

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL**

**FONTES DE AMIDO E FIBRA SOLÚVEL EM DIETAS PARA
CÃES E SEUS EFEITOS NO PROCESSO DE EXTRUSÃO,
QUALIDADE DOS KIBBLES E METABOLISMO**

Peterson Dante Gavasso Pacheco

Zootecnista

2020

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QUALIDADE DOS KIBBLES E METABOLISMO**

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UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal



CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: FONTES DE AMIDO E FIBRA SOLÚVEL EM DIETAS PARA CÃES E SEUS EFEITOS NO PROCESSO DE EXTRUSÃO, QUALIDADE DOS KIBBLES E METABOLISMO

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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado "**Avaliação da inclusão de polpa cítrica e fibra de laranja em rações para cães**", protocolo nº 017359/17, sob a responsabilidade do Prof. Dr. Aulus Cavalleri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 08 de fevereiro de 2018.

Vigência do Projeto	05/03/2018 a 17/12/2018
Espécie / Linhagem	Canina / Beagle
Nº de animais	30
Peso / Idade	12 Kg / 8
Sexo	Ambos os sexos
Origem	Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof. Flavio Prada"

Jaboticabal, 08 de fevereiro de 2018.



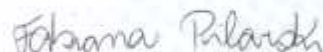
Profª Drª Fabiana Pilarski
Coordenadora – CEUA

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado "**Fontes alternativas de carboidratos sobre as variáveis de processo de extrusão, digestibilidade dos nutrientes e resposta pós-prandial de glicose e insulina de cães**", protocolo nº 017360/17, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 08 de fevereiro de 2018.

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Prof.ª Dr.ª Fabiana Pilarski
Coordenadora – CEUA

FONTES DE AMIDO E FIBRA SOLÚVEL EM DIETAS PARA CÃES E SEUS EFEITOS NO PROCESSO DE EXTRUSÃO, QUALIDADE DO KIBBLE E METABOLISMO

RESUMO – O desenvolvimento e a utilização de ingredientes novos ou alternativos são interessantes para a indústria de alimentos para animais de estimação, especialmente ingredientes derivados de fontes que promovam benefícios à saúde dos animais. Sendo assim, a presente tese está estruturada em três capítulos. O Capítulo 1 teve como objetivo apresentar uma visão geral sobre carboidratos através de uma revisão bibliográfica. O estudo presente no Capítulo 2 teve como objetivos avaliar os efeitos da inclusão de polpa cítrica (PC) e fibra de laranja (FL) em dietas para cães sobre o processo de extrusão, macroestrutura dos kibbles, digestibilidade dos nutrientes e da energia, características fecais, produtos de fermentação intestinal e palatabilidade. Uma dieta controle, sem inclusão de fonte de fibra, e quatro dietas contendo 3%, 6%, 12% de PC ou 6% de FL foram testadas. A inclusão de PC e FL reduziu a amperagem, pressão e energia mecânica específica do motor ($P < 0,05$), mas a aplicação de energia térmica específica e energia específica total não se alterou ($P > 0,05$). A macroestrutura dos kibbles foi afetada com redução na taxa de expansão, gelatinização do amido e digestibilidade *in vitro* da matéria orgânica ($P < 0,05$), enquanto a dureza e o comprimento específico dos kibbles aumentaram ($P < 0,05$) com a inclusão de ambas as fontes. A inclusão da FL não alterou a gelatinização do amido ($P > 0,05$). O consumo de fibra alimentar total aumentou ($P < 0,05$) com a adição das fontes de fibra, o que levou à redução no coeficiente de digestibilidade aparente dos nutrientes e da energia, exceto para a digestibilidade da fibra alimentar que aumentou ($P < 0,05$). A digestibilidade da fibra alimentar foi maior para a dieta com 6% de FL comparada com a dieta contendo 6% de PC ($P < 0,05$). A inclusão de PC e FL aumentou a produção de fezes e reduziu a matéria seca fecal ($P < 0,05$), mas não alterou o escore fecal ($P > 0,05$). O pH fecal diminuiu e os ácidos graxos de cadeia curta (AGCC), acetato, butirato, valerato e lactato aumentaram ($P < 0,05$) com a adição de PC. A FL induziu pH fecal mais baixo e concentrações totais de AGCC mais altas ($P < 0,05$) do que a PC. A inclusão de FL aumentou a palatabilidade da dieta, sendo preferida pelos cães em relação à dieta controle ($P < 0,05$). A FL pode ser utilizada como fonte de fibra alimentar na nutrição de cães, melhorando a saúde intestinal e a palatabilidade, sem afetar a qualidade fecal. O estudo apresentado no Capítulo 3 teve como objetivos avaliar os efeitos do grão-de-bico (GB), aveia descascada (AD), farinha de amido de feijão (AF) e ervilha (ER) como fontes de amido em dietas para cães sobre o processo de extrusão, características da ração, digestibilidade, características fecais, produtos de fermentação intestinal, palatabilidade e resposta pós-prandial de glicose e insulina. Uma dieta controle à base de arroz (AR) foi usada como referência e oito dietas foram formuladas, substituindo 50% ou 100% da dieta AR pelas fontes alternativas de amido: AD50, AD100, ER50, ER100, GB50, GB100, AF50 e AF100. As dietas foram processadas em extrusora de rosca simples. O coeficiente de digestibilidade aparente dos nutrientes foi determinado através da coleta total de fezes, utilizando seis cães por tratamento distribuídos em delineamento em blocos casualizados. Fezes frescas foram coletadas para avaliar o pH e os produtos de fermentação. As dietas formuladas com 100% das fontes de carboidrato foram utilizadas para avaliar as respostas pós-prandiais de glicose e insulina, sendo utilizado nove animais por tratamento. Os

resultados foram submetidos à ANOVA e as médias comparadas pelo teste de Tukey ($P<0,05$). A digestibilidade aparente da MS, MO e EB foi semelhante entre as dietas AR, AD50, GB50, AF50 e AF100, e menor que a AR para as dietas AD100, ER50, ER100 e GB100 ($P<0,05$). Os cães alimentados com a dieta GB100 apresentaram menor digestibilidade da proteína bruta ($P<0,05$). Os animais alimentados com AD100 apresentaram menor digestibilidade da gordura que os alimentados com AR ($P<0,05$). A produção de fezes e umidade foram maiores em cães alimentados com as dietas ER100 e GB100 comparado com a AR ($P<0,05$). O pH fecal foi menor e o lactato fecal maior para a maioria das fontes alternativas de amido ($P<0,05$). Comparado com a dieta AR, os cães alimentados com as dietas ER100 e GB100 apresentaram maiores concentrações de acetato, propionato e AGCC totais nas fezes ($P<0,05$). Os cães alimentados com as dietas à base de AD e GB apresentaram menor pico máximo de insulina e menor área total sob a curva (AUC) de insulina em comparação à dieta BR ($P<0,05$). Em conclusão, o AF pode substituir o AR, enquanto a AD e o GB podem substituir até 50% o AR sem influenciar a digestibilidade dos nutrientes. A substituição total de AR por AD, GB e ER reduziu a digestibilidade com potencial de uso em dietas de baixa energia. Ervilha e GB aumentam a formação de produtos de fermentação e podem ser usados em dietas para tratar de problemas intestinais. Dietas contendo AD e GB reduziram a secreção de glicose e insulina.

Palavras-chave: carboidratos, digestibilidade, glicose, grain-free, insulina, palatabilidade

SOURCES OF STARCH AND SOLUBLE FIBRE IN DOG FOODS AND THEIR EFFECTS ON EXTRUSION PROCESSING, KIBBLE QUALITY AND METABOLISM

ABSTRACT – The development and use of novel or alternative ingredients are interesting for the pet food industry, especially ingredients derived from sources that promote animal health benefits. Therefore, the present thesis is organized in three chapters. Chapter 1 aimed to provide an overview of carbohydrates (starch and fiber) by a literature review. The study in Chapter 2 aimed to evaluate the effects of citrus pulp (CP) and orange fiber (OF) inclusion on extrusion process, kibble macrostructure, digestibility of nutrients and energy, fecal characteristics, fermentation end products and palatability of dog foods. A control diet (CO), with no fiber source inclusion, and four diets containing 3%, 6%, 12% of CP or 6% of OF were tested. The inclusion of CP and OF reduced the motor amperage, pressure, and specific mechanical energy ($P < 0.05$) but specific thermal energy and total specific energy application did not change ($P > 0.05$). Kibble macrostructure was affected with a decreased in expansion rate, starch gelatinization and in vitro digestibility of organic matter ($P < 0.05$), whereas kibble hardness and specific length increased ($P < 0.05$) with CP and OF inclusion. OF inclusion did not change starch gelatinization ($P > 0.05$). Total dietary fiber (TDF) intake increased ($P < 0.05$) with the addition of fiber sources, which led to reduction on digestibility of nutrients and energy, excepted for TDF digestibility that increased ($P < 0.05$). TDF digestibility was higher for diet with 6% OF than 6% CP ($P < 0.05$). The inclusion of CP and OF increased feces production and reduced fecal DM ($P < 0.05$) but did not alter fecal score ($P > 0.05$). Fecal pH decreased and total short-chain fatty acids (SCFA), acetate, butyrate, valerate, and lactate increased ($P < 0.05$) adding CP. The OF induced lower fecal pH and higher total SCFA concentrations ($P < 0.05$) than CP. OF inclusion enhanced food palatability, being preferred by the dogs over the CO food ($P < 0.05$). OF can be utilized as dietary fiber source in dog nutrition, improving gut health and palatability, with no effect on fecal quality. The study presented in Chapter 3 aimed to evaluate the effects of chickpea (CP), dehulled oat (DO), faba bean starch flour (FS) and pea (PE) as starch source in dog foods on extrusion processing, kibble characteristics, digestibility, fecal characteristics, fermentation end products, palatability and postprandial glucose and insulin responses. The present study in Chapter 3 compared chickpea (CP), dehulled oat (DO), faba bean starch flour (FB) and pea (PE) incorporated to dog extruded food as the main carbohydrate sources on extrusion variables, kibble macrostructure, apparent nutrient digestibility, fecal characteristics, fermentation end-products, palatability, and postprandial responses of glucose and insulin. A control diet based on broken rice (BR) was formulated and used as a reference carbohydrate source, and eight test foods were obtained replacing 50% or 100% of the broken rice by the ingredients in study: DO50, DO100, PE50, PE100, CP50, CP100, FB50 and FB100. Foods were extruded in a single screw extruder. Coefficients of total tract apparent digestibility (CTTAD) were determined with 6 dogs per diet by total collection of feces, in a completely randomized block design. Fresh feces were used to evaluate pH and fermentation products. The diets with BR and 100% of each ingredient in study were evaluated in 9 dogs per diet to determine glucose and insulin postprandial responses. Data were submitted to ANOVA and means compared by Tukey test ($P < 0.05$). CTTAD of DM, OM and GE were similar among BR, DO50, CP50, FB50 and FB100 diets, but lower than BR for DO100, PE50, PE100, and CP100 treatments ($P < 0.05$). Dogs fed the CP100 diet had lower crude protein

digestibility ($P<0.05$). Animals fed DO100 showed lower fat digestibility than those fed BR ($P<0.05$). Feces production and moisture were greater for dogs fed PE100 and CP100 than those fed BR ($P<0.05$). Fecal pH was lower and fecal lactate higher for most of non-conventional starch sources compared to BR ($P<0.05$). Dogs fed PE100 and CP100 had higher acetate, propionate, and total volatile fatty acids concentration on feces than dogs fed BR ($P<0.05$). Dogs fed DO100 and CP100-based foods presented lower maximum insulin pick and lower total area under the curve (AUC) of insulin in comparison to BR diet ($P<0.05$). In conclusion, the FB can replace BR, while DO and chickpea can replace up to 50% of BR without influencing digestibility of nutrients. The total substitution of BR by DO, CP and PE reduced digestibility with a potential use for low energy diets. PE and CP increased fermentation end-products formation and may be used in diets to support specific intestinal conditions. Diets based on DO and CP reduced the postprandial insulin secretion in dogs.

Keywords: carbohydrate, digestibility, glucose, grain-free, insulin, palatability

CAPÍTULO 1 – Considerações gerais

1. Introdução

A população de cães no Brasil foi estimada em 55,1 milhões de animais, em 2019, segundo levantamento realizado pelo Instituto Pet Brasil (IPB, 2019). Essa estatística populacional impulsiona o crescimento da indústria *pet* no país, que obteve um faturamento total de 22,3 bilhões de reais de acordo a Associação Brasileira da Indústria de Produtos para Animais de Estimação (ABINPET, 2019). Deste faturamento, o setor *pet food* abrange 73,3%, com crescimento de 8,4% em relação ao ano de 2018. Estes dados mostram a importância da indústria de alimentos para animais de companhia e o desenvolvimento deste segmento, além da demanda dos tutores por alimentos de alta qualidade.

Com base nos dados atuais de mercado e exigência dos tutores, a indústria de alimentos para animais de companhia (*pet food*) está cada vez mais preocupada em oferecer alimentos que atendam às exigências nutricionais e que proporcionem benefícios à saúde e aos bem-estar dos animais. Neste contexto, pode-se destacar o desenvolvimento e a utilização de ingredientes novos ou alternativos, que permitam desenvolver novos atrativos para os clientes, promover benefícios para a saúde dos animais, evitar potenciais competições com recursos alimentares humanos ou potencialmente reduzir o custo das formulações.

