato and sweet potato, contain a higher proportion of amylopectin (700–800 g/kg) relative to amylose (200–300 g/kg; Pérez e Bertoft, 2010), facilitating starch gelatinization during extrusion. Furthermore, tubers contain high levels of phosphate monoesters, which are covalently bound to the amylose and amylopectin fraction (Schirmer et al. 2013). These phosphate groups contribute to higher viscosity, water binding capacity, and swelling power, contributing to a low gelatinization temperature (Hoover, 2001; Singh et al., 2003), directly influencing the gelatinization properties and retrogradation rate (Karim et al., 2007; Schirmer et al. 2013).

Increasing starch gelatinization improves kibble expansion (Bhattacharya and Choudhury, 1994). A greater EI was observed in the present study in kibbles of potato starch and sweet potato-based diets (2.52 and 2.33, respectively). The same was described in studies evaluating the inclusion of potato starch compared to corn (Domingues et al., 2019) and different types of processing on physical characteristics of kibbles of diets containing sweet potato flour (Borba et al., 2005). A higher expansion also results in kibbles with lower density (Bhattacharya and Choudhury, 1994; Domingues et al., 2019).

Besides the amylose and amylopectin ratio, kibble macrostructure also may be influenced by the TDF concentration of the diet. A high dietary fiber concentration may hinder the formation of an adequate cellular structure, as it conducts water

vapor without the formation of cells. This reduces the radial expansion, increases specific and apparent density, and promotes the formation of harder kibbles with small pores (Robin et al., 2012; Monti et al., 2016; Souza et al., 2022). These characteristics may explain the lower kibble expansion of the diet with the inclusion of pea (TDF = 170.6 g/kg) and the lower radial size, and higher density of the chickpea-based diet (TDF = 169.1 g/kg).

High density diets usually are associated with low porosity (Camire, 1990; Tiwari and Jha, 2017). Size and number of pores in a kibble are related to density, and the formation of the porous structure is also dependent on the starch content and type (Gill 2002; Ah-Hen et al., 2014). This association was observed in kibbles of diets containing brown rice and chickpea, which presented higher density and lower total pore area, average pore per area, and number of pores (only chickpea-based). In turn, it was observed in tubers-based diets kibbles with lower density and higher total pore area, average pore per area, and number of pores.

As a limitation of this study, extrusion parameters were not subjected to statistical analysis, due to the lack of repetition, making it difficult to correlate these variables with their effects on physical characteristics of the kibbles. Therefore, it is important that future studies with repeated extrusion variables measures be conducted to investigate possible changes in these parameters with the inclusion of different starch sources.

Regarding diet palatability, one of the hypotheses for the difference in the intake ratio between corn compared to the other diets was the moisture content (moisture of the diets: corn = 36.7 g/kg; brown rice = 72.4 g/kg; sorghum = 66.5 g/kg; potato = 48.9g/kg; chickpea = 46.4 g/kg, and pea = 59.0 g/kg). Moisture content is crucial for diet palatability and dogs can distinguish and choose diets with only 2

points more moisture when comparing diets with 80 and 100 g/kg moisture (Brito et al., 2010). On another hand, in studies evaluating diets with similar moisture content, dogs also preferred potato starch, pea, and chickpea, instead of the corn-based diet (Domingues et al., 2019; Pacheco et al., 2021). These results demonstrate that not only the moisture content of the diets but also other physical and chemical characteristics influence diet palatability for dogs.

It is possible that in diets with similar moisture content, the main effects of starch sources on diet palatability are their influence on kibble texture and consequently also on coating. Kibbles with lower density may increase crispness (strength and number of chewing movements) (Félix et al., 2010). In addition, density and highly porous kibbles are also associated with high oil absorption capacity (Gill 2002; Ah-Hen et al., 2014), affecting coating homogeneity. Fatty clusters tend to stick to the surface of the pet food, rendering it less appealing in terms of appearance (Samant et al., 2021). Fat distribution on kibbles is important for good food palatability, and a worse coating efficiency could reduce food acceptance by dogs (Monti et al., 2016).

However, the palatability can be influenced by several factors, such as the ingredients of the diet and its processing, the macrostructural characteristics of the kibbles, chemical composition, inclusion of different palatability enhancers, and how they all relate to sensory properties, such as aroma, texture, shape, and taste (Aldrich and Koppel 2015), demonstrating the complexity of the evaluation of palatability in dogs.

5. Conclusion

The inclusion of potato and sweet potato as starch sources results in higher kibble expansion, size, hardness, and porosity and lower density. The inclusion of starch sources with a high concentration of fiber, such as pea, results in diets with kibbles less expanded and with less porosity. In addition, moisture content and kibble texture affect diet palatability in dogs.

Credit authorship contribution statement

Gislaine Cristina Bill Kaelle: Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Taís Silvino Bastos:** Investigation. **Renata Bacila Morais dos Santos de Souza:** Investigation. **Eduarda Lorena Fernandes:** Investigation. **Simone Gisele de Oliveira:** Supervision, Writing - review & editing. **Ananda Portella Félix:** Conceptualization, Data Curation, Project administration, Writing - Review & Editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Table 1. Ingredients of the experimental diets.

Ingredients (g/kg)	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Corn	433.90	-	-	-	-	-	-
Brown rice	-	447.45	-	-	-	-	-
Sorghum	-	-	450.13	-	-	-	-
Potato	-	-	-	330.13	-	-	-
Sweet Potato	-	-	-	-	366.28	-	-
Chickpea	-	-	-	-	-	643.84	-
Pea	-	-	-	-	-	-	565.63
Poultry offal meal	267.89	264.36	255.63	293.17	293.13	262.10	227.20
Isolated swine protein	147.43	138.73	146.97	170.68	171.84	45.92	81.26
Celulose	55.00	41.43	45.30	66.81	46.20	9.12	26.05
Poultry fat	49.71	61.91	58.94	93.41	74.75	20.00	53.33
Poultry hydrolizate	20.00	20.00	20.00	20.00	20.00	5.00	20.00
Potassium chloride	7.05	7.09	5.00	6.78	8.78	3.00	7.50
Sodium chloride	5.00	5.00	4.02	5.00	5.00	2.00	5.00
Mineral-vitamin supplement[1]	3.00	3.00	3.00	3.00	3.00	2.00	3.00
Adsorvent	2.00	2.00	2.00	2.00	3.00	2.00	2.00
Choline chloride	2.00	2.00	2.00	2.00	2.00	1.00	2.00
Calcium propionate	2.00	2.00	2.00	2.00	2.00	0.80	2.00
Taurine	1.00	1.00	1.00	1.00	1.00	0.15	1.00
DL-Methionine	0.80	0.80	0.80	0.80	0.80	0.08	0.80

BHT	0.15	0.15	0.15	0.15	0.15	0.00	0.15
BHA	0.08	0.08	0.08	0.08	0.08	0.00	0.08
Citric acid	3.00	3.00	3.00	3.00	3.00	3.00	3.00

[1]enrichment per kg of product^{-1}: vitamin A (retinol), 20,000 IU; vitamin D3, 2,000 IU; vitamin E (alpha-tocopherol α), 48 mg; vitamin K3, 48 mg; vitamin B1, 4 mg; vitamin B2, 32 mg; pantothenic acid, 16 mg; niacin, 56 mg; choline, 800 mg; Zn as zinc oxide, 150 mg; Fe as ferrous sulphate, 100 mg; Cu as copper sulphate, 15 mg; I as potassium iodide, 1.5 mg; Mn as manganese oxide, 30 mg; Se as sodium selenite, 0.2 mg; antioxidant, 240 mg.

Table 2. Analyzed chemical composition (g/kg, dry matter basis) of starch sources and experimental diets.

Item	Starch sources						
	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Dry matter	887.8	887.6	877.0	857.9	915.1	912.6	881.5
Ash	25.1	14.5	23.0	3.0	17.9	29.5	28.9
Crude protein	74.5	137.7	107.3	3.7	27.8	240.3	255.4
EEAH	38.1	41.1	20.6	5.3	11.0	71.3	23.9
Gross energy, kcal/kg	4829.2	4429.9	4321.8	4217.4	4126.4	4860.7	4545.6
Total starch	700.6	767.5	753.7	960.4	786.9	452.7	476.2
Total dietary fiber	52.2	68.9	53.3	28.3	111.23	257.0	237.0
Insoluble fiber (IF)	46.8	61.2	46.9	27.6	68.3	226.9	208.6
Soluble fiber (SF)	5.5	7.7	6.4	2.1	44.0	30.1	28.4
IF:SF	8.5	7.9	7.3	13.1	1.5	7.5	7.3
Ca	0.3	0.3	0.4	0.3	1.1	1.5	0.5
P	2.4	2.9	3.9	0.7	0.6	3.8	3.3
	Diets						
	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Dry matter	963.3	927.6	933.5	951.1	970.3	953.6	941.1
Ash	79.7	87.1	81.3	77.0	81.7	89.8	84.7
Crude protein	328.4	320.8	338.3	330.5	350.1	354.9	345.0
EEAH	128.7	123.1	126.3	124.8	123.8	138.5	123.0
Gross energy, kcal/kg	4784.4	4861.6	4863.4	4959.1	4916.9	4987.1	4882.6

Total starch	304.1	373.7	353.3	350.3	288.3	291.5	269.4
Total dietary fiber	81.7	77.3	76.0	94.3	96.2	169.1	170.6
Insoluble fiber (IF)	76.4	70.8	69.1	89.1	75.1	146.4	146.9
Soluble fiber (SF)	5.3	6.5	6.9	5.2	21.1	22.7	23.7
IF:SF	14.3	10.9	10.0	17.0	3.56	6.44	6.19
Ca	15.0	14.5	13.2	13.5	15.0	15.0	12.3
P	7.9	7.9	7.8	6.9	7.7	7.7	8.3

Table 3. Extrusion variables in diets containing different starch sources for dogs.

Item	Experimental diets						
	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Preconditioner							
Temperature, ºC	65.7	55.9	76.4	80.8	68.6	85.6	63.5
Water addition, L/h	257.5	260.0	227.5	300.0	210.0	295.0	247.5
Extruder							
Screw speed, Hz	39.0	39.0	37.0	41.0	42.5	42.5	37.2
Feeding rate, Hz	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Amperage, A	56.4	60.9	54.4	67.2	67.2	48.2	63.1
SME, kW/ton/h[1]	17.27	18.65	16.68	20.60	20.60	14.76	19.33
Dryer							
Kibble temperature, ºC	76.5	56.5	60.8	84.7	72.2	58.9	52.3
Belt velocity, Hz	15	16	16	15	16	16	16
Retention time, min	25	25	25	25	25	25	25
Die							
Number of holes	25	25	25	25	25	25	25
Hole diameter, mm	4.5	4.5	4.5	4.5	4.5	4.5	4.5

Table 4. Variables of physical characteristics of kibbles and porosity in diets containing different starch sources for dogs.

Item	Experimental diets							SEM[1]	p-value
	Corn	Brown rice	Sorghum	Potato	Sweet potato	Chickpea	Pea		
Kibble characteristics									
Density, g/L	409.50^{b}	459.60^{a}	364.70^{c}	356.10^{cd}	343.70^{d}	447.20^{a}	371.70^{c}	3893	<0.001
Size, mm	8.81^{cd}	8.97^{cd}	9.89^{bc}	11.35^{a}	10.49^{ab}	8.33^{d}	10.20^{b}	0.116	<0.001
EI[2]	1.96^{cd}	1.99^{cd}	2.15^{bc}	2.52^{a}	2.33^{ab}	2.26^{b}	1.85^{d}	0.026	<0.001
Hardness, kgf/m²	9.65^{b}	8.87^{b}	8.96^{b}	13.92^{a}	10.60^{b}	4.74^{c}	10.14^{b}	0.276	<0.001
Porosity									
Total area (mm²)	35.04^{ab}	28.24^{b}	57.81^{ab}	65.48^{a}	63.49^{a}	33.04^{ab}	24.22^{b}	3805	0.003
Average pore area (mm^2)	1.17^{bc}	0.94^{c}	1.92^{a}	2.18^{a}	1.74^{ab}	1.10^{c}	0.81^{c}	0.058	<0.001
Number of pores	36.00^{cd}	41.00^{bc}	42.00^{bc}	46.00^{ab}	55.00^{a}	30.00^{d}	30.00^{d}	0.463	<0.001

[1]SEM = Standard error of mean; [2]EI = Expansion index; ^{a,b,c,d}Distinct letters indicate difference by Tukey's test (P<0.05)

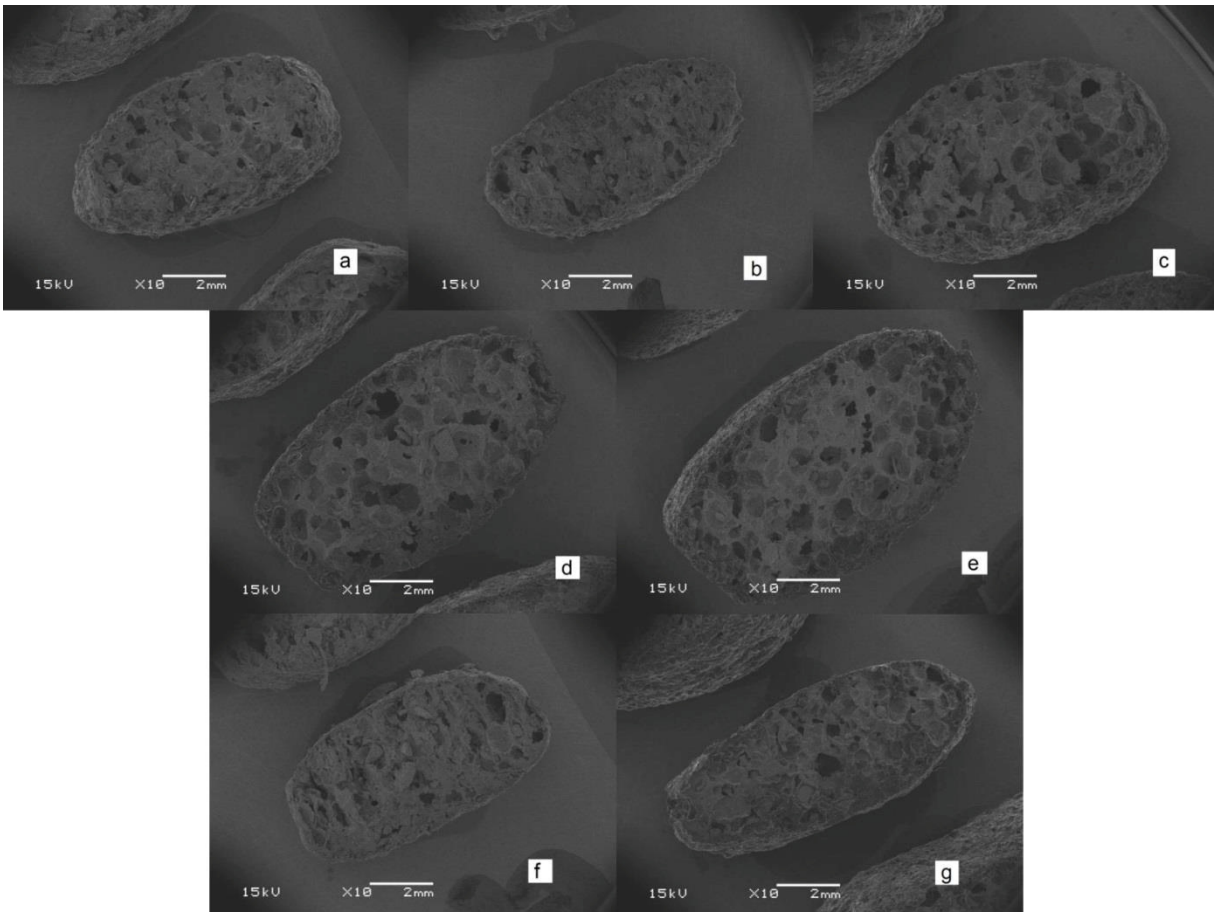

Figure 1. Scanning electron microscopy (10x magnification) of the longitudinal section of kibbles of diets with different starch sources. (a) corn; (b) brown rice; (c) sorghum; (d) potato; (e) sweet potato; (f) chickpea; and (g) pea.

Table 5. Number of first choices to diet A and intake ratio (%) of experimental diets.

Diet (A x B)	n[1]	Intake ratio[2]		p-value
		A	B	
Corn x Brown rice	14	37.80	62.20	0.039*
Corn x Sorghum	13	6.93	93.07	<0.001*
Corn x Potato	12	16.20	83.80	<0.001*
Corn x Sweet potato	11	40.49	59.51	0.102
Corn x Chickpea	7*	9.12	90.88	<0.001*
Corn x Pea	11	26.07	73.93	<0.001*

[1]Number of visits to the pot with diet B is obtained by 32-n; *number of visits to the pot with diet A differs by the chi-square test and intake ratio by the t-test (P<0.05); [2]Intake ratio: [g ingested from diet A or B/total g provided (A + B)] x 100.

CHAPTER III - DIFFERENT STARCH SOURCES RESULT IN DISTINCT RESPONSES TO DIETS DIGESTIBILITY, FECAL MICROBIOTA AND FERMENTATIVE METABOLITES, AND POSTPRANDIAL GLYCEMIC RESPONSE IN DOGS

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Different starch sources result in distinct responses on diets digestibility, fecal microbiota and fermentative metabolites, and postprandial glycemic response in dogs

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Abstract

This study aimed to evaluate the effects of different starch sources on the coefficients of total tract apparent digestibility (CTTAD) of nutrients, metabolizable energy (ME), intestinal fermentative metabolites, fecal microbiota, and postprandial glycemic response in dogs. Seven diets containing corn, brown rice, sorghum, potato starch, sweet potato flour, chickpea, and pea flour were evaluated. Fourteen adult Beagle dogs were randomly distributed in blocks (three periods). Dogs were fed the experimental diets for 15 days during three periods, totaling 6 repetitions. In general, diets with brown rice and pea had higher CTTAD of nutrients, followed by diets containing sorghum, chickpea, and potato. The diet containing sweet potato had the lowest CTTAD of nutrients. Diets containing corn and brown rice presented the highest ME content. Dogs fed the chickpea diet had lower fecal pH, ammonia concentration, and dry matter content ($P<0.05$). In general, higher fecal concentrations of short-chain fatty acids were observed in dogs fed the sweet potato and chickpea diets compared to the potato and brown rice diets ($P<0.05$). Pulses-based diets resulted in a higher fecal abundance of *Bacteroides plebeius*, *Prevotella copri*, *Blautia*, and *Turicibacter* ($P<0.05$). A higher abundance of *Faecalibacterium prausnitzii* and *Blautia* was observed in the feces of dogs fed the sweet potato diet ($P<0.05$). Blood samples collected from dogs fed diets containing corn and potato indicated a greater glycemic peak incremental concentration and maximum glycemia than the other starch sources ($P<0.05$). The time for the glycemic peak was longer for diets containing sorghum and chickpea ($P<0.05$). The results obtained in this study showed that sweet potato and pulses improve indicators of gastrointestinal functionality and help control the postprandial glycemic response in dogs.

Keywords: Cereals; Glycemia; Gut microbiota; Pulses; Total Dietary Fiber; Tuber

Abbreviations: AUC, area under the curve; BCFA, branched-chain fatty acids; CP, crude protein; CTTAD, coefficients of total tract apparent digestibility; DM, dry matter; DMf, fecal dry matter; EEHA, ether extract in acid hydrolysis; GE, gross energy; GIT, gastrointestinal tract; IF, insoluble fiber; ME, metabolizable energy; OM, organic matter; OTUs, observed taxonomic units; PCoA, principal coordinate analysis; SCFA, short-chain fatty acids; SDS, slowly digestible starch; SEM, standard error of the mean; SF, soluble fiber; TDF, total dietary fiber.

1. Introduction

Starch is the main carbohydrate present in commercial pet foods. Starch concentrations typically vary between 200 g/kg to 400 g/kg in dry dog foods (Gross et al., 2010; Domingues et al., 2019). Although starch is not an essential nutrient for dogs and cats, it can impact health in different ways depending on its type (Corsato Alvarenga and Aldrich, 2020). Starch also has an important role in the processing of extruded dog food, as it plays an essential role in extrusion (Crane et al., 2000). During this process, starch granules gelatinize, becoming soluble in water and more susceptible to enzymatic degradation (Dona et al., 2010). This influences the overall diet digestibility.

In general, according to their botanical origin, tuber-based ingredients are more digestible, especially potato starch, followed by cereals, and pulses, which are recognized as less digestible (Carciofi et al., 2008; Bajaj et al., 2018; Domingues et al., 2019). It is known that different starch sources vary according to their granular structure and the amylose:amylopectin proportion (Tester et al., 2004), which affects their digestibility and, consequently, their postprandial glycemic response in dogs (Carciofi et al., 2008). Thus, greater starch availability for digestion causes

anticipation and intensification of the glucose peak and insulin in the blood (Jenkins et al., 1998; Appleton et al., 2004).

Cereal grains such as rice, corn, and sorghum are the most used ingredients in the food industry for dogs and cats. Although these ingredients are widely used, there has been an increasing interest in alternative starch sources, such as those from tubers (potato and sweet potato) and pulses (chickpea and pea). Pulses can be considered also as a protein source, due to a greater crude protein (CP) concentration (above 200 g/kg) in their composition. However, they are usually included at lower concentrations due to the presence of oligosaccharides, which may cause excess fermentation in the large intestine and, when excessively included, soften stool (Corsato Alvarenga et al., 2020).

Although a few studies in dogs evaluated different starch sources on extrusion, digestibility, and postprandial glycemic responses (Carciofi et al., 2008; Bazzoli et al., 2015; Domingues et al., 2019; Alvarenga et al., 2021), little is known about the effects of these ingredients on the gut microbiome and fermentative metabolites in dogs. In this sense, we hypothesized that starch sources with a higher concentration of soluble fiber (SF)—such as sweet potato and pulses—may result in greater intestinal microbiota modulation, due to their higher fermentability, resulting in higher short-chain fatty acids (SCFA) production (Brito et al., 2021; Souza et al., 2021).

Based on this information, our study aimed to evaluate the coefficients of total tract apparent digestibility (CTTAD) and metabolized energy (ME) of diets containing different starch sources for dogs. In addition, the objective was to evaluate the characteristics, fermentative metabolites, and microbiota of the feces, as well as the glycemic response in dogs fed diets containing different starch sources.

2. Material and methods

All the animal procedures were approved by the Ethics Committee on Animal Use of the Agrarian Sciences Sector of the Federal University of Paraná, Curitiba, Paraná, Brazil, under protocol n. 001/2020. The study was conducted at the Research Laboratory in Canine Nutrition – LENUCAN in Curitiba, Paraná, Brazil (25º 25’ 40" S, 49º 16’ 23" W).

2.1 Animals and facilities

Fourteen adult Beagle dogs (7 males and 7 females, all of them 4 years old) were used, with a mean body weight of 10.4 ± 1.76 kg, and a mean body condition score of 5.60 ± 0.60, according to Laflamme (1997). The animals were submitted to clinical examination before and after the experimental period.

The dogs were individually housed in brickwork kennels (5 m long x 2 m wide), containing a bed and free access to fresh water. During most of the experimental period, the dogs had free access to a grassy outdoor area of 1,138 m2 for 4 h/day for voluntary exercise and socialization. During the feces collection period, the dogs were individually housed in kennels. The facilities had side wall bars that allowed visual and limited interaction with neighboring dogs. Besides, the animals received extra attention and environmental enrichment inside the kennel during this period. The temperature ranged from 16 ºC to 28 ºC, with a 12 h light-dark cycle (light from 6:00 am to 6:00 pm).

2.2 Diets

Seven diets containing different starch sources were evaluated: corn, sorghum, brown rice, potato starch, sweet potato flour, chickpea, and pea flour. The diets were formulated to have the same starch content between them (300 g/kg of

starch) and to meet the nutritional requirements for the maintenance of adult dogs, according to the European Pet Food Industry Federation (FEDIAF, 2019). The ingredients were weighed, mixed, and ground using a hammer mill fitted with a 1.0 mm screen. Diets were extruded in a twin-screw extruder (Ferraz; E-92; Ribeirão Preto, SP, Brazil) with a processing capacity of 250 kg/h. After drying, diets were coated with poultry fat and poultry hydrolysate. The ingredients of the evaluated diets are shown in Table 1 and the analyzed chemical composition of the starch sources and diets are presented in Table 2.

2.3 Digestibility test

The digestibility assay followed the total fecal collection method recommended by the Association of American Feed Control Officials (AAFCO, 2016). The diets were offered during a 15-day adaptation period followed by 5 days of total fecal collection.

The animals were fed twice a day (8:30 am and 6:30 pm) in sufficient amounts to supply the metabolizable energy (ME) requirement of adult dogs in maintenance as recommended by the National Research Council (NRC, 2006): ME (MJ/day) = 0.40 – 0.54 × body weight (kg)0.75.

Feces were collected and weighed twice a day, stored in individual plastic bags previously identified by animal and period, and stored in a freezer (-20 °C). At the end of each collection period, the feces were thawed at room temperature and homogenized separately, forming a composite sample from each animal, and dried in a forced ventilation oven (320-SE, Fanem, São Paulo, Brazil) at 55 °C for 72 hours or until reaching a constant weight. After drying, the feces and the experimental diets were ground using an 1 mm-sieve (Arthur H. Thomas Co., Philadelphia, PA, USA)

and analyzed at 105 ºC for dry matter (DM105) for 12 hours, ether extract in acid hydrolysis (EEAH, method 954.02), ash (method 942.05), calcium (method 927.02), phosphorus (method 984.27), nitrogen (N, method 954.01) and then CP was calculated as N × 6.25, according to the Association of Official Analytical Chemists (AOAC, 1995). The total dietary fiber (TDF), insoluble fiber (IF), and SF of the diets were analyzed according to Prosky et al. (1988). Gross energy (GE) was determined by an isoperibol calorimeter (Parr Instrument Co., model 1261, Moline, IL, USA).

2.4 Fecal characteristics and intestinal fermentative metabolites

Fecal characteristics were evaluated during the collection period by total DM (DMf) content, fecal output, fecal consistency by score, ammonia, and pH. Fecal pH and ammonia were analyzed in feces collected up to 15 min after spontaneous defecation on day 15 of the study.

Fecal score was always evaluated by the same researcher, assigning points from 1 to 5, being: 1 = feces are soft and have no defined shape; 2 = feces are soft and poorly formed; 3 = feces are soft, formed, and moist; 4 = feces are well formed and consistent; 5 = feces are well formed, hard and dry, according to Carciofi et al. (2009). Fecal pH was measured using a digital pH-meter (331, Politeste Instrumentos de Teste Ltda, São Paulo, SP, Brazil) using 3.0 g of fresh feces diluted in 30 mL of distilled water. Fecal ammonia concentration was determined according to Brito et al. (2010).

Stool samples for analysis of intestinal fermentative metabolites were collected up to 15 min after spontaneous defecation on day 15 of the analyses. For SCFA (acetate, butyrate, valerate, and propionate) and branched-chain fatty acids (BCFA, isovalerate and isobutyrate) determination, 10 g of stool sample was weighed and

mixed with 30 mL of 16% formic acid. This mixture was homogenized and stored in a refrigerator at 4 °C for a period of 3 to 5 days. After this period, the solutions were centrifuged at 2,500 g (2K15, Sigma, Osterode am Hans, NI, Germany) for 15 min. At the end of centrifugation, the supernatant was separated and subjected to further centrifugation. Each sample underwent three centrifugations, and at the end of the last one, part of the supernatant was transferred to a properly labeled eppendorf tube for subsequent freezing at -14 °C. Afterwards, the samples were thawed and underwent new centrifugation at 18,000 g for 15 min (Rotanta 460 Robotic, Hettich, Tuttlingen, BW, German). Both centrifugations were conducted under refrigeration (approximately 5 oC). Fecal SCFA and BCFA were analyzed by gas chromatography (Shimadzu, model GC-2014, Kyoto, Honshu, Japan), using a glass column (Agilent Technologies, HP INNO wax - 19,091N, Santa Clara, CA, United States of America) 30 m long and 0.32 mm wide. The injected volume of the supernatant was set to 1 µL. Nitrogen was used as the carrier gas with a flow rate of 3.18 mL/min. The working temperatures were 200 °C at the injector, 240 °C at the column (at a speed of 20 °C/min), and 250 °C at the flame ionization detector.

2.5 Fecal microbiota

Stool samples for the fecal microbiota analysis were collected on day 15 of the study. For fecal microbiota evaluation, approximately 2 g of sample were taken from the interior of the freshly collected stool, placed in a sterile eppendorf tube and stored in a -80 ºC freezer until the moment of analysis.