O foco marcante do uso de fontes alternativas de ingredientes pode ser observado nas dietas *grain-free* ou livre de grãos. As dietas *grain-free* são formuladas com ingredientes de qualidade, sendo as leguminosas (ervilha, feijão, grão-de-bico e lentilha) e os tubérculos (batata, batata doce e mandioca) as principais fontes de carboidrato (amido). A proposta desses alimentos é reduzir a quantidade de carboidratos presentes na sua composição, principalmente de amido. Os alimentos *grain-free* não são livres de carboidratos, mas podem apresentar, devido a composição das fontes de carboidratos utilizados, redução do teor de amido e aumento dos teores de proteína, gordura e fibra alimentar (Alvarenga e Aldrich, 2020). Isto, porém, não é regra geral e algumas dietas comerciais *grain-free* para cães podem apresentar maior quantidade de amido na sua composição nutricional em relação as dietas à base de grãos cereais tradicionais (Alvarenga e Aldrich, 2020).

Além do uso de fontes alternativas de amido, a caracterização e o desenvolvimento de novas fontes de fibras alimentares também são interessantes para a indústria de alimentos para animais de estimação, especialmente ingredientes derivados de fontes naturais e com propriedades funcionais que possam colaborar para melhorar a saúde intestinal. Atualmente, a polpa de beterraba é a mais usual fonte de fibra alimentar fermentável utilizada em alimentos para animais de companhia de alta qualidade, por apresentar fermentação moderada para cães e gatos. Contudo, essa fibra é cara, elevando o custo das formulações. Como o Brasil é o maior produtor de laranja, uma grande quantidade de resíduos é gerada durante a extração do suco da fruta, material com elevada proporção de fibra solúvel. Esses resíduos se apresentam, devido à sua composição química, como alternativa à polpa de beterraba, podendo apresentar benéficos para a saúde dos animais.

Com base no exposto, este trabalho teve como objetivos gerais compreender os efeitos da utilização de fontes alternativas de amidos e fibras solúveis em dietas para cães e os seus efeitos sobre o processo de extrusão, digestibilidade dos nutrientes e da energia, produtos de fermentação microbiana, resposta pós-prandial de glicose e insulina e palatabilidade. Para isso, essa tese está estruturada em três capítulos: Capítulo 1: Considerações gerais; Capítulo 2: Citrus pulp and orange fiber as dietary fiber sources for dogs; e Capítulo 3: Chickpea, dehulled oat, faba bean starch flour and pea as dietary starch sources for dog foods: effects on extrusion, digestibility, fermentation end-products in feces, palatability, and postprandial glucose and insulin responses.

2. Revisão de literatura

2.1. Classificação dos carboidratos

Os carboidratos são moléculas formadas por carbono, hidrogênio e oxigênio. Podem ser classificados em grupos distintos, de acordo com sua estrutura química e seu efeito fisiológico: monossacarídeos, dissacarídeos, oligossacarídeos e polissacarídeos (NRC, 2006). Os monossacarídeos são classificados como carboidratos absorvíveis, pois não necessitam da digestão enzimática para serem absorvidos no intestino, e englobam a glicose, frutose e galactose (NRC, 2006). Os

dissacarídeos são formados por duas moléculas de açúcar, facilmente digeridos pelas enzimas intestinais e incluem a lactose, sacarose e maltose (NRC, 2006; Cummings e Stephen, 2007). Os oligossacarídeos são constituídos por maior número de moléculas de açúcares, variando de três a nove moléculas. Este grupo é formado por compostos digeríveis e não digeríveis, sendo os últimos resistentes à ação das enzimas intestinais, porém, podem ser fermentados pelos microrganismos presentes no intestino grosso (NRC, 2006). Os polissacarídeos ou carboidratos complexos são àqueles que apresentem 10 ou mais unidades de açúcares em sua molécula e podem ser de origem animal e vegetal (NRC, 2006; Slavin, 2013). Os polissacarídeos de origem vegetal, como o amido (classificado como carboidrato digerível) e a fibra alimentar (carboidrato não digerível), são as duas principais classes do grupo de carboidratos complexos presentes em dietas para cães e gatos. Embora os carboidratos (amido e fibra) não sejam considerados nutrientes essenciais para cães e gatos (NRC, 2006), sua inclusão pode variar de 30 a 60% na maioria dos alimentos extrusados para animais de companhia (Kempe et al., 2004).

2.2. Fontes de amido

O amido é usualmente o nutriente em maior proporção nos alimentos para cães, podendo variar de 20 a 65% dos alimentos extrusados (Riaz e Rokey, 2012). Em função disso, os ingredientes utilizados como fonte de amido somam proporção importante nas formulações. Existe grande diversidade de fontes de amido que são utilizadas em alimentos para animais de companhia. Dependendo da sua classificação botânica, essas fontes podem ser divididas em três classes: cereais, leguminosas e tubérculos. Os grãos cereais como arroz, milho, sorgo e trigo, são as fontes de amido mais utilizadas em alimentos para cães, podendo apresentar de 58 a 83% de amido (Herrera-Saldana et al., 1990; Kempe et al., 2004; Carciofi et al., 2008; Bazolli et al., 2015). As leguminosas como ervilha, lentilha, feijão e grão-de-bico podem apresentar de 27 a 57% de amido (Carciofi et al., 2008; He e Wei, 2017), enquanto os ingredientes derivados de tubérculos como batata e mandioca apresentam valores de 95 a 97% de amido, pois normalmente se usam farinhas ou amidos purificados de tubérculos (Carciofi et al., 2008; Domingues et al., 2019). O uso de leguminosas e tubérculos como fonte de amido se popularizou recentemente, nas rações *grain-free*

(Alvarenga e Aldrich, 2020).

O amido é importante fonte de energia na forma de glicose para cães e gatos. Os cães possuem quantidades suficientes de enzimas pancreáticas (amilase) e intestinais (sacarase e maltase) capazes de hidrolisar a molécula de amido em monômeros de glicose. A glicose é fisiologicamente essencial para os animais de companhia (Laflamme et al., 2014; Verbrugghe e Hesta, 2017), pois é considerada a principal fonte de energia requerida pelo cérebro, tecidos nervosos, glóbulos vermelhos, leucócitos e outros tipos de células (McGrane, 2013). O processamento do amido, incluindo sua moagem e cozimento pela extrusão, é importante para aumentar sua digestibilidade para animais de companhia, visto que o amido cru é pouco digerível pelos cães (Murray et al., 2001; Bazolli et al., 2015).

Em alimentos adequadamente processados, o amido é o nutriente que apresenta 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% (Murray et al., 1999; Carciofi et al., 2008; Bazolli et al., 2015; Maria et al., 2017; Pacheco et al., 2018; Pezalli et al., 2019; Ribeiro et al., 2019). Porém, alguns estudos demonstram que a digestibilidade do amido pode ser alterada de acordo com a fonte utilizada (Murray et al., 1999; Twomey et al., 2002; Carciofi et al., 2008). Essa diferença no perfil de digestibilidade entre as fontes amido pode ser devido a vários fatores, como a interações amido-proteína, forma física dos grânulos, tipo de amido, a proporção de amilose e amilopectina, inibidores da digestão, processamento e tamanho das partículas após o processamento (Rooney e Pugfelder, 1986; Wolter et al., 1998; Svihus et al., 2005; Bazolli et al., 2015; Dhital et al., 2017). Esses aspectos, no entanto, são pouco estudados em alimentos para cães. Assim, foi demonstrado que o emprego de diferentes cereais (arroz, milho ou sorgo) pode resultar em dietas com diferentes digestibilidades e que resulta em diferentes concentrações de ácidos graxos de cadeia curta nas fezes dos cães, a depender da moagem e redução de partículas adotada e dos parâmetros de extrusão das rações (Bazolli et al., 2015; Peixoto et al., 2018; Ribeiro et al., 2019).

Uma questão pouco explorada, mas que se reveste de potencial importância

na produção de alimentos saudáveis e que promovam benefícios à saúde, é a modulação da digestibilidade ileal do amido com maior fermentação no intestino grosso do amido não gelatinizado ou amido resistente. Estudos demonstram que este processamento controlado, com mistura após moagem de maior granulometria e menor aplicação de energia térmica e mecânica com redução da gelatinização do amido e aumento da fração de amido resistente pode resultar em significativa elevação dos ácidos graxos de cadeia curta nas fezes, em especial do butirato (Bazolli et al., 2015), abordagem que foi explorada quanto à saúde intestinal (Peixoto et al., 2018) e as respostas pós-prandial de glicose e insulina ao consumo da ração (Ribeiro et al., 2019).

2.2.1. Arroz quebrado

O arroz (*Oryza sativa* L.) é um dos grãos cereais mais produzidos mundialmente para o consumo humano. Muitos coprodutos são originados como resultado do beneficiamento do arroz, que inclui as etapas de descascamento, brunição e separação (Carvalho et al., 2011). Dentre os coprodutos originados, o arroz quebrado é composto por grãos quebrados ou defeituosos. Esses grãos fora das especificações para o consumo humano são destinados a alimentação animal, sendo uma das principais fontes de carboidratos utilizado em dietas para animais de companhia. É considerado ingrediente de alta qualidade e com bom perfil nutricional, muito utilizado em alimentos *premium* e *super premium* para cães, por ser mais caro que o milho. Apresenta em média 72,5% de amido, 9,4% de proteína bruta, 2,3% de gordura e 1,9% de fibra alimentar; composição química semelhante ao arroz branco cru utilizado para consumo humano (TACO, 2011). Estudando a digestibilidade do ingrediente, Kempe et al. (2004) e Fortes et al. (2010) observaram que os cães aproveitam melhor os nutrientes do arroz quebrado comparado com outros cereais e coprodutos. Além disso, dietas formuladas à base de arroz apresentam digestibilidade dos nutrientes superior as outras fontes tradicionais de carboidratos como milho, sorgo, cevada e trigo (Kempe et al., 2004; Carciofi et al., 2008; Fortes et al., 2010; Bazolli et al., 2015). O arroz também é responsável por induzir elevado pico glicêmico e insulinêmico em cães comprado com outras fontes (Carciofi et al., 2008; Adolphe et al., 2015).

2.2.2. Aveia descascada

A aveia (*Avena sativa* L.) é o grão cereal menos empregado, presente em alguns alimentos *super premium* para cães. A utilização deste cereal na nutrição animal ocorre devido à sua imagem positiva e benefícios atrelados a nutrição humana, incluindo redução dos níveis de colesterol, glicose e insulina sanguíneos (Mälekia & Virtanenb, 2001; Wood, 2007; Stewart e McDougall, 2014). Dados definitivos a este respeito, no entanto, não estão disponíveis para cães. Diferente dos outros cereais, a aveia contém grandes quantidades de fibra solúvel, principalmente β -glucanos (Butt et al., 2008), além de maior teor de proteína bruta. A composição da aveia descascada cultivada na Suécia varia entre 13 – 18% de proteína, 6 – 8% de gordura, 9 – 11% de fibra alimentar e 50 – 63% de amido (Asp et al., 1992). No Brasil, a composição da aveia descascada varia de 10,4 – 10,7% de umidade, 14,5 – 16,5% de proteína, 6,3 – 7,5% de gordura, 1,7 – 2,0 de cinzas e 10,5 – 11,7 de fibra alimentar, sendo 5,7 – 7,0 fibra insolúvel e 4,6 – 4,8 fibra solúvel (Pedó e Sgarbieri, 1997). A casca do grão de aveia representa cerca de 25 a 30% da semente (Butt et al., 2008). O grão de aveia possui miolo mole e gordura distribuída por toda a semente, o que torna seu processo de moagem mais difícil que do milho e trigo (Butt et al., 2008). Em estudo com cães, a digestibilidade da aveia foi superior ao milho, cevada e trigo. Além disso, os animais alimentados com a dieta contendo aveia apresentaram maior digestibilidade aparente da matéria seca, matéria orgânica e proteína bruta comparado com as dietas com cevada, milho ou trigo (Kempe et al., 2004). Porém, no estudo supracitado, a dieta à base de aveia não mostrou ser eficiente no controle glicêmico e insulinêmico. Isto talvez se explique pelo modelo do experimento, pois a aveia não foi fonte exclusiva de amido, já que as dietas utilizadas para avaliar a digestibilidade dos ingredientes (método de substituição) foram as mesmas utilizadas para se avaliar a resposta pós-prandial de glicose e insulina.

2.2.3. Ervilha

A ervilha (*Pisum sativum* L.) é uma leguminosa cultivada mundialmente para consumo humano e animal (Dahl et al., 2012). Dados compilados por Dahl et al. (2012) mostram que a composição da ervilha pode variar de 21,2 a 32,9% de proteína bruta,

36,9 a 49% de amido, 1,2 a 2,4% de gordura, 2,3 a 3,4% de cinzas, 2,1 a 6,3 de amido resistente, 14 a 26% de fibra alimentar, 10 a 15% de fibra insolúvel e 2,0 a 9,0% de fibra solúvel. Em geral, a ervilha apresenta alto teor de proteína e fibra alimentar, com baixo teor de amido. Atualmente a ervilha é muito utilizada em alimentos para animais de companhia, principalmente em dietas terapêuticas para perda de peso e diabetes, e formulações *grain-free*. Carciofi et al. (2008) avaliaram os efeitos de diferentes fontes de amido sobre a digestibilidade e resposta pós-prandial de glicose e insulina em cães. Comparado com os grãos cereais (arroz, milho e sorgo), a dieta à base de ervilha apresentou menor digestibilidade dos nutrientes e da energia. Além disso, a resposta pós-prandial de glicose e insulina foi menor em cães alimentados com a dieta contendo ervilha comparado com as dietas com arroz, milho e sorgo. Adolphe et al. (2012) e Adolphe et al. (2015) também estudaram os efeitos da inclusão de ervilha sobre o metabolismo de glicose e insulina em cães. Em ambos os estudos, a dieta formulada com ervilha promoveu baixa liberação de glicose e insulina.