For DNA extraction, the commercial kit "ZR Fecal DNA MiniPrep®" from Zymo Research (Zymo Research, Irvine, CA, USA) was used, following the manufacturer-recommended protocol. The extracted DNA was quantified by

spectrophotometry at 260 nm using the NanoDrop® 2000 spectrophotometer (Thermo Scientific, Wilmington, VA, USA). To evaluate the extracted DNA integrity, all samples were run by electrophoresis in 1% agarose gel, stained with a 1% ethidium bromide solution and visualized with ultraviolet light in a transilluminator.

A 460-base segment of the V4 hypervariable region of the 16S rRNA gene was amplified using the universal primers 515F and 806R and the following PCR conditions: 94 °C for 3 min; 18 cycles of 94 °C for 45 sec, 50 °C for 30 sec, and 68 °C for 60 sec; followed by 72 °C for 10 min. From these amplifications, a metagenomic library was built using the commercial Nextera DNA Library Preparation Kit from Illumina® (San Diego, CA, USA). The amplifications were pooled and subsequently sequenced in the Illumina® "MiSeq" sequencer (Degnan and Ochman, 2012). The reads obtained on the sequencer were analyzed on the Quantitative Insights into Microbial Ecology (QIIMEII) platform (Caporaso et al., 2010; Caporaso et al., 2011), followed by a workflow of the low-quality sequence removal, filtration, chimeras removal, and taxonomic classification. To generate the classification of bacterial communities by operational taxonomic units (OTU) identification, 12,804 reads per sample were used, in order to normalize the data and not compare samples with different reading numbers. Sequences were classified into bacterial genera by recognizing the OTUs through identity (>97%) between sequences when compared against a database. The update named "SILVA 132" from the year 2018 of the ribosomal sequence database "SILVA database" (Yilmaz et al., 2014) was used to compare the sequences.

2.6 Postprandial response tests

The dogs were submitted to the determination of postprandial glycemic response on day 16 of each experimental period. Blood samples were taken before feeding (baseline sample, time 0) and 5, 10, 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480 minutes after feeding (adapted to Carciofi et al., 2008). Capillary blood samples were obtained by puncture of the face upper medial lip with a sterile needle (20 x 0.55 mm, Modesto et al., 2020). Blood glucose was measured using a portable glucometer (Accu-chek Active®, Jaguaré, SP, Brazil).

2.7 Calculations and statistical analysis

The organic matter (OM) was calculated by: 100 – Ash.

The DMf = (DM at 55 °C × DM at 105 °C)/100.

The CTTAD and ME were estimated according to AAFCO (2016), based on the equations:

CTTAD = (g nutrient intake - g nutrient excreted)/g nutrient intake

ME (MJ/kg) = {kJ/g GE intake - kJ/g fecal GE - [(g CP intake - g fecal CP) × (5.23 kJ/g)]}/g feed intake.

Each pair of dogs were fed one of the experimental diets per period (3), totaling 6 replicates/treatment at the end of the periods. The normality of data was analyzed by the Shapiro-Wilk test. When data and residues assumed normal distribution, they were analyzed using the PROC MIXED of SAS statistical package (version 8, SAS Institute Inc., Cary, NC, USA), considering the effects of diets, blocks (periods), and animals. Means were compared using Tukey's test ($P<0.05$). Data that did not show normal distribution were analyzed using the Kruskal-Wallis test at 5% probability.

To characterize the overall differences in fecal microbial communities among the groups, we performed principal coordinate analysis (PCoA) using Bray-Curtis dissimilarity distance. The effect of treatments on beta-diversity was evaluated among groups by PERMANOVA (Permutational Multivariate Analysis of Variance) with $P<0.05$ (Anderson, 2001).

The glycemic responses were plotted and then the glucose area under the curve (AUC) was calculated in the Origin 2020® software.

3. Results

3.1 Digestibility test

The CTTAD and ME of the diets are shown in Table 3. In general, brown rice, sorghum, and pea diets had the highest CTTAD of DM, OM, CP, and GE, while the sweet potato diet had the lowest CTTAD ($P<0.001$). Diets containing corn and brown rice showed higher ME, compared to the other treatments ($P<0.001$).

3.2 Fecal characteristics and intestinal fermentative metabolites

The results obtained for fecal characteristics and intestinal fermentative metabolites are shown in Table 4. The diet containing potato starch resulted in higher DMf than the other diets ($P<0.001$). The feces of the dogs fed with pulses had a similar pH. Fecal pH of the animals fed with chickpea was lower than those fed with diets based on cereals and tubers ($P<0.001$). Fecal ammonia concentration differed between sweet potato and chickpea diets ($P<0.001$), being similar among the other sources. In addition, sweet potato and chickpea resulted in lower DMf ($P<0.001$), with similar results between sweet potato and pea diets. However, the sweet potato diet

resulted in higher production of feces/day when compared to the diet containing brown rice ($P<0.001$). Fecal score did not differ among treatments ($P>0.05$).

The sweet potato and chickpea diets resulted in higher fecal concentrations of acetate ($P=0.009$), propionate ($P=0.001$), butyrate ($P=0.014$), and total SCFA ($P=0.001$), in comparison to potato and brown rice diets. The potato diet resulted in lower fecal concentrations of total BCFA in relation to sweet potato and chickpea diets ($P=0.007$).

The average peak area of phenols was lower in the feces of dogs consuming the diets with chickpea than the diets with brown rice, potato, and pea ($P<0.001$). However, the diets did not differ in indole production ($P>0.05$, Table 5).

3.3 Fecal microbiota

Among the bacterial genera identified, 16 were considered with significantly different abundance ($P<0.05$) among the seven treatments. There was an increase in the relative abundance of *Allobaculum*, *Bacteroides plebeius*, *Blautia*, *Prevotella*, and *Turicibacter* in the feces of dogs fed diets containing chickpea and pea ($P<0.05$). Also, dogs fed the diet containing sweet potato had the highest abundance of *Faecalibacterium prausnitzii*, followed by those fed diets containing chickpea and pea ($P<0.001$). The differences between the most abundant genera are shown in Table 6.

Regarding alpha-diversity, there was a difference only in the Chao1 index, with the lowest index for sweet potato-based diet and the highest for the diets containing cereals and potato. Chickpea and pea-based diets showed similar indexes to the other diets. Beta-diversity analysis (Figure 1) identified three main distinct profiles of the fecal microbiota ($P<0.05$): 1- pulses (chickpea + pea), 2- sweet potato, and 3- cereals (corn + brown rice + sorghum) + potato.

3.4 Postprandial response tests

Dogs fed diets containing corn and potato starch had higher maximum glycemia, higher peak glycemic increment, and higher initial AUC (0-30 min) than dogs fed other starch sources ($P<0.05$). The time until glycemic peak was longer ($P<0.001$) for the diets containing sorghum (120 min) and chickpea (60 min), with no difference ($P>0.05$) among the other treatments (average = 30 min). The results of the postprandial glycemic curve of dogs are presented in Table 7 and illustrated in Figure 2.

4. Discussion

The results obtained in this study showed the influence of different starch sources on the digestibility of the diets. The differences observed in diet digestibility may be due to several factors, among them: starch-protein interactions, fiber concentration of starch sources, crystallinity of starch granules, amylose:amylopectin ratio, and gelatinization degree during the extrusion process (Bazolli et al., 2015; Dhital et al., 2017; Martens et al., 2018; Lv et al., 2021).

As mentioned before, according to their botanical origin, tuber-based ingredients, such as potato starch, are generally more digestible, followed by cereals and pulses (Bajaj et al., 2018; Domingues et al., 2019; Quilliam et al., 2021). However, different from what was expected, in this study the diet containing potato starch showed similar digestibility to the other tested diets. The literature shows greater digestibility of macronutrients in dogs fed diets containing potato starch in comparison to diets containing corn (Domingues et al., 2019), which can be attributed to the higher digestible starch content and lower resistant starch (RS)

content in potato diet (Domingues et al., 2019). A possible increase in RS intake may lead to a decrease in nutrient digestibility (Beloshapka et al., 2014).

It would be expected that the nutritional composition of whole grains with more fiber would result in lower diet digestibility (Fouhse et al., 2016). However, in the dogs of our study higher CTTAD of nutritional fractions of the brown rice diet were observed, similar to studies evaluating brewers rice in comparison to corn (Twomey et al., 2003; Silva Jr et al., 2005; Carciofi et al., 2008). In pigs, several studies demonstrated that corn can be completely replaced by brown rice in diets for weanling piglets, growing pigs, and finishing pigs without any adverse effects on macronutrient CTTAD (Kim et al., 2017; Li et al., 2018; Kim et al., 2021), which, based on the results in this study, can be extrapolated to dog nutrition.

Pea and chickpea diets, which present a higher fiber concentration than brown rice, also resulted in relatively high CTTAD of nutrients, especially the pea diet. Besides the higher fiber concentration, pulses also present higher amylose (290-650 g/kg) concentration than cereals (200-350 g/kg) (Joshi et al., 2013; Thakur et al., 2019). Amylose is more susceptible to recrystallization after cooking than amylopectin and its linear structure tends to form double helical structures that are less available for enzymatic digestion (Wang et al., 2014). Therefore, ingredients with compositions containing higher proportions of amylose than amylopectin may result in lower DM and OM digestibility (Carciofi et al., 2008; Quilliam et al., 2021). Thus, higher DM and OM digestibility for the pea diet in relation to the corn diet was not expected. Studies have reported that diets with cereals such as rice, sorghum, and corn have higher digestibility than pulses (Carciofi et al., 2008; Corsato Alvarenga and Aldrich, 2018; Quilliam et al., 2021). Despite these results, the ME content of the

chickpea and pea diets was lower than the diets containing cereals, in agreement with the literature (Carciofi et al., 2008).

Although there is a high TDF concentration in diets containing starch sources from pulses—around 169.0 g/kg and 171.0 g/kg in chickpea and pea diets, respectively—the IF:SF ratio of these diets is around 6.19 - 6.44:1 (data proved by our study). This indicates a higher proportion of IF than SF, which results in less impact on the digestibility of nutrients (Burkhalter et al., 2001; Sabchuk et al., 2017; Souza et al., 2021). The IF fraction presents less interference in the enzymatic digestion in the gastrointestinal tract. On the other hand, the effect of the SF fraction on the diet digestibility was observed in our study through the lower CTTAD of DM, OM, CP, and lower ME content of the sweet potato diet. Besides the high TDF concentration (96.2 g/kg), the sweet potato diet presented a lower IF:SF ratio (3.56). The SF fraction may present rapid hydration and, as a consequence, increases digesta viscosity, which can reduce the interaction of digestive enzymes with nutrients and affect their absorption (Godoy et al., 2013). In addition, sweet potato has antinutritional factors, such as phenolic compounds, including tannins (1.50 g/kg, Régnier et al., 2011), which can inhibit enzymes and form complexes with carbohydrates and proteins, thus impairing the digestibility of CP (Eluagu and Onimawo, 2010; Kumari and Jain, 2012), and favoring fermentation in the gut (Wyatt and Bedford, 1998). Although phenolic compounds can be thermally sensitive and highly prone to degradation during the extrusion process (Zhang et al., 2018), it is possible that a small content of total tannins, phenols, and condensed tannins is detected after this process (Teixeira et al., 2021).

Although the differences observed among the digestibility of the diets, the starch sources evaluated did not cause adverse effects on fecal score, which

remained at 4, considered within the normal range for dogs (Félix et al., 2009). However, diets containing higher fiber concentrations (chickpea, pea, and sweet potato) resulted in greater fecal production (as-is) and reduction in DMf. The low-fermentable IF present in these ingredients increases the fecal volume (Wenk, 2001).

On the other hand, the fermentable SF in these ingredients increases stool moisture (Case et al., 2000; Sabchuk et al., 2017), due to their ability to bind water. In addition, SF fermentability enhances SCFA production, increasing the osmotic pressure in the gut. The higher osmotic pressure in the intestinal lumen reduces water absorption, increasing the amount of water that is excreted with the feces (Herschel et al., 1981). The same mechanism may happen due to low dietary CP digestibility, which was observed in dogs fed the sweet potato diet. The partially indigestible protein is fermented in the large intestine and produces nitrogenous compounds, increasing intestinal environment osmolarity (Nery et al., 2010).

These nitrogenous compounds produced by a lower CTTAD of CP are recognized as putrefactive compounds, such as indole and phenolic compounds, ammonia, and BCFA (Godoy et al., 2013). They can negatively affect intestinal functionality due to the potential toxicity of some of these compounds to the gut mucosa (Zentek et al., 2002; Celi et al., 2019). However, although sweet potato resulted in greater production of total BCFA and isovalerate than the potato diet, it resulted in similar concentrations of these metabolites in comparison to the other diets. In addition, the reduction in fecal pH and higher fecal concentration of SCFA in dogs fed the sweet potato and pulses diets may be indicative of positive effects on intestinal functionality (Zentek et al., 2002; Swanson et al., 2006; Pinna et al., 2017).

Among the SCFA, butyric acid has been associated with improvement of the epithelial barrier (Hamer et al., 2008; Huang et al., 2015) and reduction in pH of the

gut content (Pieper et al., 2014; Chen et al., 2015). Butyrate is an important energy source for colonocytes and promotes the stimulation of normal cell proliferation and the prevention of intestinal diseases (Hamer et al., 2008; Detweiler et al., 2019), presenting anti-inflammatory properties that improve intestinal homeostasis and mucosa immunity (Liu et al., 2018).

The canine gastrointestinal tract (GIT) hosts a complex and highly biodiverse microbial ecosystem (Mondo et al., 2020). However, in comparison to other omnivorous mammals, the canine gut microbiome is the most similar to humans (Coelho et al., 2018). The most predominant phyla reported in the gut of healthy dogs are Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, and Actinobacteria (Swason et al., 2010; Deng et al., 2015; Wernimont et al., 2020; Corsato Alvarenga et al., 2021). The results found in this study showed a higher abundance of genera from the phylum Firmicutes (*Allobaculum*, *Blautia*, *Catenibacterium*, *Dorea*, *Eubacterium*, *Faecalibacterium*, *Megamonas*, and *Turicibacter*), followed by Bacteroidota (*Bacteroides plebeius*, *Prevotella*, and *Prevotella copri*) in feces of dogs fed with sweet potato and pulses diets.

A greater abundance of the genus *Allobaculum* in the feces of chickpea-fed dogs may indicate positive effects on intestinal functionality, as this genus is reported to be reduced in dogs with gastrointestinal diseases (Minamoto et al., 2015). Chickpea and pea-fed dogs had a higher abundance of *Bacteroides plebeius* and *Megamonas* in their feces. *B. plebeius* and *Megamonas* are considered producers of propionate in human (Rios-Covian et al., 2017) and dog (Wernimont et al., 2020) intestines. *Megamonas* is also recognized to produce acetic acid (Wernimont et al., 2020). Both propionic and acetic acids were observed in higher concentrations in the feces of dogs fed these diets.

An increase in the fecal abundance of *Catenibacterium* was demonstrated in vitro (Yang et al., 2013) and in vivo studies in dogs (Jarett et al., 2019) and cats (Hooda et al., 2013) in the fermentation of dietary fibers. *Catenibacterium* is recognized as a producer of SCFA (Kageyama and Benno, 2000) and its abundance is lower in dogs with advanced heart failure and inflammatory bowel disease (AlShawaqfeh et al., 2017; Luedde et al., 2017; Pilla and Suchodolski, 2020; Seo et al., 2020), suggesting that the overall increase of *Catenibacterium* abundance in feces of dogs consuming pulse-based diets may be beneficial.

Furthermore, the dietary inclusion of pulses also resulted in a higher abundance of *Prevotella copri* and *Turicibacter*. *Prevotella* is a well-known fiber fermenter and SCFA producer that has been associated with gastrointestinal health (Minamoto et al., 2015; Schmidt et al., 2018; Pilla and Suchodolski, 2020). *P. copri* is known to be a succinate-producing species in humans that improves glucose homeostasis via intestinal gluconeogenesis by a yet-to-be-defined mechanism (Kovatcheva-Datchary et al., 2015; De Vadder et al., 2016). The beneficial effects of *Turicibacter spp*. on GIT functionality seem to be primarily due to its role in the serotonin (5-hydroxytryptamine) regulation levels in the gut (Félix et al., 2022). Consequently, it may influence gut motility and the host's overall health.

In addition, the increase in the relative abundance of *Blautia producta* and *Eubacterium biforme* in feces of dogs fed pulses and sweet potato diets may be also related to the improvement in gut functionality (Suchodolski et al., 2012; Alshawaqfeh et al., 2017; Pujo et al., 2021; Félix et al., 2022). *B. producta* abundance is low in dogs with acute hemorrhagic diarrhea, chronic enteropathy, and inflammatory bowel disease (Suchodolski et al., 2012). *E. biforme*, on the other hand, produces the long-

chain fatty acid 3-hydroxyoctadecaenoic acid that has anti-inflammatory effects in colitis (Pujo et al., 2021).

A higher *Faecalibacterium prausnitzii* abundance observed in the feces of dogs fed the sweet potato diet is in agreement with the high fecal concentration of butyrate in these animals. *F. prausnitzii* is known as the main butyrate producer in the gut of dogs (Duncan et al., 2002; Duncan et al., 2004), being a positive indicator of improvement of intestinal functionality (Souza et al., 2021; Félix et al., 2022). *F. prausnitzii* is abundant in the gut microbiota of healthy adults and changes in its abundance have been linked to dysbiosis in several human disorders (Miquel et al., 2013). This species is the only known of the genus *Faecalibacterium* which demonstrates anti-inflammatory activity in the colon mucosa (Ploger et al., 2012) due to the production of butyrate (Flint et al., 2012; Xu et al., 2021) and by secreting metabolites that block the transcription factor NF-κB activation, and the consequent production of the pro-inflammatory cytokine, interleukin-8 (Sokol et al., 2008).

Besides the modulation of specific bacterial genera, it is important to evaluate the richness and diversity of microorganisms (Ziese and Suchodolski, 2021). Although sweet potato resulted in a lower richness of the microbiota, as measured by the Chao1 index, the diversity did not differ (Shannon index). While the Chao1 index mainly calculates the number of taxa, given lower importance to their evenness, the Shannon index measures the quantity and evenness of their distribution in the microbiota.

Regarding postprandial glycemic responses, studies in adult dogs observed that the amount, type, and botanical origin of the starch consumed interfere with the glycemic responses (Nguyen et al., 1998; Carciofi et al., 2008; Feitosa et al., 2016; Teixeira et al., 2018). Common starch-containing pet food ingredients such as corn,

brewers rice, and potato have been shown in humans to have higher glycemic index values compared with pulses, such as pea and lentil (Foster-Powell et al., 2002; Fernandes et al., 2005). Studies with dogs show that sorghum and pulses-based diets result in lower postprandial glucose increments (Carciofi et al., 2008; Adolphe et al., 2015; Quilliam et al., 2021), which is consistent with the results observed in our study. These results may be explained by the higher fraction of slowly digestible starch (Carciofi et al., 2008) and higher fiber concentration (NRC, 2006) of these ingredients, which can reduce the gastric emptying rate, indirectly interfering with the starch hydrolysis and, consequently, the glucose absorption rate.

The lower postprandial glycemic response is beneficial mainly in cases of changes in glucose metabolism, such as obesity and diabetes mellitus. For healthy dogs, the use of starch sources that keep glycemia more constant, without generating excessive peaks, can also contribute to weight maintenance and prolonged satiety. However, these factors still need to be further investigated.

5. Conclusion

Cereals, potato starch, and pulse-based diets have similar digestibility. Diets containing sweet potato have lower digestibility and, similar to pulses, result in greater fecal volume and moisture. Besides, fermentation of the soluble fiber present in pulses and sweet potato results in lower fecal pH, higher fecal SCFA, and beneficial fecal microbiota selection, such as *Allobaculum*, *Bacteroides plebeius*, *Blautia*, and *Turicibacter* in diets containing chickpea and pea, and *Faecalibacterium* and *Blautia* in the sweet potato diet. Corn and potato are starch sources that are rapidly assimilated, resulting in a higher postprandial glycemic increment. Sorghum,

pulses, sweet potato, and brown rice result in a lower postprandial glycemic increment, and their use in diets for glycemic control for dogs may be considered.

Credit authorship contribution statement

Gislaine Cristina Bill Kaelle: Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Taís Silvino Bastos:** Investigation. **Renata Bacila Morais dos Santos de Souza:** Investigation. **Eduarda Lorena Fernandes:** Investigation. **Simone Gisele de Oliveira:** Supervision, Writing - review & editing. **Ananda Portella Félix:** Conceptualization, Data Curation, Project administration, Writing - Review & Editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Table 1. Ingredients of the experimental diets.

Ingredients (g/kg)	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Corn	433.90	-	-	-	-	-	-
Brown rice	-	447.45	-	-	-	-	-
Sorghum	-	-	450.13	-	-	-	-
Potato	-	-	-	330.13	-	-	-
Sweet Potato	-	-	-	-	366.28	-	-
Chickpea	-	-	-	-	-	643.84	-
Pea	-	-	-	-	-	-	565.63
Poultry offal meal	267.89	264.36	255.63	293.17	293.13	262.10	227.20
Isolated swine protein	147.43	138.73	146.97	170.68	171.84	45.92	81.26
Celulose	55.00	41.43	45.30	66.81	46.20	9.12	26.05
Poultry fat	49.71	61.91	58.94	93.41	74.75	20.00	53.33
Poultry hydrolizate	20.00	20.00	20.00	20.00	20.00	5.00	20.00
Potassium chloride	7.05	7.09	5.00	6.78	8.78	3.00	7.50
Sodium chloride	5.00	5.00	4.02	5.00	5.00	2.00	5.00
Mineral-vitamin supplement[1]	3.00	3.00	3.00	3.00	3.00	2.00	3.00
Adsorvent	2.00	2.00	2.00	2.00	3.00	2.00	2.00
Choline chloride	2.00	2.00	2.00	2.00	2.00	1.00	2.00
Calcium propionate	2.00	2.00	2.00	2.00	2.00	0.80	2.00
Taurine	1.00	1.00	1.00	1.00	1.00	0.15	1.00
DL-Methionine	0.80	0.80	0.80	0.80	0.80	0.08	0.80

BHT	0.15	0.15	0.15	0.15	0.15	0.00	0.15
BHA	0.08	0.08	0.08	0.08	0.08	0.00	0.08
Citric acid	3.00	3.00	3.00	3.00	3.00	3.00	3.00

[1]enrichment per kg of product^{-1}: vitamin A (retinol), 20,000 IU; vitamin D3, 2,000 IU; vitamin E (alpha-tocopherol α), 48 mg; vitamin K3, 48 mg; vitamin B1, 4 mg; vitamin B2, 32 mg; pantothenic acid, 16 mg; niacin, 56 mg; choline, 800 mg; Zn as zinc oxide, 150 mg; Fe as ferrous sulphate, 100 mg; Cu as copper sulphate, 15 mg; I as potassium iodide, 1.5 mg; Mn as manganese oxide, 30 mg; Se as sodium selenite, 0.2 mg; antioxidant, 240 mg.

Table 2. Analyzed chemical composition (g/kg, dry matter basis) of starch sources and experimental diets.

Item	Starch sources						
	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Dry matter	887.8	887.6	877.0	857.9	915.1	912.6	881.5
Ash	25.1	14.5	23.0	3.0	17.9	29.5	28.9
Crude protein	74.5	137.7	107.3	3.7	27.8	240.3	255.4
EEAH	38.1	41.1	20.6	5.3	11.0	71.3	23.9
Gross energy, kcal/kg	4829.2	4429.9	4321.8	4217.4	4126.4	4860.7	4545.6
Total starch	700.6	767.5	753.7	960.4	786.9	452.7	476.2
Total dietary fiber	52.2	68.9	53.3	28.3	111.23	257.0	237.0
Insoluble fiber (IF)	46.8	61.2	46.9	27.6	68.3	226.9	208.6
Soluble fiber (SF)	5.5	7.7	6.4	2.1	44.0	30.1	28.4
IF:SF	8.5	7.9	7.3	13.1	1.5	7.5	7.3
Ca	0.3	0.3	0.4	0.3	1.1	1.5	0.5
P	2.4	2.9	3.9	0.7	0.6	3.8	3.3
	Diets						
	Corn	Brown rice	Sorghum	Potato	Sweet Potato	Chickpea	Pea
Dry matter	963.3	927.6	933.5	951.1	970.3	953.6	941.1
Ash	79.7	87.1	81.3	77.0	81.7	89.8	84.7
Crude protein	328.4	320.8	338.3	330.5	350.1	354.9	345.0
EEAH	128.7	123.1	126.3	124.8	123.8	138.5	123.0
Gross energy, kcal/kg	4784.4	4861.6	4863.4	4959.1	4916.9	4987.1	4882.6

Total starch	304.1	373.7	353.3	350.3	288.3	291.5	269.4
Total dietary fiber	81.7	77.3	76.0	94.3	96.2	169.1	170.6
Insoluble fiber (IF)	76.4	70.8	69.1	89.1	75.1	146.4	146.9
Soluble fiber (SF)	5.3	6.5	6.9	5.2	21.1	22.7	23.7
IF:SF	14.3	10.9	10.0	17.0	3.56	6.44	6.19
Ca	15.0	14.5	13.2	13.5	15.0	15.0	12.3
P	7.9	7.9	7.8	6.9	7.7	7.7	8.3

Table 3. Coefficients of total tract apparent digestibility (CTTAD, g/kg of dry matter) means and metabolizable energy (ME, MJ/kg of dry matter) of diets containing different starch sources.

Item	Corn	Brown rice	Sorghum	Potato	Sweet potato	Chickpea	Pea	SEM	p-value
CTTAD (g/kg)									
DM	0.796^{bc}	0.847^{a}	0.837^{ab}	0.804^{abc}	0.777^{c}	0.818^{abc}	0.844^{a}	0.005	<0.001
OM	0.843^{bc}	0.891^{a}	0.877^{ab}	0.847^{bc}	0.824^{c}	0.859^{ab}	0.883^{a}	0.004	<0.001
CP	0.863^{ab}	0.890^{a}	0.892^{a}	0.880^{a}	0.825^{c}	0.834^{bc}	0.875^{a}	0.005	<0.001
EEA	0.920	0.929	0.915	0.923	0.908	0.906	0.922	0.003	0.0591
GE	0.857^{bcd}	0.897^{a}	0.877^{abc}	0.846^{cd}	0.831^{d}	0.860^{bcd}	0.883^{ab}	0.004	<0.001
ME (MJ/kg)	18.727^{a}	18.788^{a}	17.641^{bc}	17.251^{c}	17.467^{bc}	17.375^{c}	17.913^{b}	0.099	<0.001

Abbreviations = SEM: standard error the mean; DM: dry matter; OM: organic matter; CP: crude protein; EEA: ether extract acid; GE: gross energy; a, b, c, d, e distinct letters indicate difference by Tukey's test ($P<0.05$)

Table 4. Fecal characteristics and intestinal fermentation products means of dogs fed with diets containing different starch sources.

Item	Corn	Brown rice	Sorghum	Potato	Sweet potato	Chickpea	Pea	SEM[1]	p-value
Fecal characteristics									
Dry matter, g/kg	421.7^{b}	428.7^{b}	409.1^{b}	463.9^{a}	324.0^{cd}	293.9^{d}	339.2^{c}	9.420	<0.001
pH	7.13^{bc}	7.64^{a}	7.41^{ab}	7.47^{ab}	7.00^{cd}	6.45^{e}	6.67^{de}	0.070	<0.001
Fecal output[2]	96.2^{abc}	79.5^{c}	92.0^{abc}	87.6^{bc}	139.2^{a}	124.8^{ab}	104.0^{abc}	4.110	<0.001
Ammonia, g/kg	0.092^{ab}	0.091^{ab}	0.110^{ab}	0.102^{ab}	0.149^{a}	0.055^{b}	0.089^{ab}	0.001	<0.001
Score[3]	4	4	4	4	4	4	4	-	-
Short-chain fatty acids (SCFA, µmol/g)									
Acetate	72.883^{ab}	59.952^{b}	55.742^{ab}	46.442^{b}	89.635^{a}	82.928^{ab}	70.242^{ab}	4.008	0.010
Propionate	25.598^{ab}	18.793^{b}	23.307^{ab}	18.213^{b}	30.765^{ab}	35.552^{a}	29.925^{ab}	1.580	0.001
Butyrate	12.550^{ab}	9.505^{b}	10.227^{ab}	7.702^{b}	17.538^{a}	14.867^{a}	13.163^{ab}	0.919	0.014
Valerate	4.652	5.538	7.953	5.955	10.003	9.352	7.570	0.655	0.079
Total SCFA	111.04^{ab}	88.25^{bc}	89.27^{bc}	68.19^{c}	142.19^{a}	133.35^{a}	113.33^{ab}	6.118	0.001
Branched-chain fatty acids (BCFA, µmol/g)									
Isovalerate	5.615^{ab}	4.497^{bc}	5.522^{ab}	3.977^{c}	5.900^{a}	4.810^{abc}	4.453^{bc}	0.151	0.001
Isobutyrate	4.148^{ab}	4.088^{b}	3.667^{b}	3.647^{b}	4.550^{ab}	6.038^{a}	5.225^{ab}	0.199	0.003
Total BCFA	9.76^{ab}	8.58^{ab}	9.19^{ab}	7.62^{b}	10.45^{a}	10.85^{a}	9.68^{ab}	0.252	0.007

[1]SEM: standard error of the mean; [2]Fecal output = g feces produced as-is/animal/day; [3]Median fecal consistency score analyzed by Kruskal Wallis ($P > 0.05$);

Table 5. Median of peak area of the most abundant volatile organic compounds present in the feces of dogs fed different starch sources.