2.2.4. Grão-de-bico

O grão-de-bico (*Cicer arietinum* L.) ocupa o terceiro lugar entre as leguminosas mais produzidas no mundo, sendo a Índia o maior produtor com 65% da produção global (Merga e Haji, 2019). O uso do grão-de-bico em alimentos para cães é recente, com o surgindo das dietas *grain-free*. De acordo com a Tabela Brasileira de Composição de Alimentos, o grão-de-bico apresenta 21,2% de proteína, 5,4% de gordura, 12,4% de fibra alimentar, 57,9% de amido (por diferença) e 3,2% de cinzas (TACO, 2011). Resultados semelhantes foram reportados por Costa et al. (2006), contudo, esses valores podem variar de acordo com a cultivar (Singh et al., 2004; Jukanti et al., 2012). Até o momento, não há estudos na literatura avaliando os efeitos da inclusão de grão-de-bico como única e principal fonte de amido na nutrição de cães.

2.2.5. Farinha de amido de feijão

Entre as leguminosas, o feijão (*Vicia faba* L.) é a cultura mais produzida no mundo para consumo humano (Merga e Haji, 2019). É fonte de amido pouco utilizada nos alimentos para animais de companhia. Contudo, devido seu elevado valor

nutricional, estudos estão sendo desenvolvidos para avaliar os efeitos da inclusão deste ingrediente na nutrição de cães (Foster et al., 2012; Alvarenga et al., 2020). Antes de ser transformado em farinha, os feijões faba (*Vicia faba* L.) são limpos, para remover todo matérias estranho, descascados e moídos. De acordo com a empresa fabricante (Vestkorn Milling AS, Tau, Noruega), a farinha de amido de feijão tem cerca de 18 a 20% de proteína, 60 a 70% de amido, 1 a 1,5% de gordura, 2 a 4% de fibra bruta, 2,5 a 3,0% de cinzas e 33% de amilose. Em um estudo com cães, Foster et al. (2012) avaliaram a inclusão de 25% de feijão navy (*Phaseolus vulgaris* L.). O ingrediente apresentava 24% de proteína, 3% de gordura e 64% de carboidratos. A dieta contendo feijão foi bem aproveitada pelos animais, que apresentaram a mesma digestibilidade dos nutrientes e da energia comparado à dieta controle. Os autores também não observaram aumento de flatulência ou alterações na consistência fecal. Recentemente, Alvarenga et al. (2020) avaliaram a utilização de diferentes inclusões (0, 10, 20 e 30%) de feijão faba (*Vicia faba* L.) em alimentos para cães. O ingrediente utilizado continha cerca de 32% de proteína bruta, 1,57% de gordura, 3,51% de cinzas e 8,11 de fibra alimentar, sendo 6,31 fibra insolúvel e 1,80 fibra solúvel. O teor de amido não foi apresentado. A inclusão gradual de feijão promoveu aumento linear na produção de fezes e redução linear do score fecal, de 3,77 para 3,53. Apesar dessa redução, a consistência se manteve dentro da ideal. A digestibilidade foi semelhante entre as dietas, porém, a inclusão de feijão promoveu redução linear na digestibilidade da matéria seca, matéria orgânica e energia bruta, enquanto a digestibilidade da fibra alimentar aumentou linearmente. A digestibilidade da proteína e gordura não foi alterada. Com relação ao estudo de palatabilidade, a dieta controle foi preferida em relação as dietas com 10% e 30% de feijão, enquanto o consumo da dieta com 20% foi semelhante a controle.

2.3. Fontes de fibra alimentar

A fibra alimentar é classificada como polissacarídeo não digerível, pois os animais monogástricos, incluindo os cães e gatos, não possuem enzimas digestivas capazes de hidrolisar as ligações β -glicosídicas presentes nas moléculas de açúcar da fibra alimentar (Verbrugghe e Hesta, 2017). Porém, uma fração da fibra pode ser fermentada por microrganismos presentes cólon dos animais, produzindo alguns

gases (dióxido de carbono, hidrogênio e metano), ácidos graxos de cadeia curta (acetato, propionato e butirato) e lactato (NRC, 2006). A fibra alimentar pode ser classificada com base na sua fermentabilidade em fermentável, moderadamente fermentável ou não fermentável, bem como em sua solubilidade em insolúvel e solúvel (Holscher, 2017). A fração insolúvel da fibra é composta por polissacarídeos da parede celular (celulose e hemicelulose) e polissacarídeos não-estruturais (lignina), e pode ser moderadamente ou não fermentável, enquanto a fração solúvel, que corresponde aos polissacarídeos não celulósicos (beta-glucano, pectinas, gomas e mucilagem) é em geral fermentável (Nair et al., 2010; Verbrugghe e Hesta, 2017).

Os efeitos das fibras insolúveis, moderadamente ou não fermentáveis, estão relacionados com a redução do valor energético da dieta (Kawauchi et al., 2011), contribuindo com a perda de peso, possíveis efeitos na saciedade (Loureiro et al., 2017), controle de bola de pelo em gatos (Loureiro et al., 2014), aumento do volume e umidade das fezes (Kawauchi et al., 2011; Fischer et al., 2012; Loureiro et al., 2017), redução do tempo de trânsito intestinal e aumento na frequência de defecação (Burrows et al., 1982; Loureiro et al., 2017). Podem prevenir e aliviar a constipação (Ho et al., 2012). Com relação a digestibilidade, tanto fontes de fibra não fermentável quanto fermentável promovem alguma redução na digestibilidade aparente dos nutrientes. Em cães, a inclusão de fibras não fermentáveis promove grande redução na digestibilidade da matéria seca e da energia bruta, com pouco efeito na digestibilidade da gordura e proteína (Kawauchi et al., 2011; Kröger et al., 2017; Sabachuk et al., 2017; Detweiler et al., 2019).

Em relação as fibras solúveis e fermentáveis, os seus efeitos estão relacionados com aumento da viscosidade da digesta, retardando a migração e a absorção dos nutrientes pelas células intestinais (Nair et al., 2010; Dai e Chau, 2017). Como resultado, estas fontes podem reduzir os níveis de colesterol, glicose e insulina no sangue (Mälkkia e Virtanenb, 2001; Wood, 2007) e a digestibilidade dos nutrientes, principalmente da gordura e da proteína (Fahey Jr et al., 1990a,b; Diez et al., 1997; Cole et al., 1999; Burkhalter et al., 2001). Além disso, esses ingredientes geralmente são fermentados pela microbiota presente no cólon, originando ácidos graxos de cadeia curta (acetato, butirato e propionato), gases e lactato (NRC, 2006). O butirato é considerado a principal fonte de energia para os colonócitos, pois desencadeia uma

cascata de efeitos benéficos que melhoram a estrutura da barreira intestinal (Peng et al., 2009; Wang et al., 2012; Peixoto et al., 2018) e reduzem inflamações (Knudsen et al., 2018; Theodoro et al., 2019), melhorando a saúde dos animais. Além disso, os produtos oriundos da fermentação reduzem o pH do lúmen do cólon, inibindo o desenvolvimento de patógenos e estando relacionados à possível aumento de saciedade em cães (Bosch et al., 2009).

As frações insolúvel e solúvel podem ser encontradas em diferentes proporções nos ingredientes fibrosos. Portanto, os efeitos fisiológicos e metabólicos decorrentes do uso de fibra estão relacionados com o tipo e nível de inclusão da fonte de fibra (Swanson et al., 2001). Por exemplo, a polpa de beterraba é a principal fonte de fibra comumente utilizada em alimentos *premium* e *super premium* para cães, devido a proporção desejável de fibra insolúvel e solúvel que essa fonte apresenta (De-Godoy et al., 2013). Contudo, alguns estudos mostram que a polpa de beterraba não parece ser muito eficiente para promover aumento nas concentrações de butirato nas fezes de cães (Muir et al., 1996; Fischer et al., 2012; Wambacq et al., 2016; Maria et al., 2017). Sendo assim, fontes alternativas de fibras estão cada vez mais sendo pesquisadas como possíveis substitutos para a polpa de beterraba, um ingrediente de elevado custo.

As principais fontes de fibras utilizadas em alimentos para animais de companhia são obtidas a partir dos coprodutos gerados da indústria de alimentos humanos ou para animais, embora ingredientes específicos também sejam produzidos, como a celulose. Os ingredientes fibrosos mais utilizados são a polpa de beterraba, casca de soja, fibra de cana, fibra de ervilha, celulose e farelo de trigo. No entanto, fontes obtidas a partir de outros cereais além do trigo, tubérculos, casca de outras leguminosas além da soja e coprodutos de frutas também podem ser potenciais fontes de fibra alimentar. A caracterização e o desenvolvimento de novas fontes de fibras alimentares são interessantes para a indústria de alimentos para animais de estimação, principalmente ingredientes derivados de fontes naturais e com propriedades funcionais que melhorem a saúde intestinal dos animais (Swanson et al., 2001).

O Brasil é o maior produtor mundial de laranja (*Citrus sinensis*), sendo a maior parte desta produção destinada ao processamento e produção de suco (IBGE, 2020).

O estado de São Paulo é o principal produtor, responsável por 77,5% da produção total nacional. Em 2019, a produção nacional foi de 17,6 milhões de toneladas, crescimento de 5,6 em relação ao ano anterior (IBGE, 2020). Durante o processamento do suco, expressiva quantidade de resíduos são gerados, sendo destinado a nutrição animal (Kasapidou et al., 2015). A polpa cítrica é o principal coproduto gerado, porém, outros ingredientes podem ser obtidos durante a etapa de processamento do suco, como a fibra de laranja.

2.3.1. Polpa cítrica

A polpa cítrica é obtida a partir do resíduo sólido que permanece após etapa de prensagem da laranja para a extração do suco. A polpa cítrica compreende cerca de 50 a 70% do peso da fruta in natura e é composta pela casca (60 – 65%), tecidos internos (30 – 35%) e sementes (0 – 10%) (Tayengwa e Mapiye, 2018), incluindo ainda os resíduos sólidos retidos após a filtração do suco. O processo de produção da polpa cítrica inclui as etapas de adição de óxido de cálcio (para liberação de água e ajuste do pH), moagem, secagem (reduzir a umidade para estabilização microbiológica), peletização, resfriamento e armazenamento (Yamanaka, 2005). A polpa cítrica peletizada é ingrediente utilizado como fonte de energia na nutrição de ruminantes (Belibasakis e Tsirgogianni, 1996; Tayengwa e Mapiye, 2018). A inclusão de polpa cítrica peletizada foi previamente estudada em ensaios *in vitro* (Sunvold et al., 1995a) e *in vivo* em cães (Sunvold et al., 1995b). No estudo *in vitro*, os autores observaram elevado desaparecimento de matéria orgânica e elevada produção dos ácidos graxos de cadeia curta, quando incubada com inóculo fecal de cães (Sunvold et al., 1995a). No estudo *in vivo*, os cães alimentados com dietas extrusadas suplementadas com 14,45% de polpa cítrica apresentaram digestibilidade aparente dos nutrientes e da energia, bem como produção e teor de umidade das fezes semelhantes quando comparado aos animais alimentados com dieta suplementada com 12,48% de polpa de beterraba (Sunvold et al., 1995b).

2.3.2. Fibra de laranja

A fibra de laranja é um ingrediente novo, composto pelo resíduo sólido obtido do processo de filtração do suco de laranja. É composta, principalmente, pelas

paredes das vesículas de suco e segmentos da fruta, que são posteriormente lavados com água e álcool para se extrair os açúcares solúveis e secos. Por se tratar de um ingrediente em desenvolvimento, não há estudos na literatura avaliando os efeitos da inclusão desta matéria prima na nutrição animal. Apresenta cerca de 11% de proteína, 3,2% de gordura, 2,9% de cinzas, 2,5% de amido e 73,8% de fibra alimentar, sendo 42,4% fibra insolúvel e 31,4% fibra solúvel. A fração solúvel é composta principalmente por pectina (27,6% do ingrediente).

2.4. Estrutura do amido

O amido é um polissacarídeo produzido durante a fotossíntese que serve de reserva de energia para as plantas (Englyst, 1989). É armazenado nas plantas na forma de grânulos, que são compostos por dois polímeros de glicose que apresentam diferentes estruturas e propriedades: amilose e amilopectina (Jenkins e Donald, 1995; Tester et al., 2004). A amilose é molécula formada por cadeia linear de glicose unidas por ligações glicosídicas α -1,4, com baixo peso molecular, enquanto a amilopectina apresenta cadeia ramificada, contendo cadeias de ligações α -1,4 ligadas umas às outras por ligações α -1,6, com maior peso molecular (Zhang et al., 2019).