Item	Corn	Brown rice	Sorghum	Potato	Sweet potato	Chickpea	Pea	p-value*
Phenol	1.7426[ab]	1.9123[a]	1.7744[ab]	1.7989[a]	1.7227[ab]	1.5751[b]	1.8212[a]	<0.001
Indole	0.3033	0.8917	0.4700	0.9167	0	0	0	0.3619

[a,b]Distinct letters indicate difference by Kruskal-Wallis (P>0.05)

Table 6. Relative abundance (%) of the most abundant bacterial genera in feces of dogs fed different starch sources.

Genera	Corn	Brown rice	Sorghum	Potato	Sweet potato	Chickpea	Pea	SEM[1]	p-value
Allobaculum	0.52^{b}	0.68^{b}	0.64^{b}	0.45^{b}	1.02^{a}	1.25^{a}	0.84^{ab}	0.066	0.005
Bacteroides	20.97^{a}	20.57^{a}	15.94^{ab}	21.47^{a}	7.79^{b}	9.45^{b}	6.55^{b}	1.354	<0.001
Bacteroides plebeius	0.52^{b}	0.39^{b}	0.45^{b}	0.49^{b}	0.54^{b}	3.34^{a}	2.54^{a}	0.271	<0.001
Blautia producta	1.06^{b}	0.85^{b}	1.34^{b}	1.32^{b}	2.21^{a}	1.44^{ab}	2.24^{a}	0.259	0.008
Catenibacterium	0.13^{b}	0.06^{c}	0.06^{c}	0.02^{c}	0.07^{c}	0.37^{a}	0.45^{a}	0.032	<0.001
CF231	1.58^{ab}	2.46^{a}	0.81^{b}	1.21^{b}	0.01^{c}	0.16^{c}	0.05^{c}	0.229	0.021
Dorea	1.01^{a}	1.26^{a}	1.36^{a}	1.42^{a}	1.85^{a}	0.42^{b}	0.42^{b}	0.109	<0.001
Eubacterium biforme	0.63^{b}	0.29^{c}	0.76^{b}	0.28^{c}	1.89^{a}	1.29^{a}	1.80^{a}	0.138	<0.001
Faecalibacterium	1.84^{c}	1.60^{c}	1.73^{c}	1.98^{c}	16.72^{a}	3.14^{b}	2.72^{b}	0.837	<0.001
Fusobacterium	22.55^{b}	30.94^{a}	22.43^{b}	27.92^{ab}	25.38^{ab}	7.35^{c}	6.62^{c}	1.505	<0.001
Megamonas	0.27^{b}	0.18^{b}	0.14^{b}	0.37^{b}	0.29^{b}	16.25^{a}	12.37^{a}	1.062	<0.001
Peptococcus	0.57^{bc}	0.49^{c}	1.43^{a}	0.48^{c}	1.13^{a}	0.29^{c}	0.41^{c}	0.079	<0.001
Prevotella	5.04^{a}	5.13^{a}	6.14^{a}	7.29^{a}	0.24^{b}	5.63^{a}	4.73^{a}	0.391	<0.001
Prevotella copri	6.39^{b}	3.94^{b}	1.25^{bc}	1.33^{bc}	0.10^{c}	20.59^{a}	24.93^{a}	1.52	<0.001
Sutterella	3.31^{b}	5.90^{a}	5.06^{a}	5.67^{a}	3.01^{b}	1.54^{c}	2.93^{b}	0.279	<0.001
Turicibacter	1.73^{b}	0.40^{c}	0.84^{c}	0.32^{c}	0.62^{c}	3.21^{a}	2.54^{a}	0.21	<0.001

[1]SEM = standard error the mean; a, b, c, d, e distinct letters indicate difference by Tukey's test (P<0.05)

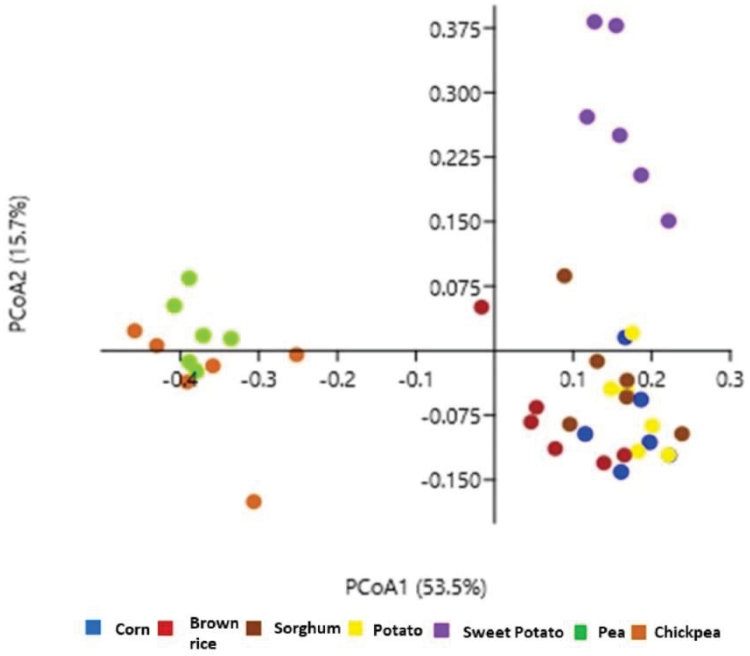

Figure 1. Principal coordinate analysis (PCoA) of bacterial communities in stool samples from dogs fed different starch sources by the Bray-Curtis dissimilarity. The PERMANOVA test identified differences ($P<0.05$) among three groups: 1- pulses (chickpea + pea), 2 - sweet potato, and 3- cereals (corn + brown rice + sorghum) + potato.

Table 7. Postprandial glycemic response means in dogs fed diets containing different starch sources.

Item	Corn	Brown rice	Sorghum	Potato	Sweet potato	Chickpea	Pea	SEM[1]	p-value
Area under the glucose curve (mg/dL/min)									
Total (0-480 min)	35.748^{b}	35.583^{bc}	36.057^{ab}	36.656^{a}	34.844^{c}	36.541^{a}	34.857^{c}	325.6	0.002
Initial (0-30 min)	2.388^{ab}	2.340^{bc}	2.304^{c}	2.472^{a}	2.235^{c}	2.292^{c}	2.262^{c}	36.3	0.001
Final (30-480 min)	33.360^{b}	33.243^{bc}	33.753^{ab}	34.184^{a}	32.609^{c}	34.249^{a}	32.595^{c}	303.1	0.002
Glycemia (mg/dL)									
Mean concentration	75.4^{ab}	74.7^{b}	75.1^{b}	77.0^{a}	72.8^{c}	76.0^{ab}	73.0^{c}	0.67	0.001
Maximum	86.8^{a}	79.6^{b}	79.0^{b}	87.8^{a}	79.6^{b}	82.2^{b}	78.0^{b}	1.76	0.001
Peak incremental	15.2^{a}	8.3^{b}	9.6^{b}	19.0^{a}	10.6^{b}	11.1^{b}	9.4^{b}	1.72	<0.001
Time to peak (min)	30^{c}	30^{c}	120^{a}	30^{c}	30^{c}	60^{b}	30^{c}	14.4	<0.001

[1]SEM = standard error the mean; a, b, c, d, e distinct letters indicate difference by Tukey's test (P<0.05)

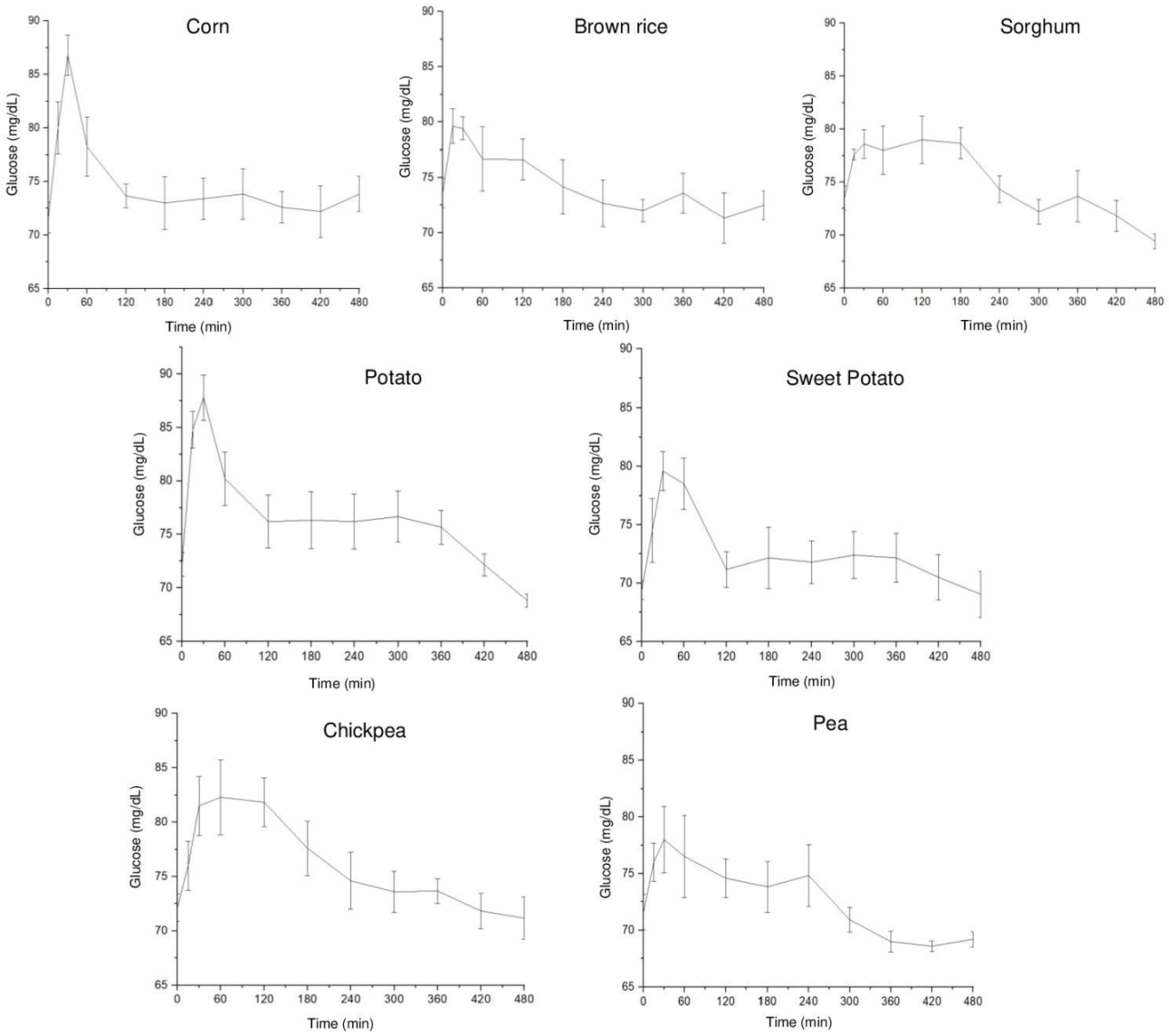

Figure 2. Postprandial glycemic curves of dogs fed diets containing different starch sources.

CHAPTER IV - HIGH-PROTEIN DRIED DISTILLERS GRAINS IN DOG DIETS: DIET DIGESTIBILITY AND PALATABILITY, INTESTINAL FERMENTATION PRODUCTS, AND FECAL MICROBIOTA

This chapter is written in accordance with the guidelines for authors in Journal of Animal Science (JAS).

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High-protein dried distillers grains in dog diets: Diet digestibility and palatability, intestinal fermentation products, and fecal microbiota[1]

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Lay summary

Considering the constant search for novel ingredients in animal nutrition and the increasing use of corn to produce ethanol, dried distillers grains with (DDGS) or without (DDG) solubles can potentially be used in dog food. Previous studies show that DDGS and DDG can contribute mainly with protein and fiber to the diets and that their fibrous fraction can potentially be fermented by the gut microbiota. However, DDGS and DDG may present variable digestibility in dogs. Besides, we did not find studies evaluating the nutritional effects of high-protein DDG (HPDDG) in dogs. This study evaluated the effects of HPDDG on diet digestibility and palatability and on variables related to the intestinal functionality of adult dogs. Our results demonstrated that HPDDG can be used in extruded diets for dogs due to its high digestibility and palatability. Besides, the HPDDG evaluated may result in a modulation of the gut microbiota, favoring bacteria considered beneficial for gut health.

Teaser text

This study identified a high digestibility of nutrients of high-protein dried distillers grains (HPDDG) in dogs. Additionally, the dietary inclusion of HPDDG enhanced diet palatability and modulated the fecal microbiota of dogs, favoring bacteria considered beneficial for gut health.