Os grânulos de amido são formados por regiões amorfas e semicristalinas, dispostas de forma alternada, denominadas anéis de crescimento (Pérez e Bertoft, 2010). A estrutura cristalina do grânulo de amido é atribuída principalmente à amilopectina, devido a sua formação de cadeia dupla hélice, que se compactam formando estrutura de composição lamelar cristalina e amorfa. As regiões de formação da dupla hélice da amilopectina ficam dentro das regiões cristalinas, enquanto as regiões amorfas são formadas pelos pontos de ramificações da molécula de amilopectina (Jenkins e Donald, 1995). Com base na estrutura e no comprimento das cadeias de amilopectina, a cristalinidade deste polímero de glicose pode ser classificada em tipos A, B e C, por meio de difração aos raios-X (Jenkins e Donald, 1995). Estes padrões de cristalinidade são variáveis de acordo com a fonte vegetal. A estrutura tipo A é encontrada na maioria dos cereais (arroz, milho, trigo e aveia), a tipo B é característica dos tubérculos (batata e mandioca) e a estrutura tipo C, uma mistura dos tipos A e B, está presente nas leguminosas como ervilha, grão-de-bico e feijão (He e Wei, 2017).

Em geral, o grânulo de amido normal ou natural, como no arroz, milho, trigo e batata possui cerca de 20% a 30% de amilose e 70% a 80% de amilopectina (Tester et al., 2004; Pérez e Bertoft, 2010; Zhang et al., 2019). Contudo, a proporção destes polissacarídeos pode variar de acordo com a origem botânica do amido (Tester et al., 2004). Entre os cereais, o arroz apresenta 17% de amilose e 83% amilopectina, enquanto a aveia pode apresentar uma variação de 16 – 29% de amilose e 71 – 84% de amilopectina (Ratnayake e Jackson, 2003). Grande variação nestas proporções pode ocorrer, também, entre as cultivares de cada espécie (Ratnayake e Jackson, 2003; Bertoft, 2017). Essa mesma variação no teor de amilose também pode ser observada na classe das leguminosas (Wani et al., 2016; He e Wei, 2017). O teor de amilose na ervilha e no grão-de-bico pode variar, respectivamente, de 24 a 49% (Hoover et al., 2010) e de 30,4 a 35% (Hughes et al., 2009; Hoover et al., 2010). O feijão faba (*Vicia faba*) apresenta também grande variação na composição de amilose, podendo variar de 17 a 42% (Hoover et al., 2010).

2.5. Efeito dos carboidratos no metabolismo de glicose e insulina

Além de ser a principal fonte de glicose presente nas dietas, o amido é considerado o principal nutriente responsável por alterar as respostas pós-prandiais de glicose e insulina em cães (Nguyen et al., 1998). Desta forma, as concentrações pós-prandiais de glicose e insulina no sangue podem ser manipuladas pela seleção de fontes de amidos com diferentes padrões de digestibilidade (Dhital et al., 2017). No entanto, existe poucos estudos na literatura avaliando as respostas metabólicas de glicose e insulina frente a diferentes fontes de carboidratos em cães (Kempe et al., 2004; Carciofi et al., 2008; Adolphe et al., 2012; Adolphe et al., 2015; Rankovic et al., 2020).

O metabolismo do amido pode ser influenciado por fatores como o tipo de amido, a origem botânica, a quantidade de amilose e amilopectina, o processamento e a quantidade ingerida (Nguyen et al., 1998; Englyst et al., 2007; Toutounji et al., 2019). Esses fatores, no entanto, são pouco explorados na nutrição de cães. Atualmente, existe interesse em se compreender a relação entre os diferentes tipos de carboidratos dietéticos e as respostas glicêmicas e insulinêmicas, como possível

forma de se controlar o aumento da resistência à insulina em cães obesos (Brunetto, et al., 2011) ou idosos (Ribeiro et al., 2019).

Neste quesito, o amido pode ser classificado em amido rapidamente digerível, amido lentamente digerível e amido resistente, de acordo com a taxa e a extensão de glicose que é liberada e mensurada por meio do uso de enzimas sob condições *in vitro* (Englyst et al., 1992). Resumidamente, o amido rapidamente digestível consiste naquele que é convertido em glicose em um tempo máximo de 20 minutos, promovendo rápida liberação de glicose e, conseqüentemente, de insulina no sangue. O amido lentamente digerível é aquele que é completamente digerido em glicose após um tempo de 120 minutos de digestão enzimática, promovendo liberação lenta e gradual de glicose e insulina. O amido resistente é aquela fração que resiste a etapa de digestão no intestino delgado, podendo ser fermentado no cólon (Englyst et al., 1992). No entanto, não se sabe ao certo ainda como esta classificação *in vitro*, estabelecida para seres humanos, se relaciona com o metabolismo de carboidratos em cães.

O amido resistente pode ser ainda classificado em cinco categorias de acordo com a causa da resistência à digestão. Em humanos, essa classificação é muito utilizada para entender a relação entre a taxa de liberação de glicose e a resposta glicêmica em alimentos ricos em amidos, com base no tempo de digestão (Raigond et al., 2015). O amido resistente tipo 1 (AR1) é fisicamente inacessível à digestão devido a presença da matriz proteica e parede celular em volta do amido, encontrado em grãos e legumes inteiros ou parcialmente moídos. O amido resistente tipo 2 (AR2) é resistente à digestão devido a conformação e estrutura do amido, presente em grânulos de amido da batata crua, bananas verdes e fontes de amido com estrutura tipo B e C. O amido resistente tipo 3 (AR3) é o amido retrogradado (fisicamente modificado), formado após o cozimento seguido de resfriamento dos alimentos. O amido resistente tipo 4 (AR4) é quimicamente modificado, enquanto o amido resistente tipo 5 (AR5) é aquele formado pela complexação amilose-lipídeo durante o processamento de alimentos (Raigond et al., 2015; Dhital et al., 2017). Estudos em cães já demonstraram que menor gelatinização do amido com maior fração de amido resistente tipo 2 em dieta à base de milho resulta em menor secreção de insulina pós-prandial em cães idosos (Ribeiro et al., 2019). No entanto, faltam ainda estudos com

este tipo específico de apresentação do amido para melhor compreensão de suas consequências metabólicas.

Diferentes fontes de cereais como aveia, arroz, cevada, milho e trigo foram avaliados em cães por Kempe et al. (2004). No estudo, os ingredientes foram previamente cozidos e adicionado em um nível de inclusão de 30% em um alimento comercial com 20,98% de amido, obtendo-se dietas que apresentaram em média 34,54% de amido. A única diferença observada no metabolismo de glicose, entre as fontes, ocorreu 30 minutos após a refeição, quando a dieta com cevada induziu maior liberação de glicose. Já aos 60 minutos, não havia mais diferenças entre os demais tratamentos. Aos 90 minutos, a dieta com aveia (32,21% amido, 30,44% proteína e 19,93% gordura) promoveu maior liberação de glicose comparada com a dieta comercial (20,98% amido, 36,91% proteína e 24,75% gordura). Aos 30 minutos, a concentração de insulina foi menor para as dietas com trigo e milho em comparação a aveia, cevada e arroz. Aos 60 minutos, a curva de insulina foi maior para a dieta com aveia. Estes dados, no entanto, não são coerentes com a composição química e o esperado para estes ingredientes em relação à estudos com seres humanos. O método empregado não foi o mais correto, uma vez que foram adicionados a uma dieta comercial já preparada, resultando em alimentos não balanceados.

Em estudo que comparou seis fontes de amido (quirera de arroz, milho, sorgo, ervilha, lentilha e farinha de mandioca) em dietas extrusadas para cães, foi observado que a resposta imediata de glicose e insulina (área abaixo da curva < 30min) foi maior para quirera de arroz, milho e farinha de mandioca, e que a resposta mais tardia de insulina e glicose (área abaixo da curva acima de 30 min) maior nas dietas à base de sorgo, lentilha e ervilha (Carciofi, et al., 2008). Adolphe et al. (2012) também observaram que a ervilha induz menor liberação de glicose e insulina ao longe de 180 min após a refeição, além de um baixo índice glicêmico em cães em relação ao arroz e o milho. Resultados semelhantes foram observados quando uma dieta formulada com ervilha foi comparada com outra à base de arroz (Adolphe et al., 2015).

Recentemente, a resposta glicêmica, insulinêmica e o índice glicêmico de diferentes alimentos comerciais extrusados e formulados com diferentes categorias de fontes de amido (grãos tradicionais, *grain-free*, grão integrais e vegano) foram avaliados em cães (Rankovic et al., 2020). A concentração de glicose foi menor para

a dieta *grain-free* (104,7 mmol/L) comparado com a dieta com grãos tradicionais (177,2 mmol/L) após 150 minutos. A resposta insulinêmica não diferiu entre as dietas. Esta observação, no entanto, é confundida pelo fato de diferentes formulações de produtos comerciais terem sido comparadas, vários ingredientes e teores nutricionais diferiram, além da fonte de amido, incluindo o próprio teor de amido dos alimentos que foi diferente. Não é possível, dessa forma, se obter informações sobre os ingredientes em si.

2.6. Efeito dos carboidratos na extrusão e qualidade dos kibbles

A extrusão é um processo no qual um conjunto de ingredientes, previamente misturados e moídos, é cozido, sanitizado, texturizado e formatado na presença de umidade, temperatura, pressão e cisalhamento, em um curto espaço de tempo (Riaz, 2000; Tran et al., 2008). Esse processo inclui as etapas de pré-condicionamento, extrusão, corte, secagem e recobrimento, sendo que cada uma desempenha uma função específica tanto no cozimento quanto na formação dos kibbles (Riaz, 2000; Rokey, et al. 2010). É uma tecnologia amplamente utilizada na produção de alimentos para animais de companhia, organismos aquáticos e seres humanos devido a sua versatilidade, alta produção, baixo custo relativo e eficiência energética (Yeh e Jaw, 1999).

O pré-condicionamento faz parte de qualquer sistema de extrusão. Essa etapa ocorre no interior do pré-condicionador, um equipamento composto por um sistema de barras cilíndricas e um conjunto de pás dispostas radialmente que giram a uma velocidade variável (Riaz, 2000). Tem como objetivos básicos promover a hidratação, mistura e o aquecimento das partículas da matéria-prima. Nesta etapa, a água e o vapor são adicionados à ração farelada para que ocorra o início do cozimento do amido (Pacheco et al., 2018). Além de melhorar a estabilidade da extrusora e a qualidade do produto, o pré-condicionamento da ração farelada antes da etapa de extrusão reduz o desgaste do equipamento causado pelas partículas abrasivas da matéria-prima (Riaz, 2000; Rokey et al., 2010).

Em seguida, a matéria-prima pré-condicionada entra no canhão da extrusora, um tubo composto por um sistema de rosca (ou parafuso) sem fim, que gira a uma velocidade ajustável, equipada com anéis de retenção (Riaz, 2000). É nesta etapa que

o ocorre a principal transformação da ração pré-condicionada, pois a taxa de compressão é aumentada para auxiliar na mistura de água e vapor. Isso faz com que ocorra aumento da pressão e cisalhamento, transformando a massa amorfa em um fluido viscoelástico. A maior parte do aumento de temperatura no canhão da extrusora é proveniente da energia mecânica dissipada pela rotação da rosca, transmitindo força de cisalhamento, mas esta pode ser auxiliada pela injeção direta de vapor (Rokey et al., 2010). Além disso, a configuração da rosca pode ser alterada, escolhendo elementos de rosca diferentes como as roscas cortadas (ou interrompidas) ou adicionando anéis de retenção maiores, que irão dificultar a passagem da massa pela rosca. Todos esses fatores afetam o transporte do material através da rosca, influenciando a quantidade de energia mecânica que é adicionada através do parafuso. A velocidade da rosca também é variável utilizada para controlar a transferência de energia mecânica durante o processo (Riaz, 2000; Rokey et al., 2010).

Ao final do canhão extrusor existe uma matriz que desempenha duas funções: restrição e formatação. A matriz fornece restrição ao fluxo da massa, fazendo com que no interior da extrusora se desenvolvam pressão e cisalhamento necessários. A matriz também molda o produto extrusado. O design da matriz também é responsável por promover expansão, uniformidade e aparência do kibbles, através da área aberta da matriz (Ganjyal e Hanna, 2004; Rokey et al., 2010). Além disso, a matriz pode apresentar diferentes formatos (circular, triangular, etc.) e quantidades de furos abertos. Um conjunto de facas girando em velocidade constante e variável também é utilizado em conjunto com a matriz. As lâminas das facas ficam bem rentes a matriz e devem ser bem afiadas, para evitar a formação de finos que se quebram durante a secagem. Em seguida, a ração é transportada para um secador. O principal objetivo da secagem é reduzir a umidade do produto, aumentando sua estabilidade microbiológica. Os dois tipos de secadores mais utilizados são o horizontal e o vertical (Riaz, 2000; Rokey et al., 2010). Considerando que a gordura é um lubrificante e reduz a eficiência de transferência de energia mecânica, esta não é incorporada à massa, mas sim aspergida por recobrimento nos kibbles após secagem. Durante a etapa de recobrimento também são adicionados palatilizantes, para aumentar a aceitação do produto.

Durante o processo de extrusão, diversas alterações físicas e químicas ocorrem nos nutrientes, alterando sua qualidade nutricional (Singh e Smith, 1997; Rokey et al., 2010; Bordoloi e Ganguly, 2014). Essas modificações são dependentes da quantidade de energia específica total que é transferida para a massa durante o processo de extrusão (Monti et al., 2016; Pacheco et al., 2018). A energia específica total é composta pela soma das implementações da energia mecânica específica (EME) e energia térmica específica (ETE). A EME é aplicada através do cisalhamento ou atrito da massa no canhão extrusor, enquanto a ETE é adicionada a partir da adição de vapor e água, que podem ser aplicados no condicionador e/ou extrusor (Riaz, 2000).

Além de ser importante fonte de energia para os animais, o amido é o principal nutriente que auxilia o processo de extrusão e interfere na qualidade estrutural dos kibbles. Quando submetidos a adequada temperatura e quantidade de água, por tempo suficiente, os grânulos de amido incham e as regiões cristalinas são destruídas, fenômeno conhecido como gelatinização (Srichuwong et al., 2005; Ratnayake e Jackson, 2008). O amido gelatinizado torna-se solúvel em água e mais suscetível à degradação enzimática do que o amido cru (Murray et al., 2001; Dona et al., 2010). Além disso, a extrusão resulta na expansão do amido, alterando a estrutura e a qualidade do produto extrusado (Crane et al., 2000; Monti et al., 2016). A taxa de expansão é uma medida do grau de expansão do produto extrusado e é mensurada como a razão entre os diâmetros da seção transversal do extrusado e matriz (Karkle et al., 2012). Cinco etapas estão envolvidas no processo de expansão: transformações ordem-desordem, nucleação, inchaço do extrudado, crescimento da bolha e colapso da bolha. Primeiro, a elevada temperatura, pressão e força de cisalhamento dentro da extrusora induzem a transformação de amidos em uma massa viscoelástica. Em seguida, a nucleação da bolha ocorre em locais onde pequenas bolhas de ar estão presas dentro da massa amilácea. Essas bolhas crescem devido à evaporação da umidade (flash-off), após a massa ter saído da matriz da extrusora. A força que impulsiona o crescimento da bolha é a alta pressão do vapor superaquecido gerado pela vaporização da umidade nos núcleos, que supera a resistência mecânica da massa viscoelástica. Por fim, o crescimento das bolhas cessa

quando a matriz viscoelástica se torna vítrea após o resfriamento (Moraru e Kokini, 2003).

Por outro lado, embora a fibra tenha importante papel na nutrição de cães, auxiliando na perda de peso, cinética do bolo alimentar e saúde gastrointestinal, a adição de fibra afeta negativamente a aparência e a qualidade estrutural do extrusado. Estudos avaliando os efeitos da adição de fibras na qualidade dos kibbles em alimentos para cães são escassos. Até o momento, existe um estudo desenvolvido por Monti et al. (2016) mostrando que a inclusão gradual de fibra de goiaba, em uma formulação para cães, reduziu a gelatinização do amido, gerando produtos menos expandidos e mais duros. Embora os efeitos das fibras sejam poucos explorados em dietas para cães, existem vários estudos mostrando os efeitos da sua adição em alimentos para humanos (Robin et al., 2012). Assim como observado por Monti et al. (2016), a incorporação de fibra altera a textura dos alimentos, reduzindo a expansão e aumentando a dureza (Robin et al., 2012).

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CAPÍTULO 2 – Citrus pulp and orange fiber as a dietary fiber sources for dogs¹

¹ Artigo redigido conforme as normas da Animal Feed Science and Technology

Citrus pulp and orange fiber as a dietary fiber sources for dogs

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Abbreviations: ANOVA, analysis of variance; CPP, citrus pulp pellet; CO, control diet; CTTAD, coefficient of total tract apparent digestibility; OF, orange fiber; SCFA, short-chain fatty acids; SME, specific mechanical energy; STE, specific thermal energy; TDF, total dietary fiber; TSE, total specific energy; TVFA, total volatile fatty acids.

Abstract

Citrus pulp pellets (CPP) and orange fiber (OF) are coproducts of the citrus juice industry. OF is a novel dietary fiber source that contains elevated amounts of soluble fiber and has not yet been studied in dog nutrition. The objectives of this study were to evaluate the effects of CPP and OF inclusion on the extrusion parameters, kibble macrostructure, the coefficient of total tract apparent digestibility (CTTAD) of nutrients, fecal characteristics, fermentation end products, and palatability of dog foods. A control diet (CO) with no fiber source inclusion was formulated, and the experimental diets were composed of 30 g/kg, 60 g/kg and 120 g/kg CPP or 60 g/kg OF inclusion (on an as-fed basis). The extrusion process was carried out in a single-screw extruder, and processing parameters were recorded for each diet to determine energy balance and kibble macrostructure. Thirty beagle dogs were used to evaluate the CTTAD of nutrients and the fermentation products in feces. The results were analyzed by analysis of variance (ANOVA), and means were compared by polynomial and orthogonal contrasts. The inclusion of CPPs and OF reduced motor amperage, mass pressure, and specific mechanical energy transference ($P < 0.05$). Kibble macrostructure was affected by a decrease in expansion and an increase in hardness ($P < 0.05$). Starch gelatinization was reduced by CPP ($P < 0.05$) but not by OF inclusion ($P > 0.05$). Total dietary fiber (TDF) intake increased ($P < 0.05$) with the addition of CPP, with a linear reduction in CTTAD of nutrients and energy ($P < 0.05$). The OF treatment, however, presented similar CTTAD values of DM and CP to those of the CO diet ($P > 0.05$). The TDF CTTAD was higher for 60 g/kg OF than for 60 g/kg CPP ($P < 0.05$). The fecal pH decreased, and the contents of total short-chain fatty acids (SCFA), acetate, butyrate, valerate, and lactate increased with CPP and OF inclusion ($P < 0.05$), but the diet supplemented with OF induced the production of feces with a lower pH and higher total SCFA concentrations than those of feces produced from CPP inclusion the treatment with the same dose of CPP ($P < 0.05$). The OF diet presented a higher preference than the CO diet by dogs ($P < 0.05$). The CPPs and OF were fermentable fiber sources, increasing SCFA and butyrate contents in feces. The novel ingredient OF was more fermentable than CPP, with a limited impact on kibble formation, starch cooking, and CTTAD of nutrients and showing good acceptance on diets by dogs.

Keywords: butyrate, digestibility, extrusion, fermentation products, palatability, soluble fiber.

1. Introduction

Characterization and development of novel dietary fibers are of interest in the pet food industry, especially ingredients derived from natural sources and with functional properties on gut health. The potential health benefits of fiber intake depend on their physicochemical properties, including fermentability, solubility and viscosity development (Holscher, 2017). Insoluble and low- or nonfermentable fiber sources have primary effects on reducing the energy value of the diet (Kawauchi et al., 2011) and hairball control in cats (Loureiro et al., 2014), with possible effects on satiety (Loureiro et al., 2017), although this result is still controversial. In addition, this type of dietary fiber may increase fecal bulk (Kawauchi et al., 2011; Fischer et al., 2012; Loureiro et al., 2017), decrease gastric transit time and increase frequency of defecation (Burrows et al., 1982; Loureiro et al., 2017).

The soluble fiber fraction is considered the most effective to improve gut health by inducing physiological and structural changes, mainly through modulation of microbiota composition and metabolic activity (Maria et al., 2017; Cui et al., 2019). Among the potential benefits of soluble and fermentable fiber intake are the production of short-chain fatty acids (SCFA), especially butyrate, after microbial fermentation in the colon (Topping and Clifton, 2001). Butyrate is an important substrate for colonocyte energy metabolism and a growing factor for a healthy colon epithelium, improving the structure of the intestinal barrier (Jiminez et al., 2017; Peixoto et al., 2018) and reducing inflammation (Tan et al., 2014; Theodoro et al., 2019).

In this context, citrus pulp pellets (CPP) and orange fiber (OF) are coproducts generated by citrus juice extraction with potential use as natural soluble fiber sources for pet food. In addition to containing soluble and insoluble fiber, orange coproducts may also contain natural antioxidants such as ascorbic acid, flavonoids and carotenoids, essential oils, and minerals (Barros et al., 2012; Sharma et al., 2017). CPP were previously evaluated by Sunvold et al. (1995a), who described high *in vitro* organic matter disappearance and elevated SCFA levels when incubated using dog

fecal inoculum. Furthermore, dogs fed extruded diets supplemented with CPP presented similar nutrient and energy digestibility, fecal production, and moisture content to dogs fed diets supplemented with beet pulp (Sunvold et al., 1995b). The OF used in the current study is a novel ingredient in development with elevated amounts of soluble fibers that has not been previously studied in dogs.

Fibers are a highly structured, unexpandable material that possesses water holding capacity, so their inclusion in recipes modifies the material flow into the extruder barrel, altering extrusion parameters and cellular structure formation of extruded products (Karkle et al., 2012). In a study with dog formulations, fiber source addition increased electric energy consumption and kibble hardness and reduced starch cooking and kibble expansion (Monti et al., 2016). For this reason, it is advisable to study the effects of the inclusion of fiber sources on extrusion traits and kibble formation, allowing for the informed development of suitable product processing.

Considering that CPP and OF present high amounts of total dietary fiber, it is expected that their inclusion in dog formulations alters extrusion processing and kibble formation, reduces nutrient digestibility, and increases fermentation products on the feces of dogs. Therefore, the objectives of the present study were to evaluate the effects of CPP and OF inclusion on extrusion processing, kibble macrostructure, total tract apparent digestibility of nutrients and energy, fecal characteristics and fermentation end products, and the palatability of dog foods.

2. Materials and Methods

The study was performed at the Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, Brazil. All procedures with animals followed the ethical principles adopted by the Brazilian College of Animal Experimentation and were previously approved by the Ethics Committee on the Use of Animals (protocol number 017359/17).

2.1. Fiber ingredients

The fiber ingredients used were provided by Citrosuco Paulista S.A. (Matão, Sao Paulo, Brazil). CPP and OF are coproducts of orange processing to produce orange juice. CPP are a traditional ingredient mainly used as ruminant feedstuff

(Belibasakis and Tsirgogianni, 1996) and are composed of orange seeds, flavedo, albedo and central core, including the solids retained after juice filtration obtained after fruit pressing to extract the juice. This material represents approximately 50% of the fruit weight (as-is), with elevated moisture. The CPP was mixed with hydrated lime to neutralize free acids, heated to remove moisture and pelletized. The OF is a new ingredient in development that has not yet been studied in animals. This ingredient is composed of the material obtained by the juice filtration process, mainly comprising the walls of juice vesicles and fruit segments. The OF was washed with alcohol to extract soluble sugars and then dried. The analyzed chemical compositions of the CPP and OF samples used in the study are presented in Table 1.

Table 1. Analyzed chemical composition (g/kg, DM basis) of the fiber sources used in the study.

Item	Fiber sources ¹	
	Citrus pulp pellets	Orange fiber
Moisture	89.8	106.4
Ash	74.7	28.9
Crude fat	63.7	32.3
Crude fiber	148.6	199.1
Crude protein	67.9	110.3
Starch	60.5	25.5
Total dietary fiber	516.8	737.9
Insoluble dietary fiber	442.9	401.0
Soluble dietary fiber	73.9	313.6
Cellulose	226.8	253.2
Lignin	28.2	47.9
Pectin (as calcium pectate)	73.9	276.0
Soluble carbohydrates ²	216.4	65.1
Gross energy (kcal/g)	4.06	4.14

¹ CPP, citrus pulp pellet; OF, orange fiber. Fiber sources were provided by Citrosuco Paulista S.A. (Matão, SP, Brazil). ² Calculated as total mass minus ash, crude fat, crude protein, starch, and total dietary fiber contents.

2.2. Diet formulation and experimental design

Five experimental diets (Table 2) were formulated to supply the nutritional requirements of adult dogs according to FEDIAF (2018). A control diet (CO) without fiber source inclusion was formulated based on maize and poultry byproduct meal. Then, fiber sources were added to the CO formula replacing maize to create three inclusions of CPP at 30 g/kg (3CPP), 60 g/kg (6CPP) and 120 g/kg (12CPP), and one inclusion of OF at 60 g/kg (6OF). Adjustments to chicken byproduct meal and poultry fat inclusions were made to obtain similar nutrient compositions in the final diets.

Table 2. Ingredients composition of the dog foods with different inclusions of dietary fiber.