Abstract

This study aimed to evaluate the effects of high-protein dried distillers grains (HPDDG) on palatability and metabolizable energy (ME) of the diet, apparent total tract digestibility (ATTD) of nutrients and energy, intestinal fermentation products, and fecal microbiota in dogs. Four diets containing 0, 70, 140, and 210 g/kg of

HPDDG were manufactured. To evaluate the ME and the ATTD of macronutrients of HPDDG itself, an additional test diet was manufactured containing 70% of the control diet formula (0 g/kg) and 300 g/kg of HPDDG. Fifteen adult Beagle dogs were distributed in a randomized block design, with two periods of 15 days each (n=6). The HPDDG digestibility was obtained using the Matterson substitution method. For the palatability test, 16 adult dogs were used, comparing the diets: 0 vs. 70 g/kg of HPDDG and 0 vs. 210 g/kg of HPDDG. The ATTD of HPDDG were: dry matter = 85.5%, crude protein = 91.2%, and acid hydrolyzed ether extract = 84.6% and the ME content was 5041.8 kcal/kg. The ATTD of macronutrients and ME of the diets and the fecal dry matter, score, pH, and ammonia of the dogs did not differ among treatments (P>0.05). There was a linear increase in the fecal concentrations of valeric acid with the inclusion of HPDDG in the diet (P<0.05). *Streptococcus* and *Megamonas* genera reduced linearly (P<0.05), and *Blautia*, *Lachnospira*, *Clostridiales*, and *Prevotella* genera showed a quadratic response to the inclusion of HPDDG in the diet (P<0.05). Alpha-diversity results showed an increase (P<0.05) in the number of OTUs and Shannon index and a trend (P=0.065) for a linear increase in the Chao-1 index with the dietary inclusion of HPDDG. Dogs preferred the 210 g/kg diet over the 0 g/kg HPDDG diet (P<0.05). These results demonstrate that the HPDDG evaluated does not affect the utilization of nutrients in the diet, but it may modulate the fecal microbiome of dogs. In addition, HPDDG may contribute to diet palatability for dogs.

Key words: Co-product, ethanol, food preference, microbial diversity, total dietary fiber.

List of Abbreviations

AAFCO, Association of American Feed Control Officials

ADF, Acid detergent fiber

AOAC, Association of Official Analytical Chemists

ATTD, Apparent total tract digestibility

BCFA, Branched-chain fatty acids

BCS, Body condition score

CP, Crude protein

DDG, Dried distillers grains

DDGS, Dried distillers grains with solubles

DM, Dry matter

DMf, Fecal dry matter

EEAH, Ether extract in acid hydrolysis

FEDIAF, European Pet Food Industry Federation

GE, Gross energy

HPDDG, High-protein dried distillers grains

L, linear

ME, Metabolizable energy

NDF, Neutral detergent fiber

NRC, National Research Council

OM, Organic matter

OTU, operational taxonomic units

Q, quadratic

SCFA, Short-chain fatty acids

SEM, standard error of the mean

INTRODUCTION

Due to the high concentration of crude protein (CP, 25-43%), lipids (10 to 13%), vitamins, and minerals (Spiehs et al., 2002; Salim et al., 2010), dried distillers grains with (DDGS) or without (DDG) solubles, obtained after the fermentation of grains (such as corn) to produce ethanol, are promising substitutes for conventional protein sources for pet food. DDGS and DDG may also be good sources of essential amino acids, such as lysine (0.9-1.2 g/100g), methionine (0.6-0.82 g/100g), and tryptophan (0.2 g/100g, de Godoy et al., 2009; Rho et al., 2017). Besides corn protein, DDGS and DDG also contain residual yeast protein, which is used during the fermentation process, contributing to their amino acid concentrations and profile (Belyea et al., 2004).

Additionally, DDGS and DDG present a fiber fraction (5.4–10.4% of crude fiber and 30-55% of total dietary fiber), which along with the yeast wall, may have prebiotic action, serving as a substrate for microbial fermentation in the gut (Silva et al., 2016; Iram et al., 2020). Consequently, there may be positive effects on intestinal functionality through the modulation of gut microbiota and the production of important metabolites for intestinal homeostasis, such as short-chain fatty acids (SCFA) (Tramontano et al., 2018). Furthermore, the residual yeast in DDGS may positively influence diet palatability (Martins et al., 2014; Kaelle et al., 2022).

Some studies evaluated the effects of DDGS on diet digestibility and intestinal metabolites in different species such as broilers (Fries-Craft and Bobeck, 2019), laying hens (Trupia et al., 2016), swine (Curry et al., 2019; Cristobal et al., 2020; Rodriguez et al., 2020), and dogs (Silva et al., 2016; Risolia et al., 2019). However, no studies evaluating the effects of DDGS and DDG on the intestinal microbiota of dogs were found. In addition, all studies found in dogs evaluated DDGS

with protein contents ranging from 20 to 30%, being this the first study to evaluate a high-protein DDG (HPDDG, above 40% CP) as an ingredient in dog diets.

Therefore, we hypothesized that increasing dietary inclusion of HPDDG promotes beneficial gut health effects and does not negatively affect apparent total tract digestibility. In this context, we aimed to assess the impact of increasing dietary concentrations of HPDDG on diet digestibility and palatability, fecal characteristics, intestinal fermentation products, and fecal microbiota in dogs. Additionally, we aimed to evaluate the digestibility of the HPDDG itself in dogs.

MATERIAL AND METHODS

All animal care and experimental procedures were approved by the Animal Use Ethics Committee of the Agrarian Sciences sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol n. 001/2021. Experiments I and II presented identical diets, facilities, and health conditions of the animals.

Experiment I: Digestibility, fecal characteristics, intestinal fermentation products, and fecal microbiota.

Digestibility assay

Animals and housing

Seven male and eight female adult dogs (five years old), weighing 11.8 ± 1.31 kg were used. The dogs' body condition score (BCS) was assessed at the beginning and at the end of the trial, on a scale of one to nine, according to Laflamme (1997). The dogs had a mean BCS of 5.4 ± 0.2. The animals underwent clinical examination before and after the experimental period.

During most of the diet adaptation period, dogs had free access to an outdoor area of 1,137.84 m^2 under supervision for four h/d for voluntary exercise and

socialization with other experimental dogs and people. During the fecal collection period, the dogs were individually housed in covered masonry kennels (5 m long × 2 m wide) with a bed and access to water ad libitum. The facilities had side wall grates allowing visual and limited interaction with neighboring dogs. The ambient temperature ranged from 15 °C to 26 °C with a 12 h light-dark cycle (light from 600 h to 1800 h).

Ingredient and experimental diets

Diets were formulated to meet the nutritional needs of adult dogs, according to the European Pet Food Industry Federation (FEDIAF, 2019). Four diets containing increasing concentrations of HPDDG were evaluated: 0, 70, 140, and 210 g/kg, replacing soybean meal. Other macro ingredients, such as corn, poultry by-product meal, and poultry fat suffered small adjustments to keep the diets isonutritive. To evaluate the digestibility of the HPDDG itself, a diet containing 700 g/kg of the control diet formula (0 g/kg) and 300 g/kg of HPDDG, called “test diet”, was manufactured, totaling five experimental diets. The ingredients were weighed, mixed, and ground using a hammer mill fitted with a 1.0 mm screen. Diets were extruded in a single screw extruder (Ferraz; E-130; Ribeirão Preto, SP, Brazil) with a processing capacity of 250 kg/h. After drying, diets were coated with poultry fat and liquid palatant.

The HPDDG was produced by FS Fueling Sustainability (Mato Grosso, Brazil) from corn. The HPDDG processing consists primarily of the removal of the excess pericarp from corn followed by fermentation and distillation to obtain ethanol. After these phases, the insoluble solids were separated from the liquid containing soluble solids by centrifugation and forwarded to the drying process. The ingredients

and chemical composition of the diets and HPDDG are shown in Tables 1 and 2, respectively.

Digestibility and metabolizable energy determination

The digestibility assay followed the total fecal collection method described by the recommendations of the Association of American Feed Control Officials (AAFCO, 2016). The diets were provided for two 15-day periods, each with an adaptation phase (10 days) and a total feces collection phase (five days). Every three dogs were fed one of the experimental diets per period, totaling six repetitions/diet after the two periods. Water was provided ad libitum. Dogs were fed twice a day (830 h and 1630 h) in an amount sufficient to meet their metabolizable energy (ME) requirement for maintenance according to the following equation recommended by the National Research Council (NRC, 2006):

$$ME(kcal\ intake/dog\ /day) = 130\ x\ \ body\ weight\ (kg)^{0.75}$$

Chemical analyses

During the fecal collection phase of each period, feces were collected at least twice a day, weighed, identified by animal and period, and stored in a freezer at -20 °C. At the end of each collection period, the feces were thawed, homogenized separately, forming a composite sample from each animal, and dried in a forced ventilation oven at 55°C (320-SE, Fanem, São Paulo, Brazil) for 72 h. After drying, the diets and feces were ground in a 1 mm sieve in a mill (Arthur H. Thomas Co., Philadelphia, PA, USA) for chemical analysis.

In diets and feces, the following contents were determined: dry matter at 105 ºC (DM105), ether extract in acid hydrolysis (EEAH method 954.02), ash (method 942.05), crude fiber (CF, method 962.10), calcium (method 927.02), phosphorus (method 984.27), nitrogen (N, method 954.01) and then CP was calculated as N × 6.25 according to the Association of Official Analytical Chemists (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Ankom Technology and AOAC method 2001.11. Gross energy (GE) was determined by an isoperibol calorimeter (Parr Instrument Co., model 1261, Moline, IL, USA). Amino acid content of HPDDG was analyzed according to the AOAC method 994.12, except for tryptophan (method 988.15). Chemical analyzes were conducted in duplicate and repeated when the results varied more than 5%.

Fecal characteristics and intestinal fermentation products

On the 15th day of each period, fresh fecal samples (within 15 minutes of defecation) were collected and analyzed for DM content (DMf), pH, ammonia, SCFA, branched-chain fatty acids (BCFA), and sialic acid. Fecal score was also evaluated considering a 5-point scale: 1 = pasty, shapeless feces; 2 = soft and unshaped feces; 3 = soft, shaped, and moist feces; 4 = well-formed and consistent feces; 5 = well-formed, hard, and dry feces (Carciofi et al. 2009).

Fecal pH was measured with a digital pH meter (331, Politeste Instrumentos de Teste Ltda, São Paulo, SP, Brazil) using 3.0 g of fresh feces diluted in 30 mL of distilled water. Ammonia concentration**s** were determined according to Brito et al. (2010).

For SCFA and BCFA analyses, 10 g of fecal sample was mixed with 30 mL of 16% formic acid. This solution was homogenized and stored at 4 °C for three to

five days. Before the analysis, these solutions were centrifuged at 2500 gx (2K15, Sigma, Osterode am Hans, NI, Germany) for 15 min. After centrifugation, the supernatant was separated and subjected to further centrifugation. Each sample underwent three centrifugations, and at the end of the last one, part of the supernatant was transferred to an identified Eppendorf tube for subsequent freezing at -20 °C. Afterward, the samples were thawed and centrifuged again at 18000 gx for 15 mins (Rotanta 460 Robotic, Hettich, Tuttlingen, BW, Germany). Fecal SCFA and BCFA were analyzed by gas chromatography (SHIMADZU, model GC2014, Kyoto, Honshu, Japan) using a 30-mm long and 0.32-mm wide glass column (Agilent Technologies, HP INNO wax-19091 N, Santa Clara, CA, United States of America). Nitrogen was used as the carrier gas with a flow rate of 3.18 mL/min. Working temperatures were 200 °C at injection, 240 °C at the column (at a 20 °C/min rate), and 250 °C at the flame ionization detector.

Fecal microbiota

On the 15th day of each period, fresh fecal samples were collected to evaluate the fecal microbiota. For evaluation of fecal microbiota, approximately 2 g of sample was taken from the interior of the freshly collected stool, placed in a sterile eppendorf tube, and stored in a -80 ºC freezer until the moment of the analysis.

For DNA extraction from the samples, the commercial kit "ZR Fecal DNA MINIPREP" from Zymo Research was used, following the protocol recommended by the manufacturer. The extracted DNA was quantified by spectrophotometry at 260 nm using the NANODROP 2000 spectrophotometer (ThermoScientific). To evaluate the integrity of the extracted DNA, all samples were run by electrophoresis in 1%

agarose gel, stained with a 1% ethidium bromide solution, and visualized with ultraviolet light in a transilluminator.

A 460-base segment of the V4 hypervariable region of the 16S rRNA gene was amplified using the universal primers 515F and 806R and the following PCR conditions: 94°C for 3 min; 18 cycles of 94°C for 45 sec, 50°C for 30 sec, and 68°C for 60 sec; followed by 72°C for 10 min. From these amplifications, a metagenomic library was built using the commercial Nextera DNA Library Preparation Kit from ILLUMINA. The amplifications were pooled and sequenced in the ILLUMINA "MiSeq" sequencer (Degnan & Ochman, 2012). The reads obtained on the sequencer were analyzed on the QIIME (Quantitative Insights into Microbial Ecology) platform (Caporaso et al., 2010), following a workflow from removing low-quality sequences and removing chimeras and taxonomic classification. To generate the classification of bacterial communities by OTU identification, 29600 reads per sample were used to normalize the data and not compare samples with a different number of reads. Sequences were classified into bacterial genera by recognizing operational taxonomic units (OTUs) through identity (>97%) between sequences when compared against a database. The update named "SILVA 132" from the year 2018 of the ribosomal sequence database "SILVA database" (Yilmaz et al., 2014) was used to compare the sequences.

Calculation and statistics analysis

The DMf was calculated as:

$$DMf\ (\%) = \frac{DM55\ x\ DM105}{100}$$

Organic matter (OM) was calculated as 100 – Ash and fecal output was calculated as g feces as-is/animal/day.

Based on the laboratory results, the apparent total tract digestibility (ATTD) and ME of diets were calculated according to the Association of American Feed Control Official (AAFCO 2016):

$$ATTD\ (\%) = \frac{g\ nutrient\ intake - g\ nutrient\ excretion}{g\ nutrient\ intake}\ x\ 100$$

$$ME\ (kcal/g) = \frac{\{(kcal\ GE\ intake - kcal\ GE\ fecal)\ - [(g\ CP\ intake - g\ CP\ fecal\ )\ x\ 1.25\ kcal/g]\}}{g\ feed\ intake}$$

The ATTD of nutrients and ME of the HPDDG were estimated using the substitution method proposed by Matterson et al. (1965). The concentration**s** of digestible nutrients (protein and fat) and digestible energy of the HPDDG were calculated as:

$$Digestible\ nutrients\ or\ energy\ (\%\ or\ kcal) = \%\ \text{of nutrient or kcal of GE} \times \frac{\text{ATTD of nutrient or energy}}{100}$$

Data were analyzed for normality using the Shapiro-Wilk test. When data and residues assumed normal distribution, they were analyzed using the PROC MIXED of SAS statistical package (version 8, SAS Institute Inc., Cary, NC, USA), considering diets as a fixed effect, and blocks (periods) and dogs as random effects. Linear and quadratic contrasts were evaluated, with six repetitions per treatment. Significance was considered when $P < 0.05$ and a trend when $0.05 < P < 0.10$. Data that did not show normal distribution were analyzed using the Kruskal Wallis test at 5% probability, except for microbiome data, that were transformed for centered log-ratio and then analyzed as previous described using PROC MIXED.