Ingredients (g/kg, as-fed basis)	Experimental diets ¹				
	CO	3CPP	6CPP	12CPP	6OF
Corn, grain	562.9	532.1	501.3	439.7	501.3
Chicken by-product meal	303.8	304.7	305.6	307.3	305.6
Citrus pulp pellets (CPP)	-	30.0	60.0	120.0	-
Orange fiber (OF)	-	-	-	-	60.0
Chicken fat	93.5	93.7	93.8	94.2	93.8
Palatant enhancer ²	20.0	20.0	20.0	20.0	20.0
Vitamin-mineral premix ³	5.0	5.0	5.0	5.0	5.0
Common salt	5.0	5.0	5.0	5.0	5.0
Potassium chloride	4.4	4.1	3.9	3.3	3.9
Choline chloride	3.2	3.2	3.2	3.2	3.2
DL-Methionine	1.0	1.0	1.0	1.0	1.0
Mold inhibitor ⁴	1.0	1.0	1.0	1.0	1.0
Antioxidant ⁵	0.3	0.3	0.3	0.3	0.3
Mean geometric diameter (µm)	275	261	264	256	262
Geometric standard deviation (µm)	1.29	1.30	1.32	1.31	1.33

¹ CO, control diet with no fiber source added; 3CPP, addition of 30 g/kg citrus pulp pellet; 6CPP, addition of 60 g/kg citrus pulp pellet; 12CPP, addition of 120 g/kg citrus pulp pellet; 6OF, addition of 60 g/kg orange fiber. ² D'TECH 10L, Palatabilizante Líquido, SPF do Brasil Indústria e Comércio Ltda., Descalvado, Brazil. ³ Rovimix, DSM Produtos Nutricionais Brasil

S.A., Jaguaré, Brazil. Added per kg of food: Vitamin A, 18,750 IU; Vitamin D3, 1,500 IU; Vitamin E, 125 IU; Vitamin K3, 1,5 mg; Vitamin B1, 5 mg; Vitamin B2, 16.25 mg; Pantothenic Acid, 37.5 mg; Vitamin B6, 7.5 mg; Vitamin B12, 45 mcg; Vitamin C, 0,125 g; Nicotinic Acid, 0.0625; Folic Acid, 0.75 mg; Biotin, 0.315 mg; Choline, 0.625 g; Iron, 0.1 g; Copper, 9.25 mg; Manganese, 6.25 mg; Zinc, 0.15 g; Iodine, 1.875 mg; Selenium, 0.135 mg. ⁴ Mold-Zap Citrus, Alltech do Brasil Agroindustrial Ltda., Araucária, Brazil. ⁵ Banox, Alltech do Brasil Agroindustrial Ltda., Araucária, Brazil.

To evaluate the implications of fiber source inclusion on extrusion processing and kibble formation, an extrusion study was conducted following a completely randomized design (all diets were extruded on the same day). For statistical comparisons, the experimental unit (treatment repetition) was used to establish the processing information and material samples that were taken every 15 min during the extrusion process. At least four samplings per treatment were obtained for processing parameter evaluation. Kibble characteristic evaluation was analyzed in a completely randomized design, and each kibble was considered an experimental unit with 20 repetitions (kibbles) analyzed per treatment.

The *in vivo* study included nutrient digestibility, fecal formation and fecal fermentation end product evaluation. This evaluation was organized in a randomized complete block design with five diets, three blocks of 10 dogs each, and two dogs per diet in each block, totaling six dogs per diet and 30 dogs in total. The blocking factor was time, due to the impossibility of handling the 30 dogs at the same time in the laboratory. The experimental unit was an individual dog. The palatability comparison was conducted with 37 dogs in a completely randomized design according to the two-pan method.

2.3. Diet preparation and extrusion processing

The ingredients of each diet were weighed individually for each batch, mixed in a horizontal single shaft mixer with a capacity of 450 kg/batch directly coupled into the mill, and ground in a high-speed hammer mill fitted with a 0.8 mm size screen sieve and having a 1000 kg/h capacity (Sistema Tigre de Mistura e Moagem, Tigre, Sao Paulo, Brazil). Extrusion processing was carried out on a single-screw extruder (MEX-250, Manzoni Industrial Ltda., Campinas, Brazil) with a production capacity of 250 kg/h,

equipped with a differential diameter cylinder preconditioner with a volume capacity of 60 liters. The preconditioner shaft speed in the small and large cylinders was set at 60 and 30 rpm, respectively, resulting in an average retention time of dough in the preconditioner of 180 seconds. An extruder screw profile typical for pet foods was used: first section – single flight screw and no steam lock; second section – single flight screw and small steam lock; third section – double flight uncut screw and small steam lock; fourth section – double flight uncut screw and medium steam lock; fifth section – double flight cut cone screw.

For operation conditions, steam and water application was implemented only into the preconditioner. The extruder screw was fixed at 622 rpm for all treatments. The extruder die was equipped with a probe to measure the mass temperature at the center of the product flow. A circular die with one opening of 7.0 mm in diameter was used. The extruder knife speed was set at 922 rpm for all treatments. After extrusion, the extrudates were dried in a dual pass dryer at 110 °C for 24 min. After a stable processing condition (approximately 30 min), the extrusion parameters were recorded, and samples from the preconditioner, extruder and dryer were collected four times every 15 min for each treatment and properly stored for later analysis (experimental unit).

The specific mechanical energy (SME, kW-h/ton), specific thermal energy (STE, kW-h/ton) and total specific energy (TSE, kW-h/ton) implementations were calculated with recorded parameters for each treatment repetition using the equation proposed by Riaz (2000) as described in more detail by Pacheco et al. (2018). As a complementary analysis of processing, the *in vitro* digestibility of organic matter (Hervera et al., 2007) and starch gelatinization (Sá et al., 2013) were determined for each treatment repetition through enzymatic methods previously described.

2.4. Digestibility test

The total tract apparent digestibility of nutrient evaluation was carried out through the quantitative collection of feces without urine, according to the recommendations of FEDIAF (2018). Thirty beagle dogs aged 3.2 ± 0.7 years old and weighing 12.4 ± 1.5 kg were used. The health of the animals was previously evaluated by physical examinations. Each 15-d digestibility trial included a 10-d food adaptation

phase and a 5-d total collection of feces. During the adaptation period, dogs were housed in 1.5 × 4.0 m kennels with a solarium and were released daily into a collective playground for exercise and socialization. In the fecal collection period, dogs were individually housed in 1 × 1 × 1 m stainless steel metabolic cages. The amount of food for individual dogs was calculated to satisfy the metabolizable energy requirement (NRC, 2006; ME, kcal/d = 110 × Body Weight^{0.75}) and was offered twice daily (10 h and 16 h). Offered and refused amounts were weighed, and the intake was recorded. Feces were quantitatively collected and weighed at each feeding time and immediately frozen at -20 °C. At the end of the digestibility period, feces were thawed, homogenized, pooled by dog, predried in a forced-air oven (MA035, Marconi, Piracicaba, Brazil) at 55 °C for 72 h, and stored for chemical analysis.

The fecal quality was scored on a 1 – 5 scale (Carciofi et al., 2008): 0 = watery liquid that can be poured; 1 = soft, unformed; 2 = soft, malformed stool that assumes the shape of its container; 3 = soft, formed, and moist stool that retains its shape; 4 = well-formed and consistent stool that does not adhere to the floor; and 5 = hard, dry pellets in the form of small and hard masses. During the fecal collection phase, samples of fresh feces were collected within 15 min after excretion (cages were continuously observed) on three consecutive days to measure fecal pH and the fermentative end-products short-chain fatty acids (SCFA; acetate, propionate and butyrate), branched-chain fatty acids (BCFA; isobutyrate, valerate and isovalerate), lactate and ammonia.

2.5. Raw material particle size distribution and kibble macrostructure evaluation

The mean geometric diameter (MGD) and geometric standard deviation (GSD) of the raw material mixture after grinding were calculated using 100 g of samples in a sieve agitator with a coupled hammer (ABRT 820, Bronzinox, Sao Paulo, Brazil). An agitation time of 10 min and 12 screen sieve sizes were used: 0.841 mm, 0.595 mm, 0.500 mm, 0.420 mm, 0.354 mm, 0.297 mm, 0.250 mm, 0.210 mm, 0.177 mm, 0.149 mm, 0.105 mm, 0.74 mm, and 0.53 mm plus plates (Zanotto and Bellaver, 1996). The results were integrated into software (Granucalc, Embrapa Suínos e Aves, Concordia, Brazil).

Kibble characteristics and macrostructure were analyzed in kibble samples collected from the dryer. The radial expansion ratio, specific length and piece density were determined by measuring the length, diameter, and mass of 20 representative extrudates, as described by Karkle et al. (2012). Hardness was analyzed in 20 kibble pieces (first stabilized at the same moisture in an oven at 35 °C for 24 h) using a texture analyzer (TAX/T2I, Stable Micro Systems, Godalming, UK) equipped with a load cell of 50 kgf and a cone probe.

2.6. Chemical analysis of ingredients, diets, and feces

Samples of ingredients, diets and predried feces were ground in a cutting mill using a 1 mm screen sieve size (MA680, Marconi, Piracicaba, Brazil). The chemical composition was determined according to official methods of the AOAC (1995) for dry matter (method 934.01); crude fat was determined by acid hydrolysis (method 954.02); ash content, by muffle furnace incineration (method 942.05); crude fiber (method 962.09) and crude protein (method 990.03) using a LECO nitrogen/protein analyzer (FP-528, LECO Corporation, Saint Joseph, USA); and total and insoluble dietary fiber by the enzymatic-gravimetric method (method 991.43). Soluble dietary fiber was calculated as total fiber minus insoluble fiber. Organic matter (OM) was calculated as DM minus ash. Gross energy (GE) was determined in a bomb calorimeter (IKA C2000 Basic, IKA-Werke GmbH & Co., KG, Staufen, Germany). The total starch content was determined using an enzymatic method (Hendrix, 1993). The pectin content (as calcium pectate) was measured according to Pearson (1976). Neutral detergent fiber (aNDFom), acid detergent fiber (ADFom) and lignin (sa) were determined according to Van Soest et al. (1991). The cellulose content was calculated by subtracting lignin from ADFom, while hemicellulose was calculated by subtracting ADFom from aNDFom.

Fecal pH was measured using a digital pH meter (DM20, Digimed Analítica Ltda., São Paulo, Brazil) immediately after collection by mixing 2 g of fresh feces with 6 ml of ultrapure water. The concentrations of fecal SCFA and BCFA were analyzed by gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan) according to Erwin et al. (1961). Briefly, 10 g of feces was mixed in 30 ml of formic acid solution at 4.2 N (1:3 w/v), precipitated at 4 °C for 72 h, and centrifuged (5,000 G at 15 °C for 15 min). Lactic acid was measured according to Pryce (1969) by mixing 3 g of feces with

9 ml of ultrapure water and subsequent evaluation with a colorimetric method (Spectrophotometer Quick-Lab, Drake, Sao José do Rio Preto, Brazil). Ammonia was assessed in the extracts prepared for SCFA according to Vieira (1980) in a nitrogen distillation system (Tecnal TE-036/1, Tecnal, Piracicaba, Brazil).

2.7. Palatability study

The CO, 6CPP and 6OF diets were compared for dog palatability, following the preference tests: CO *versus* 6CPP, CO *versus* 6OF, and 6CPP *versus* 6OF. These comparisons were performed at Panellis Latin America (Descalvado, Sao Paulo, Brazil) using a qualified panel with 37 dogs of different breeds, sexes, and body weights. The first choice (first product consumed) and preferred product (product consumed in greater amount) were determined using the two-pan method (Griffin, 2003). Dogs were housed individually and tested with two consecutive meals. Due to the differences in body weight, the results were calculated as the relative consumption of each diet, and the mean intake of the two meals was compared.

2.8. Statistical analysis

The results of the extrusion parameters were analyzed as a complete randomized design with four replications (sampling time) per treatment. The kibble characteristics were evaluated in a complete randomized design, with 20 experimental units (individual kibble) per treatment. Data on apparent digestibility and fecal parameters were analyzed in a completely randomized block design, with three blocks of 10 dogs and 6 experimental units (dogs) per treatment. The blocking factor was considered the time, as at the laboratory, it was not possible to evaluate the 30 dogs in the same period. All data were tested for homogeneity of variances and normality of errors and then submitted to ANOVA. The fecal score was analyzed by the Kruskal-Wallis test. When differences were found by the F-test, polynomial contrasts were used to compare means according to the CPP inclusion (0 g, 30 g, 60 g, and 120 g per kg), and orthogonal contrasts were used to compare CO *versus* 6OF and 6CPP *versus* 6OF. Data were evaluated using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). In the palatability study, the first preference was evaluated through the Chi² test,

and the food intake ratio was evaluated by Student's t-test. Statistical significance was considered at the level of 5% ($P < 0.05$).

3. Results

3.1. Fiber sources and experimental diets

The CPP contained higher values for ash, fat, and starch than the OF and lower values of TDF and insoluble fiber (Table 1). The OF presented elevated values of soluble fiber and pectin compared to those of the CPP. All experimental diets had similar ash, acid-hydrolyzed fat, crude protein, gross energy, and moisture content values. As expected, the starch content decreased (from 371 g/kg to 296 g/kg), and the TDF increased (from 61 g/kg to 121 g/kg) with graded levels of fiber sources (Table 3).

Table 3. Analyzed chemical composition (g/kg, DM basis) of the dog foods with different inclusions of dietary fiber.

Item	Experimental diets ¹				
	CO	3CPP	6CPP	12CPP	6OF
Moisture	57.4	48.4	56.8	45.8	56.6
Ash	77.4	79.9	81.9	84.1	79.1
Acid-hydrolyzed fat	162.5	161.4	162.0	162.0	163.1
Crude protein	271.5	273.6	274.1	270.0	272.3
Starch	370.9	340.9	325.1	295.8	321.7
Total dietary fiber	61.2	74.2	90.0	121.3	101.6
Insoluble dietary fiber	61.2	56.6	54.8	72.7	64.5
Soluble dietary fiber	0.0	17.6	35.2	48.6	37.1
Ca	16.3	18.2	18.7	19.4	17.3
P	12.4	13.4	13.3	12.9	13.2
Gross energy (kcal/g)	5.02	5.00	4.92	4.92	4.94

¹ CO, control diet with no fiber source added; 3CPP, addition of 30 g/kg citrus pulp pellet; 6CPP, addition of 60 g/kg citrus pulp pellet; 12CPP, addition of 120 g/kg citrus pulp pellet; 6OF, addition of 60 g/kg orange fiber.

3.2. Extrusion variables and kibble macrostructure

During the extrusions, the preconditioner discharge mass temperature was kept at approximately 84.1 ± 0.9 °C for all diets. The extruder motor amperage and the mass pressure before the extruder die decreased quadratically as CPP inclusion in the formulation increased ($P < 0.05$; Table 4). This, however, can be a result of the unintentional reduction in the product rate out of the extruder, which was lower for the 6CPP diet than the other diets. A reduction in amperage was also observed after 60 g/kg inclusion of OF ($P < 0.05$). The mass temperature before extruder diet increased quadratically with increasing CPP inclusion and was also higher for the 6OF diet than for the CO diet ($P < 0.05$). The bulk density after the extruder increased quadratically with increasing CPP inclusion ($P < 0.05$) but was not altered for the 6OF diet in comparison to the CO diet ($P > 0.05$). The mass in-barrel moisture presented a statistically significant variation, but the differences between treatments were too small to induce any significant effect.

The SME application reduced quadratically with increasing CPP inclusion ($P < 0.05$), following the reduction in motor amperage. The OF inclusion also promoted a reduction in SME compared to CO ($P < 0.05$). The STE application was similar in all treatments ($P > 0.05$), which combined with the reduction in SME implementation might explain the quadratic reduction in TSE application after CPP inclusion in the formulations ($P < 0.05$).

The kibble hardness increased linearly with increasing CPP inclusion ($P < 0.001$), as well as the inclusion of OF compared to that of the CO diet kibbles ($P < 0.05$), without significant differences between fiber sources ($P > 0.05$). The CPP and OF inclusions promoted a reduction in expansion rate and increases in specific length ($P < 0.05$), all together signaling a shift toward longitudinal rather than radial expansion, with the production of harder kibbles. Following the lack of effect on bulk density, OF inclusion also did not change kibble piece density ($P > 0.05$). Starch gelatinization was reduced linearly with CPP addition ($P < 0.05$) but was not altered by OF inclusion ($P > 0.05$). As expected, the *in vitro* digestibility of the OM was reduced after both fiber sources were included in the formulation ($P < 0.05$).

Table 4. Processing variables, kibble characteristics and macrostructure of dog foods with different sources of dietary fiber.

Item	Experimental diets ¹					SEM ²	P- value	Polynomial ³		Orthogonal	
	CO	3CPP	6CPP	12CPP	6OF			Linear	Quad	COx6OF	6CPPx6OF
<i>Extruder</i>											
Motor amperage (A)	39.9	38.8	37.7	39.4	38.7	0.18	<0.001	0.024	<0.001	<0.001	<0.001
Pressure (MPa)	20.4	20.3	18.8	22.1	19.6	0.31	0.002	ns	0.001	ns	ns
Mass Temperature (°C)	123.8	129.8	130.3	142.5	130.0	1.53	<0.001	<0.001	0.040	ns	0.008
Throughput (kg/h)	191.3	190.0	183.5	190.3	183.8	1.33	0.127	ns	ns	0.002	ns
Bulk density (g/L)	377.7	383.5	381.5	401.0	380.0	2.17	<0.001	<0.001	0.011	ns	ns
In-barrel moisture (%)	22.8	22.2	22.3	22.5	22.5	0.07	0.002	ns	0.001	ns	ns
<i>Energy Balance (kW-h/ton)</i>											
SME	12.9	11.3	10.2	12.3	11.6	0.24	<0.001	0.048	<0.001	<0.001	0.005
STE	35.8	32.5	30.5	33.3	33.9	0.88	0.122	ns	ns	ns	ns
TSE	48.7	43.8	40.7	45.3	45.5	0.98	0.005	ns	0.012	ns	ns
STE/SME ratio	2.8	2.9	2.9	2.8	2.9	0.08	0.778	ns	ns	ns	ns
<i>Kibble macrostructure</i>											
Hardness (N)	29.0	31.5	35.3	41.4	33.6	0.68	<0.001	<0.001	ns	<0.001	ns
Expansion rate	2.6	2.5	2.4	2.1	2.4	0.02	<0.001	<0.001	<0.001	<0.001	ns
Piece density (g/cm ³)	0.40	0.39	0.40	0.42	0.40	0.002	<0.001	<0.001	<0.001	ns	ns
Specific length (cm/g)	1.9	2.2	2.2	2.3	2.2	0.01	<0.001	<0.001	0.021	<0.001	ns

Starch gelatinization (%)	94.5	93.7	91.5	90.7	94.2	0.37	<0.001	<0.001	ns	ns	<0.001
<i>In vitro</i> digestibility of the OM (%)	85.3	84.4	83.3	80.9	82.5	0.38	<0.001	<0.001	0.005	<0.001	0.029

¹ CO, control diet with no fiber source added; 3CPP, addition of 30 g/kg citrus pulp pellet; 6CPP, addition of 60 g/kg citrus pulp pellet; 12CPP, addition of 120 g/kg citrus pulp pellet; 6OF, addition of 60 g/kg orange fiber. ² SEM = standard error of the mean ($n = 20$). ³ Linear or quadratic effect of CPP inclusion.

3.3. Nutrient intake and coefficient of total tract digestibility of nutrients

Nutrient intake and CTTAD of nutrients are reported in Table 5. All diets were well accepted by the dogs, with no episodes of refusals, vomiting or diarrhea. The inclusion of dietary fiber sources did not change the intakes of dry matter, organic matter, crude protein, or fat ($P > 0.05$). However, starch intake decreased linearly ($P < 0.05$) while TDF intake increased linearly ($P < 0.05$) with increasing CPP inclusion. Dogs fed the CO diet also had higher starch ($P < 0.05$) and lower TDF ($P < 0.05$) intakes than those fed the 6OF diet. No difference in nutrient intake was observed between the 6CPP and 6OF diets ($P > 0.05$).

The CTTAD of DM, OM, CP, fat and GE decreased linearly with increasing CPP addition ($P < 0.05$), while the TDF CTTAD increased linearly ($P < 0.05$). Starch digestibility also increased, despite the numerically small difference between diets ($P < 0.05$). In comparison to the CO diet, the 6OF diet presented lower CTTAD values of OM, fat, starch, and GE ($P < 0.05$), a higher CTTAD value of TDF ($P < 0.001$) and similar CTTAD values of DM and CP ($P > 0.05$). Comparing both fiber sources at the same inclusion level (6CPP vs. 6OF), similar CTTAD values of DM, OM, CP, fat, starch and GE were observed ($P > 0.05$), while a higher CTTAD value of dietary fiber was verified for the 6OF diet ($P < 0.05$).

Table 5. Nutrient intake and coefficients of total tract apparent digestibility of nutrient of diets with different sources of dietary fiber to dogs.

Item	Experimental diets ¹					SEM ²	P-value	Polynomial ³		Orthogonal	
	CO	3CPP	6CPP	12CPP	6OF			Linear	Quad	COx6OF	6CPPx6OF
<i>Nutrient intake (g/dog/day)</i>											
Dry matter	178.35	183.48	181.30	183.38	176.18	3.26	0.952	ns	ns	ns	ns
Organic matter	174.58	177.40	176.47	176.02	171.98	3.13	0.988	ns	ns	ns	ns
Crude fat	30.84	31.04	31.13	31.13	30.44	0.55	0.995	ns	ns	ns	ns
Crude protein	53.67	52.75	52.68	51.48	50.85	0.95	0.917	ns	ns	ns	ns
Starch	70.23	65.73	62.48	56.84	59.35	1.42	0.027	0.002	ns	0.014	ns
Total dietary fiber	11.67	14.74	17.30	23.31	18.97	0.82	<0.001	<0.001	ns	<0.001	ns
<i>Coefficients of total tract apparent digestibility</i>											
Dry matter	0.853	0.833	0.833	0.797	0.835	0.47	0.001	<0.001	ns	ns	ns
Organic matter	0.892	0.875	0.871	0.835	0.871	0.43	<0.001	<0.001	ns	0.016	ns
Fat	0.941	0.917	0.914	0.909	0.919	0.44	0.015	0.002	ns	0.019	ns
Crude protein	0.887	0.868	0.869	0.829	0.869	0.31	<0.001	<0.001	ns	ns	ns
Starch	0.998	0.998	0.999	0.999	0.998	0.01	<0.001	<0.001	ns	0.002	ns
Gross Energy	0.898	0.882	0.881	0.846	0.876	0.40	<0.001	<0.001	ns	0.011	ns
Total dietary fiber	0.177	0.211	0.311	0.388	0.378	2.20	0.0004	0.001	ns	0.001	0.028

¹ CO, control diet with no fiber source added; 3CPP, addition of 30 g/kg citrus pulp pellet; 6CPP, addition of 60 g/kg citrus pulp pellet; 12CPP, addition of 120 g/kg citrus pulp pellet; 6OF, addition of 60 g/kg orange fiber. ² SEM = standard error of the mean ($n = 36$). ³ Linear or quadratic effect of CPP inclusion.

3.4. Fecal characteristics and fermentation end products

The increasing inclusion of CPP linearly increased fecal production and quadratically reduced fecal DM ($P < 0.05$; Table 6). The inclusion of OF also increased the fecal production and fecal moisture content in comparison to dogs fed the CO diet ($P < 0.05$). Despite these differences, all dogs produced adequately shaped feces with similar scores ($P > 0.05$). Fecal pH decreased linearly with increasing CPP inclusion and was lower for dogs fed the 6OF diet than for those fed the CO diet ($P < 0.05$). When comparing the fiber sources, the fecal production, DM content and score were similar ($P > 0.05$), but fecal pH was lower in dogs fed the 6OF diet than in those fed the 6CPP ($P < 0.05$).

Dogs fed diets containing increasing CPP showed a linear increase in the fecal concentrations of acetate, butyrate, total SCFA, valerate, and total volatile fatty acid (VFA) ($P < 0.05$; Table 6), while the lactate concentration increased quadratically ($P < 0.05$). Higher acetate, butyrate, total SCFA and total VFA concentrations ($P < 0.05$) were also verified in the feces of dogs fed the 6OF diet compared to those in the feces of dogs fed the CO diet. Comparing the feces of dogs fed the 6CPP and 6OF diets, a lower valeric acid concentration but higher total SCFA and VFA concentrations were verified for dogs fed the 6OF diet ($P < 0.05$), which, in conjunction with the lower fecal pH from this diet than from the CPP diet, indicated that OF is more fermented in dog colon than CPP.

3.5. Palatability study

The diet formulated with OF was preferred by dogs, although there was no difference in the first choice between the CO and 6OF diets (35 vs. 65 %, respectively; $P > 0.05$), the proportion of intake was greater for the 6OF diet than for the CO diet (64 vs. 36 ± 38 %; $P < 0.05$). No differences were detected for palatability between the CO and 6CPP diets (first choice: 41 vs. 59 %; intake ratio: 41 vs. 59 ± 36 %, respectively; $P > 0.05$) or between the 6CPP and 6OF diets (first choice: 55 vs. 45 %; intake ratio: 49 vs. 51 ± 39 %, respectively; $P > 0.05$).

Table 6. Fecal characteristics and fermentation products of dogs fed diets with different sources of dietary fiber.

Item	Experimental diets ¹					SEM ²	P- value	Polynomial ³		Orthogonal	
	CO	3CPP	6CPP	12CPP	6OF			Linear	Quad	COx6OF	6CPPx6OF
<i>Feces characteristics</i>											
g/dog/day (as-is)	66.5	78.7	85.3	116.2	84.5	3.90	<0.001	<0.001	ns	0.045	ns
Fecal DM (g/kg)	393.9	390.7	357.0	321.5	342.9	0.61	<0.001	<0.001	0.031	<0.001	ns
Score ⁴	4.0	4.0	4.0	4.0	4.0	-	-	ns	ns	ns	ns
pH	6.51	6.19	5.98	5.76	5.83	0.055	<0.001	<0.001	ns	<0.001	0.037
<i>Fermentation products (mMol/g of DM)</i>											
Acetic acid	271.6	309.3	385.0	440.4	426.7	15.07	<0.001	<0.001	ns	<0.001	ns
Propionic acid	151.4	156.9	167.6	185.6	191.7	5.62	0.099	ns	ns	ns	ns
Butyric acid	48.7	65.6	78.1	90.6	82.8	2.95	<0.001	<0.001	ns	<0.001	ns
Total SCFA	471.7	523.5	630.7	716.6	701.2	21.99	<0.001	<0.001	ns	<0.001	0.050
Isobutyric acid	9.3	9.2	9.1	8.6	8.2	0.38	0.799	ns	ns	ns	ns
Isovaleric acid	17.8	16.5	14.7	14.0	12.3	0.63	0.241	ns	ns	ns	ns
Valeric acid	2.6	2.5	3.4	4.2	2.3	0.53	0.005	0.004	ns	ns	0.030
Total BCFA	26.7	28.2	27.2	26.7	22.8	1.32	0.339	ns	ns	ns	ns
Total volatile fatty acids	498.4	551.7	657.9	743.4	724.0	22.04	<0.001	<0.001	ns	<0.001	0.037
Ammonia (mMol/kg of DM)	159.0	151.2	161.6	151.6	145.9	4.53	0.830	ns	ns	ns	ns
Lactate (mMol/kg of DM)	1.6	2.2	3.1	4.6	2.7	0.20	<0.001	<0.001	0.019	<0.001	ns

¹ CO, control diet with no fiber source added; 3CPP, addition of 30 g/kg citrus pulp pellet; 6CPP, addition of 60 g/kg citrus pulp pellet; 12CPP, addition of 120 g/kg citrus pulp pellet; 6OF, addition of 60 g/kg orange fiber.