Experiment II: Palatability

Palatability assay

Sixteen adult Beagle dogs (the same eight females and seven males that were used in experiment I, including one more male), aged five years, weighing 11.9 ± 1.35 kg, were used. The animals were kept under the same conditions previously described.

Each palatability test was conducted for two days and compared the diets 0 vs. 70 g/kg HPDDG and 0 vs. 210 g/kg HPDDG. Once a day (800 h), each dog received two bowls containing the diets to be compared. Each diet was offered 30% higher than the ME requirements recommended by the NRC (2006) for adult laboratory dogs. As soon as one of the diets was consumed entirely, both bowls were removed, and the leftovers were quantified. On the second day, the position of feeders was changed to prevent side bias. Preference was determined by calculating the intake ratio between diets and by recording the first choice (first diet eaten by the animal).

Calculation and statistics analysis

The intake ratio of each diet was calculated by the following equation:

$$Intake\ ratio = \frac{g\ ingested\ of\ diet\ A\ or\ B}{total\ g\ consumed\ (A+B)}$$

The experiment followed a completely randomized design, totaling 32 repetitions (16 dogs x 2 days). First choice data were analyzed by the chi-square test and intake ratio by the paired Student's t-test, using a significance level of 5%. Data were analyzed using the SAS statistical package (version 8, SAS Institute Inc., Cary, NC, United States of America).

RESULTS

All dogs remained healthy and consumed all diets. No adverse effects of the diets, such as vomiting, diarrhea, or coprophagia, were observed.

Experiment I: Digestibility, fecal characteristics, intestinal fermentation products, and fecal microbiota.

There was no difference (P>0.05) in food intake among diets (mean = 202 ± 13.67 g/animal/day). The inclusion of increasing concentrations of HPDDG did not change the ATTD and ME of the diets (P>0.05, Table 3). The results of ATTD of macronutrients, ME, and digestible nutrient content of the HPDDG are shown in Table 4.

For fecal characteristics, there was no change in the variables evaluated (P>0.05, Table 5). There was only a linear increase in fecal valeric acid concentration**s** in dogs fed diets containing HPDDG (P<0.05). The other SCFA and BCFA did not differ among dietary treatments (P>0.05, Table 5).

Among the 127 bacterial genera identified, six present different relative abundance among the treatments (P<0.05). There was a linear reduction in *Streptococcus* and *Megamonas* with the inclusion of HPDDG in the diet (P<0.05). The genera *Blautia*, *Lachnospira*, and *Clostridiales* showed a quadratic increase with the inclusion of HPDDG (P<0.05). In addition, the genera *Prevotella* showed a quadratic response to the inclusion of HPDDG in the diet (P<0.05).

For alpha-diversity results, there was a linear increase (P<0.05) in the number of OTUs, a quadratic effect for the Shannon index, and a trend (P=0.065) for a linear increase in the Chao-1 index with the inclusion of HPDDG in the diet.

Differences on the most abundant genera (frequency greater than 1%) and diversity data are shown in Table 6.

Experiment II: Palatability

For the palatability test, there was no difference in first choice and intake ratio when comparing 0 **vs.** 70 g/kg HPDDG diets (P>0.05). However, dogs consumed more of the 210 g/kg HPDDG compared with the 0 g/kg HPDDG diet (P<0.05). There was no difference for first choice in both palatability tests (Table 7).

DISCUSSION

The HPDDG evaluated in the present study had a high ATTD of macronutrients in adult dogs. We highlight the CP ATTD (91.2%), which was similar to the CP digestibility of corn gluten meal 60, reported in dogs (92.1%, Carciofi et al., 2008). Besides, the CP digestibility found was higher than that observed by other authors evaluating conventional DDGS in dogs (76.0%, Silva et al., 2016) and evaluating HPDDG in growing pigs (ileal digestibility = 65.5%, Rho et al., 2017).

A possible limitation to the use of high dietary concentrations of DDGS in dog foods is its fibrous content, which may reduce nutrient digestibility (Pedersen et al., 2014). This was observed in studies evaluating the use of DDGS in dog diets containing 180 g/kg DDGS with 53.5% NDF concentration (Silva et al., 2016) and 200 g/kg DDGS with 60.5% NDF concentration (Risolia et al., 2019). The authors (Silva et al., 2016; Risolia et al., 2019) found a reduction in the ATTD of nutrients and energy with the dietary increase of DDGS. In our study, however, the dietary inclusion of up to 210 g/kg HPDDG did not affect the digestibility of macronutrients, not differing of the control diet, that presented 18.48% soybean meal. This result may

be attributed at least in part due to the lower fiber concentration of HPDDG evaluated (38.0% NDF). In addition, the results obtained may also had been influenced by the raw material composition and processing methods, such as the presence of solubles in DDGS, but not in the HPDDG, fermentation process, and residual yeast concentration (Liu et al., 2011).

The fibrous fraction of HPDDG can also affect fecal characteristics such as consistency, volume, and moisture. However, no changes were observed in stool production, fecal score, and DMf with the dietary inclusion of HPDDG. Similar results on fecal consistency were described in previous studies in dogs with DDGS (Silva et al., 2016; Risolia et al., 2019). In turn, the soluble fiber fraction present in dried distillers grains co-products may have a prebiotic action, serving as a substrate for microbial fermentation (Silva et al., 2016; Iram et al. 2020). As a result, compounds such as SCFA are produced, which are essential for intestinal homeostasis and function (Tramontano et al. 2018; Félix et al., 2022). The increase in SCFA production may inhibit the development of microorganisms with pathogenic potential (Alexander et al., 2019).

The major SCFA generated in the colon are acetate and propionate, while the further metabolic activity of gut microbiota will produce butyrate (condensation of two molecules of acetyl-CoA) and valerate (condensation of an acetyl-CoA and a propionyl-CoA molecule) (Gio-Batta et al., 2020). In the present study, we observed an increase only in the fecal concentrations of valerate in dogs fed with HPDDG. However, little is known about the effects of valerate on intestinal functionality in dogs. There are some indications from studies in humans that valerate induces growth inhibition and terminal differentiation of human colon carcinoma (Hinnebusch et al., 2002). In broilers, the dietary supplementation of glycerol esters of valeric acid

positively affects the morphology of the intestinal mucosa and reduces the incidence of necrotic enteritis (Onrust et al., 2018), suggesting that valerate may beneficially support intestinal health.

One study evaluating the effects of dietary inclusion of DDGS found an increase in total SCFA concentrations in the feces of dogs (Risolia et al. 2019), indicating a possible prebiotic effect of DDGS. However, this result was not observed in the present study. The primary explanation is that the HPDDG presents lower fiber content than the conventional DDGS. The literature shows that the proportions of SCFA produced in the gut may change depending on dietary fiber sources and content (de Godoy et al., 2015; Harris et al., 2020). The second hypothesis for this result is that SCFA are rapidly absorbed in the intestinal lumen. Thus, most of them are fully absorbed before reaching the distal colon, reducing their concentrations in feces (Strompfová et al., 2017; Lima et al., 2020; Félix et al., 2022).

The fact that the fiber fraction of HPDDG was fermented by the gut microbiota of dogs may be supported by the shifts in the fecal microbiota found. Of the changes observed, the reduction in *Streptococcus* and the increase in *Blautia* relative abundance in dogs fed HPDDG may be related to the improvement in gut functionality (Suchodolski et al., 2012; Alshawaqfeh et al., 2017; Félix et al., 2022).

Furthermore, the genus *Prevotella* is a well-known fiber fermenter and SCFA producer that have been associated with gastrointestinal health (Minamoto et al., 2015; Schmidt et al., 2018; Pilla and Suchodolski, 2020). Another possible indicator of improvement in intestinal functionality is the increase in *Clostridiales* with the addition of 210 g/kg HPDDG. This genus belonging to the phylum Firmicutes has a crucial role in preventing permeable bowel syndrome, which occurs when the permeability of the intestinal barrier is altered, causing excessive inflammation

(Suchodolski et al., 2012), and it is reduced in dogs with gastrointestinal disorders (Minamoto et al., 2015).

In addition to the modulation of specific bacterial genera, one of the leading indicators of eubiosis of the gut microbiota is the greater richness and diversity of microorganisms (Ziese and Suchodolski, 2021), as observed in the present study in dogs fed HPDDG. Studies that compared the fecal microbiota of healthy dogs with animals with chronic enteropathies observed lower microbial alpha-diversity in dogs with gastrointestinal disorders (Suchodolski et al., 2012; Isaiah et al., 2017; Félix et al., 2022). A large and diverse microbial community creates an equally extensive metabolic activity that complements mammalian enzymes in the gastrointestinal tract and provides essential metabolites such as vitamins, SCFA, some polyamines, indoles, and secondary bile acids (Suchodolski, 2016; Rowland et al., 2018; Ziese and Suchodolski, 2021).

A healthy and stable gut microbiome can simultaneously act pro- and anti-inflammatory, maintaining a balance to prevent excessive inflammation and still being able to respond promptly to infections (Tizard and Jones, 2018). Furthermore, the composition of the gut microbiota also has significant effects on immune function and on the regulation of local antibody production (Pilla and Suchodolski, 2020). It also plays a vital role in the development of the gastrointestinal tract, protects the host from pathogens, and improves intestinal barrier function (Suchodolski, 2016; Rowland et al., 2018; Ziese and Suchodolski, 2021). In this way, HPDDG has components that may improve intestinal functionality in dogs, helping to prevent intestinal dysbiosis.

It is known that diet acceptance is one of the most important factors that pet food companies consider when including a new ingredient or producing a new dog

food. In the present study, the addition of 210 g/kg HPDDG improved diet palatability. Similar results were observed in dogs fed diets containing 180 g/kg DDGS (Silva et al., 2016), in weaned piglets fed diets containing different concentrations of DDGS (Gaines et al., 2007; Spencer et al., 2007), and in cats fed a diet containing 5% corn-fermented protein with 52.62% CP (Kilburn-Kappeler et al., 2022). One factor that may contribute to HPDDG and DDGS palatability is the residual yeast in the product, which contributes to the high concentration of glutamic acid of this ingredient (71.4 g/kg). Additional studies in dogs have also demonstrated an improvement in diet palatability after the dietary inclusion of yeast products (Martins et al., 2014; Lin et al., 2019; Kaelle et al., 2022).

The results of the current study indicate that HPDDG may be a promising option as a highly digestible and palatable protein source for dog diets. Moreover, HPDDG provides fermentable fiber capable of modulating the gut microbiota, favoring intestinal functionality of dogs.

CONCLUSIONS

Dietary inclusion of up to 210 g/kg HPDDG did not alter the ME of the diet and ATTD of macronutrients or the fecal consistency of dogs when compared to the control diet. In turn, the reduction of fecal concentrations of *Streptococcus* and *Megamonas* and the increase of *Blautia* and *Clostridiales* in dogs fed HPDDG indicates potential beneficial effects of this ingredient on intestinal functionality. In addition, the inclusion of 210 g/kg HPDDG improved diet palatability.

Disclosures

The authors state that there is no conflict of interest.

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Table 1. Ingredient composition of experimental diets.

Ingredient, g/kg	HPDDG, g/kg				
	0	70	140	210	300
Corn	535.22	544.36	553.52	553.79	374.65
Soybean meal[1]	184.83	114.14	43.36	0.00	129.38
Poultry offal meal	176.89	180.89	184.90	168.41	123.82
Poultry fat	52.23	43.25	34.26	27.74	36.56
Liquid palatant	20.00	20.00	20.00	20.00	14.00
Cellulose	14.78	9.78	4.79	0.00	10.34
Sodium chloride	5.00	5.00	5.00	5.00	3.50
Mineral-vitamin supplement[2]	4.00	4.00	4.00	4.00	2.80
Choline chloride	2.00	2.00	2.00	2.00	1.40
Calcium propionate	2.00	2.00	2.00	2.00	1.40
Potassium chloride	1.77	3.36	4.95	5.84	1.24
Adsorbent	1.00	1.00	1.00	1.00	0.00
BHT	0.15	0.15	0.15	0.15	0.11
BHA	0.08	0.08	0.08	0.08	0.05
HPDDG	0.00	70.00	140.00	210.00	300.00

[1]460 g crude protein/kg;

[2]enrichment per kg of product^{-1}: vitamin A (retinol), 20,000 IU; vitamin D3, 2,000 IU; vitamin E (alpha-tocopherol), 48 mg; vitamin K3, 48 mg; vitamin B1, 4 mg; vitamin B2, 32 mg; pantothenic acid, 16 mg; niacin, 56 mg; choline, 800 mg; Zn as zinc oxide, 150 mg; Fe as ferrous sulphate, 100 mg; Cu as copper sulphate, 15 mg; I as potassium iodide, 1.5 mg; Mn as manganese oxide, 30 mg; Se as sodium selenite, 0.2 mg; antioxidant, 240 mg.

Table 2. Analyzed chemical composition of the high-protein dried distillers grains (HPDDG) and of the experimental diets.

Item	HPDDG	HPDDG, g/kg			
		0	70	140	210
Dry matter, g/kg	927.70	930.00	931.30	930.10	931.20
Crude protein, g/kg	447.00	266.60	263.70	269.30	262.10
Acid-hydrolyzed ether extract, g/kg	138.00	95.40	96.00	94.30	94.60
Ash, g/kg	27.10	78.60	71.60	71.70	72.60
Crude fiber, g/kg	112.00	14.10	14.90	15.40	14.30
Neutral detergent fiber, g/kg	380.00	nd	nd	nd	nd
Calcium, g/kg	<0.01	14.80	12.50	12.80	12.30
Phosphorus, g/kg	5.30	8.90	8.10	8.20	8.20
Gross energy, kcal/kg	5412.0	4756.9	4838.2	4882.1	4904.5

Amino acid content of HPDDG (g/kg dry matter): aspartic acid = 28.4, threonine = 15.7, serine = 20.9, glutamic acid = 71.4, proline = 36.6, glycine = 17.0, alanine = 30.2, cystine = 10.5, valine = 24.2, methionine = 10.2, isoleucine = 17.4, leucine = 52.0, tyrosine = 19.1, phenylalanine = 22.2, lysine = 14.3, histidine = 13.2, arginine = 18.3, tryptophan = 3.30.

Table 3. Means of apparent total tract digestibility (ATTD, %) and metabolizable energy (ME, kcal/kg) of diets containing increasing concentrations of high-protein dried distillers grains (HPDDG).

Item	HPDDG, g/kg				SEM[1]	P-value[2]	
	0	70	140	210		L	Q
ATTD, %							
Dry matter	80.0	80.1	80.5	82.0	0.80	0.664	0.640
Organic matter	83.8	83.8	83.9	84.9	0.65	0.841	0.724
Crude protein	80.5	80.2	80.3	81.2	0.71	0.965	0.866
Ether extract	89.8	88.7	88.1	86.6	0.54	0.462	0.147
Gross Energy	83.6	83.7	84.1	85.5	0.67	0.631	0.612
ME, kcal/kg	3952.3	4025.4	4079.3	4166.6	35.32	0.106	0.258

[1]SEM = standard error of the mean;

[2]Probabilities for linear (L) and Quadratic (Q) effects.

Table 4. Means of apparent total tract digestibility (ATTD, %), metabolizable energy (ME, kcal/kg), and digestible nutrients (%) of high-protein dried distillers grains (HPDDG) in dogs.

Item	ATTD and ME	Digestible nutrients and energy[1]
Dry matter	85.5	85.5
Organic matter	87.4	85.0
Crude protein	91.2	40.8
Acid-hydrolyzed ether extract	84.6	11.7
Gross energy	87.4	4727.9
ME	5041.8	-

[1]Digestible nutrients and energy (% for nutrients or kcal for energy) = (Nutrient/100) × ATTD of HPDDG.

Table 5. Means of fecal characteristics and intestinal fermentation products of dogs fed with diets containing high-protein dried distillers grains (HPDDG).

Item	HPDDG, g/kg				SEM[1]	P-value	
	0	70	140	210		L	Q
Dry matter, %	36.97	36.41	37.81	37.07	0.553	0.548	0.582
pH	6.57	6.66	6.60	6.54	0.082	0.978	0.951
Fecal output[3]	110.13	111.55	112.17	111.10	2.638	0.831	0.982
Ammonia, g/kg	0.061	0.069	0.072	0.068	0.003	0.307	0.750
Score[4]	4	4	4	4	-	-	-
Short-chain fatty acids (SCFA), µmol/g							
Acetate	141.86	151.13	146.63	154.14	3.916	0.647	0.327
Propionate	49.41	59.80	57.13	57.49	2.418	0.356	0.289
Butyrate	7.39	9.33	7.24	7.50	0.493	0.618	0.290
Valerate	2.52	2.85	2.71	3.27	0.104	0.275	0.031
Total SCFA	201.19	223.10	213.70	222.39	6.258	0.537	0.257
Branched-chain fatty acids (BCFA), µmol/g							
Isovalerate	0.86	0.90	0.88	0.81	0.020	0.792	0.734
Isobutyrate	2.05	2.26	2.02	2.42	0.080	0.896	0.326
Total BCFA	2.92	3.16	2.90	3.23	0.083	0.970	0.431

[1]SEM: standard error of the mean;

[2]L = linear; Q = quadratic;

[3]Fecal output = g feces produced as-is/animal/day;

[4]Median fecal consistency score analyzed by Kruskal Wallis (P >05);

Table 6. Means of the relative abundance (%) of the main bacterial genera in the feces of dogs fed with diets containing high-protein dried distillers grains (HPDDG).

Item	HPDDG, g/kg				SEM[1]	P-value[2]	
	0	70	140	210		L	Q
Genera							
Prevotella	26.76	32.73	39.21	24.44	4.598	0.233	0.009
Streptococcus	2.94	0.89	0.15	0.24	0.399	0.001	0.180
Blautia	3.28	2.87	4.94	7.69	0.853	0.013	0.009
Clostridiales	2.82	2.35	1.90	3.14	0.308	0.833	0.010
Lachnospira	2.02	1.66	1.58	1.97	0.242	0.776	0.033
Megamonas	3.74	3.68	0.65	1.22	0.670	0.003	0.865
Faecalibacterium	4.70	9.12	7.07	11.13	1.439	0.125	0.317
Fusobacterium	7.69	11.72	5.37	10.27	1.000	0.802	0.663
Bacteroides	5.98	7.02	3.71	9.62	1.340	0.340	0.342
Eubacterium	2.06	1.11	6.36	6.63	1.211	0.101	0.413
Erysipelotrichaceae	3.15	2.00	3.81	3.06	0.884	0.250	0.986
Ruminococcaceae	2.08	1.91	1.71	2.60	0.247	0.403	0.410
Peptostreptococcaceae	1.20	1.19	2.06	1.68	0.461	0.150	0.672
Phascolarctobacterium	1.07	0.99	0.72	0.75	0.145	0.723	0.404
Turicibacter	0.79	0.28	1.41	0.99	0.296	0.434	0.314
Alpha-diversity							
Shannon	5.35	5.92	5.69	5.71	0.921	0.221	0.041
Chao-1	1975.0	2061.1	1948.7	2360.0	65.259	0.064	0.188
OTUs[3]	956.9	1010.0	991.5	1158.1	29.725	0.024	0.303

[1]SEM: standard error of the mean; [2]L = linear; Q = quadratic; [3]Operational taxonomic units

Table 7. Number of first visits to the food bowl and intake ratio (± standard error) of dogs fed diets with (diet B) or without (diet A) high-protein dried distillers grains (HPDDG).

Diet A x B, g/kg HPDDG	First choice diet A[1]	Intake ratio diet A[2]	P-value
0 x 70	16	0.46 ± 0.061	0.276
0 x 210	16	0.36 ± 0.065	0.023*

[1]Number of visits to the food bowl with diet B is obtained as 32 – n

[2]Intake ratio: g intake of diet A or B/ total g consumed (A + B)

*Intake ratio differs by Student's t-test ($P<0.05$)

CAPÍTULO V – CONSIDERAÇÕES FINAIS

A inclusão do amido durante o processo de produção das dietas extrusadas é essencial para que ocorra sua gelatinização e sobre a qualidade dos extrusados formados e palatabilidade final da dieta. O primeiro estudo desenvolvido, o qual envolveu as fontes de amido e sua relação com as características físicas dos extrusados evidenciem que os componentes associados aos grânulos de amido influenciam sobre esses aspectos. Os amidos de tubérculos, como batata e batata-doce, contêm maior proporção de amilopectina em relação à amilose, facilitando a gelatinização do amido durante a extrusão. Esse aumento na gelatinização do amido melhora a expansão dos *kibbles.*

No entanto, a presença de diferentes componentes, como a presença expressiva de fibra em fontes como a batata-doce e leguminosas, pode interferir sobre a macroestrutura dos extrusados. Uma alta concentração de fibra alimentar pode dificultar a formação de uma estrutura celular adequada, reduzindo a expansão radial, aumentando a densidade e promovendo a extrusados mais densos e com poros pequenos e de menor número. Esses aspectos podem refletir negativamente sobre a textura e palatabilidade final da dieta.

Em adição, a presença de fibras (de caráter solúvel e insolúvel) e macromoléculas associadas aos grânulos de amido, como proteínas, pode influenciar a digestibilidade da dieta. Tal efeito foi encontrado no segundo estudo desenvolvido, no qual a dieta contendo batata-doce apresentou menor digestibilidade dos macronutrientes da dieta. Contudo, essa fração fibrosa presente principalmente na batata-doce e nas leguminosas, tem potencial para promover e manter a funcionalidade intestinal dos cães.

Por fim, a grande variabilidade em termos de perfil nutricional de coprodutos é evidenciada nos estudos avaliando o DDGS na nutrição animal. No entanto, o avanço nas tecnologias de processamento possibilitou a obtenção de ingredientes de melhor qualidade. O terceiro estudo dessa tese evidenciou o potencial do HPDDG como substituto de fontes proteicas convencionais em fórmulas destinadas para animais de companhia. Além de suas características nutricionais, o HPDDG apresenta propriedades funcionais capazes de modular a microbiota intestinal.

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APÊNDICE – DIRETRIZES PARA AUTORES

Os capítulos II, III e IV foram apresentados de acordo com as diretrizes para autores dos respectivos jornais em que serão submetidos. As instruções para autores podem ser acessadas pelos links abaixo:

Animal Feed Science and Technology:
https://www.elsevier.com/journals/animal-feed-science-and-technology

Journal of Animal Science:
https://academic.oup.com/jas/pages/general_instructions

ANEXO I – CERTIFICADO DE COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 001/2020, referente ao projeto de pesquisa **"Avaliação de diferentes fontes de amido na dieta de cães"**, sob a responsabilidade de **Ananda Portella Félix** – 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 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em 28/02/2020.

Finalidade	Pesquisa
Vigência da autorização	Abril/2020 até Junho/2020
Espécie/Linhagem	*Canis lupus familiaris* (canino)
Número de animais	16
Peso/Idade	15 kg/4 anos
Sexo	Macho e fêmea
Origem	Laboratório de Estudos em Nutrição Canina da Universidade Federal do Paraná, Curitiba/Paraná, BRA.

CERTIFICATE

We certify that the protocol number 001/2020, regarding the research project **"Evaluation of differents sources of starch in dog diets"** under **Ananda Portella Félix** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 2 of invasiveness, in session of 28/02/2020.

Puporse	Research
Validity	April/2020 until June/2020
Specie/Line	*Canis lupus familiaris* (canine)
Number of animals	16
Wheight/Age	15 kg/4 years
Sex	Male and female
Origin	Laboratório de Estudos em Nutrição Canina of the Federal University of Paraná, Curitiba/Paraná, BRA.

Curitiba, 13 de março de 2020

Simone Tostes de Oliveira Stedile
Coordenadora CEUA-SCA

ANEXO II – CERTIFICADO DE COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 001/2021, referente ao projeto de pesquisa **"Avaliação nutricional do resíduo seco de destilaria com solúveis (DDGS) em cães",** sob a responsabilidade de **Ananda Portella Félix** – 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 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em 05/02/2021.

Finalidade	Pesquisa
Vigência da autorização	Fevereiro/2021 até Abril/2021
Espécie/Linhagem	*Canis lupus familiaris* (canino)
Número de animais	16
Peso/Idade	15 kg/6 anos
Sexo	Machos e fêmeas
Origem	Laboratório de Estudos em Nutrição Canina da Universidade Federal do Paraná, Curitiba/PR.

*A autorização para início da pesquisa se torna válida a partir da data de emissão deste certificado.

CERTIFICATE

We certify that the protocol number 001/2021, regarding the research project **"Nutrition Assessment distillers dried grains with solubles (DDGS) in dogs"** under **Ananda Portella Félix** – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 1 of invasiveness, on 2021, February 5th.

Purpose	Research
Validity	February/2021 until April/2021
Specie/Line	*Canis lupus familiaris* (canine)
Number of animals	16
Weight/Age	15 kg/6 anos
Sex	Male/Female
Origin	Laboratory of Studies in Canine Nutrition at Veterinary Hospital of the Federal University of Paraná, Curitiba/PR, Brazil.

*The authorization to start the research becomes valid from the date of issue of this certificate.

Curitiba, 05 de fevereiro de 2021

Simone Tostes de Oliveira Stedile
Coordenadora CEUA-SCA

ANEXO III – COMPROVANTE DE SUBMISSÃO ARTIGO (CHAPTER III)

Animal Feed Science and Technology

Different starch sources result in distinct responses to diets digestibility, fecal microbiota and fermentative metabolites, and postprandial glycemic response in dogs

--Manuscript Draft--

Manuscript Number:	ANIFEE-D-23-00389
Article Type:	Research Paper
Section/Category:	Non-ruminant terrestrial animal species
Keywords:	Cereals; Glycemia; Gut microbiota; Pulses; Total Dietary Fiber; Tuber
Corresponding Author:	Gislaine Kaelle Federal University of Parana BRAZIL
First Author:	Gislaine Kaelle
Order of Authors:	Gislaine Kaelle
	Taís Silvino Bastos
	Renata Bacila Morais dos Santos de Souza
	Eduarda Lorena Fernandes
	Simone Gisele de Oliveira
	Ananda Portella Félix
Abstract:	The study evaluated the effects of different starch sources on the coefficients of total tract apparent digestibility (CTTAD) of macronutrients, metabolizable energy (ME), intestinal fermentative metabolites, fecal microbiota, and postprandial glycemic response in dogs. Seven diets containing corn, brown rice, sorghum, potato starch, sweet potato flour, chickpea, and pea flour were evaluated. Fourteen adult Beagle dogs were randomly distributed in blocks (periods) and were fed the experimental diets for 15 days during three periods, totaling 6 repetitions. In general, diets with brown rice and pea had higher CTTAD of nutrients, followed by diets containing sorghum, chickpea, and potato. The diet containing sweet potato had the lowest CTTAD of nutrients. Diets containing corn and brown rice presented the highest ME content. Dogs fed the chickpea diet had lower fecal pH, ammonia concentration, and dry matter content ($P<0.05$). Generally, higher fecal concentrations of short-chain fatty acids were observed in dogs fed the sweet potato and chickpea diets compared to the potato and brown rice diets ($P<0.05$). Pulse-based diets resulted in a higher abundance of Bacteroides plebeius, Prevotella copri, Blautia, and Turicibacter in feces ($P<0.05$). A higher abundance of Faecalibacterium prausnitzii and Blautia was observed in the feces of dogs fed the sweet potato diet ($P<0.05$). Blood samples collected from dogs fed corn and potato diets indicated a greater increase in glycemic peak concentration and maximum glycemia than the other starch sources ($P<0.05$). The period until the glycemic peak was longer for diets containing sorghum and chickpea ($P<0.05$). The results obtained in this study showed that sweet potato and pulses improve indicators of gastrointestinal functionality, and help controlling the postprandial glycemic response in dogs.
Suggested Reviewers:	Luciano Trevizan, Dr Professor, Federal University of Rio Grande do Sul l.trevizan@ufrgs.br Professor Trevizan is an outstanding researcher in companion animal nutrition
	Gabriel Pacheco, Dr Professor, IFFAR gfepacheco@hotmail.com Professor Gabriel is an outstanding researcher in companion animal nutrition.
Opposed Reviewers:	

ANEXO IV – COMPROVANTE DE PUBLICAÇÃO ARTIGO (CHAPTER IV)

JOURNAL ARTICLE ACCEPTED MANUSCRIPT

High-protein dried distillers grains in dog diets: diet digestibility and palatability, intestinal fermentation products, and fecal microbiota

Gislaine Cristina Bill Kaelle, Taís Silvino Bastos, Eduarda Lorena Fernandes, Renata Bacila Morais dos Santos de Souza, Simone Gisele de Oliveira, Ananda Portella Félix ✉

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