² SEM = standard error of the mean ($n = 36$).

³ Linear or quadratic effect of CPP inclusion.

⁴ Fecal scored on a 1 – 5 scale: 0 = watery liquid, which can be poured; 1 = soft, unformed; 2 = soft, malformed stool, which assumes shape of container; 3 = soft, formed, and moist, which retains shape; 4 = well-formed and consistent stool, which does not adhere to the floor; and 5 = hard, dry pellets, which are small and a hard mass.

4. Discussion

In the present study, two different dietary fiber sources obtained from orange processing for juice extraction were evaluated regarding their implications for diet processing in an extrusion system and intake by dogs. In general, the results suggested that both ingredients induce alterations in extrusion processing and kibble formation, which should be considered to establish adequate processing conditions. Due to the relevant soluble fiber fraction, the ingredients were fermented, especially increasing the butyrate content of feces, a desirable characteristic, especially when combined with the positive influence of OF on food palatability.

Although fibers are commonly included in dog formulations, the implications of this type of ingredient on extrusion processing and kibble formation are poorly explored in the scientific literature (Monti et al., 2016). Considering that small variations in processing conditions may affect extrusion variables and product quality (Ding et al., 2005), to properly evaluate the effects of CPP and OF, the operational variable feed rate, preconditioner temperature, screw configuration and speed, die design, in-barrel moisture, and knife speed were kept constant, so any measured variation was related to the food formulation. The observed decreases in amperage (or torque), die pressure and SME application with fiber inclusion were previously reported after apple pomace was added to extruded snacks (Karkle et al., 2012). These authors attributed these results to the high sugar content present in the fruit fiber studied. Sugars induce these changes due to their water binding capacity, holding large amounts of moisture, reducing starch hydration, and interfering with gelatinization and viscosity formation. This may result in limited processing with relatively low expansion and poor cellular structure formation (Pitts et al., 2014). On the other hand, a previous study about guava fiber, an insoluble fiber source in dog food formulations, showed the opposite result with increased amperage, SME application and mass pressure (Monti et al., 2016). The CPP and OF had some residual sugars, as can be observed in Table 1, which were calculated as soluble carbohydrates. Additionally, these ingredients present considerable amounts of soluble fiber, differing in this regard to guava fiber. Therefore, enhanced moisture holding capacity due to the soluble carbohydrates and soluble fiber content may explain the difference between the two fruit coproducts. In a study of citrus

fiber in extruded products for humans, this reduction in mass resistance to flow and SME application was already described (Pitts et al., 2016).

Nevertheless, the product temperature before the die increased to 142.5 °C, showing an increase in shear strength in the extruder barrel after CPP or OF inclusion. This finding agrees with the literature, as the temperature was shown to increase after fiber inclusion in dog food formulations (Monti et al., 2016). This effect might also be secondary to the water holding capacity of the soluble fiber and remaining sugar present on CPP and OF. Water acts as a plasticizer inside the extruder barrel, reducing the resistance to mass flow and the friction between the raw material, screw shaft and extruder barrel (Chen et al., 2010). Water injection was kept constant, with a small variation of less than 1% (from 22.2 to 22.8%) among diets (although statistically significant, its implications in processing were small). Therefore, it is possible to assume that the moisture retention by soluble fiber resulted in increased shear and temperature values (Jin et al., 1994). This limitation in available moisture may also explain the reduction in starch gelatinization observed after CPP inclusion, as starch hydration, swelling, and melting are necessary for proper cooking (Schirmer et al., 2015). Starch gelatinization, however, was not affected by OF. One possible explanation is the relatively small amount of soluble carbohydrates in this raw material, which corresponded to only 30% of the amount observed in CPP.

It is a general concept that fiber inclusion interferes with cellular formation, promoting axial expansion and limiting radial expansion, with an overall increase in piece and bulk density and kibble hardness (Robin et al., 2012; Monti et al., 2016). These effects were clearly observed for CPP inclusion in the current study. The bulk density is an important product quality attribute for commercial pet food production and is regularly measured during extrusion because of its great impact on the volume of food filling the package (Chessari and Sellaheewa, 2001). The final size, shape, and texture of the kibbles depends on the temperature and pressure differential inside the barrel and the atmosphere, inducing the flash-off of the mass moisture, which in turn deforms the molten starch mass, resulting in cellular formation and kibble expansion (Moraru and Kokini, 2003). Possible factors that might explain the relatively poor texture attributes of fiber-supplemented recipes are the effect of fiber conducting moisture out of the kibble, reducing cellular formation, the proportional reduction in

starch content, replacement by fiber, and the limitation on starch transformation (swelling, melting, and gelatinization) that fiber may induce (Karkle et al., 2012; Monti et al., 2016). In the present study, although OF increased the hardness and reduced the expansion rate and specific length of the kibble, this fiber source did not induce reductions in bulk and piece density. This might be explained by the lack of interference of OF on starch gelatinization, which was similar to the CO diet, allowing for adequate moisture flash-off and expansion. It is possible to suggest that this was a consequence of the lower amount of soluble carbohydrates (sugars) in OF than in CPP, as its soluble fiber content was in fact higher.

The observed reduction in CTTAD of nutrients with the inclusion of CPP or OF was expected, as it is well described after the inclusion of fibrous ingredients in formulations for dogs (Fahey Jr. et al., 1990; de-Oliveira et al., 2012; Sabchuck et al., 2017). In the few studies about CPP, Sunvold et al. (1995b) observed comparable CTTAD values of DM, CP, and fat in diets supplemented with cellulose, beet pulp or CPP for dogs. Brambillasca et al. (2013) also reported relatively low digestibility of DM, OM, and CP in dogs fed CPP; however, the authors only added the ingredient to a kibble diet and did not formulate and process a diet with CPP, which was fed *in natura* mixed with commercial kibbles, so their results are not very comparable with the current study. The reductions in DM, OM and GE CTTAD values were explained by fiber not being digested but excreted in feces (Kawauchi et al., 2011). The change in fat CTTAD was only apparent due to a relatively high endogenous fat excretion or explained by a possible interference of soluble fiber, increasing the digesta viscosity in the gut, reducing micelle formation and enzyme substrate interaction (Dégen et al., 2007). The reduction in fat digestibility also contributed to the reduction in GE digestibility.

When OF was included in the formulation, the same effects of CPP on the CTTAD of nutrients were observed, but the CP digestibility was not reduced and was similar to that of the CO diet. This reduced effect of OF on CP digestibility is reinforced by the fact that CPP inclusion increased the fecal concentration of valerate, suggesting an increase in protein fermentation in the colon, an effect not observed after OF inclusion. This is an interesting outcome of OF, as fibers are commonly added in low-energy formulations to reduce DM and GE digestibility (Loureiro et al., 2017; Kawauchi

et al., 2011); however, it is not desired to reduce CP digestion and absorption due to the importance of this nutrient to animal health.

On the other hand, a linear increase in TDF digestibility was observed when CPP were added to the formulations. The fermentation characteristics of soluble fiber sources can be observed by their TDF CTTAD; the more fermented the raw material is, the higher the reduction in the recovery of feces (Fahey et al., 1990; Fischer et al., 2012; Maria et al., 2017; Donadelli and Aldrich, 2019). This finding indicated elevated fermentability of the CPP dietary fiber by the colon microbiota of dogs. The OF showed even higher TDF fermentability than that of the CPP in the present study, as observed by the higher TDF CTTAD of the 6OF diet than of the 6CPP diet. This was possibly explained by a combination of the higher TDF content in OF than in CPP, which was 43% higher than that in CPP, combined with the differences in raw material sources. The CPP include the fruit seeds, flavedo, albedo, central core, and the solids of juice filtration, but OF is only composed of the material obtained by the juice filtration process, mainly constituting the walls of juice vesicles and fruit segments. These differences reflected 4.24 times more soluble fiber and 3.73 times more pectin in OF than in CPP, as can be observed in Table 1. Pectin, elevated in OF, is characterized as highly fermentable by intestinal microbiota (Fukunaga et al., 2003), a characteristic also observed in *in vitro* studies with dog fecal inoculum (Sunvold et al., 1995a, 1995b; Panasevich et al., 2015).

The observed increase in fecal production and moisture content are a consequence of the physicochemical properties of the dietary fiber, since this component is indigestible and is excreted in feces with a relatively high fecal bulk. Fiber also has water holding capacity, retaining moisture, and when fiber is fermented, it increases the number of osmotic particles in the colon that altogether increase fecal water content (Dai and Chau, 2017). These effects on fecal moisture and output are well documented for dogs fed diets containing different fermentable fiber sources, such as beet pulp (Fahey et al., 1990; Donadelli and Aldrich, 2019), citrus pulp (Sunvold et al., 1995b), guar gum (Diez et al., 1998) or pectin (Silvio et al., 2000). Despite this alteration in moisture, the fecal score was not altered, remaining ideal for dogs.

Due to their heterogeneous characteristics, different fiber sources present different effects on the gut bacterial community and host (Holscher, 2017). The

microbiota composition and metabolic activity are differently affected, resulting in variable concentrations of the different fermentation products (Sunvold et al., 1995b; Maria et al., 2017). The lowering of fecal pH and increase in SCFA observed in the present study are important indicators of bacterial fermentation in the colon. Lactate, a major organic acid produced from the fermentation of dietary fibers that can be further metabolized to acetate, propionate, and butyrate (Bourriaud et al., 2005), also increased with CPP and OF inclusion. Among the SCFA, butyrate is the primary substrate that controls colonic mucosal homeostasis, is the preferred energy source for colonic epithelium cells (Topping and Clifton, 2001), may affect cell differentiation and contribute to reducing inflammation (Jiminez et al., 2017; Theodoro et al., 2019), suggesting that it is important to improve the barrier function of the gut (Jiminez et al., 2017). Therefore, butyrate is associated with improved intestinal structure and health (Tan et al., 2014; Peixoto et al., 2018). Not all fermentable fiber sources, however, induce increases in butyrate, as observed for beet pulp (Fischer et al., 2012; Wambacq et al., 2016; Maria et al., 2017), whose fermentation pattern resulted in a relatively substantial increase in acetate and propionate. Due to this result, the elevation in fecal butyrate observed after CPP and OF intake in the present study is relevant and deserves future study in dogs regarding their impact on microbiota composition and intestinal inflammation and health. Reinforcing this point, pectin, particularly elevated in OF, has been considered a potential prebiotic in studies with animal models and humans (Fukunaga et al., 2003; Larsen et al., 2019).

The linear increase in fecal valerate levels after the inclusion of CPP may reflect the interference of this ingredient with the CP CTTAD, as already discussed, but also have other potential implications. Although the role of valeric acid in gut health is still poorly understood, recent studies have suggested its action on stimulating the growth of intestinal epithelium and reducing the incidence of necrotic enteritis in broilers (Onrust et al., 2018), inhibiting histone deacetylase activity, which has implications in a variety of disease pathologies (Yuille et al., 2018) and protecting the gastrointestinal system of patients subjected to radiation injuries (Li et al., 2020). This opens opportunities to study the possible implications of this fiber source on the gut health of dogs.

There are many aspects to consider when interpreting palatability results, such as ingredients, food processing, kibble macrostructure, added palatants, food chemical composition, and how all of these relate to sensory properties such as aroma, texture, shape and taste (Aldrich and Koppel, 2015). The OF and CPP were adequately accepted by dogs, as they did not reduce food preferences. In addition, the 6OF diet was preferred in comparison with the CO diet, so this orange coproduct seems to present some organoleptic properties that improved diet acceptability by dogs. The texture attributes were less impacted by OF than by CPP, as its addition did not reduce the overall expansion (bulk and specific density), so this might also account for diet acceptance. Few studies have evaluated the palatability effects of fibrous raw material addition to dog diets, but in general, a lower acceptance in comparison to that of diets not supplemented with fiber has been described (Koppel et al., 2015). Thus, considering the importance of the palatability attributes to the commercial performance of the diets, the results obtained pointed favorably to the use of CPP and especially OF in extruded foods for dogs.

5. Conclusion

CPP and OF differed in their nutritional composition, especially in their total, insoluble and soluble dietary fiber fractions, which were higher for OF than for CPPs, and in their soluble carbohydrate content, which was lower for OF. Both orange coproducts did not show a noticeable impact on extrusion processing parameters, but their inclusion negatively impacted kibble characteristics, with lower expansion and higher hardness. CPP inclusion additionally reduced starch gelatinization. CPP reduced the total tract apparent digestibility of nutrients and energy, but OF impacted nutrient digestibility less than CPP did, as it did not reduce the apparent digestibility of DM and CP. Both fiber sources were fermented, increasing dietary fiber apparent digestibility of the diets, reducing fecal pH and inducing elevation of short-chain fatty acids in feces. The level of butyrate was especially increased, with possible implications for gut health that deserve future investigations. The OF, in comparison with CPP, was more fermentable, as observed by higher dietary fiber apparent digestibility, lower fecal pH and higher total short-chain fatty acid levels in feces at the

same level of inclusion in diets. Additionally, OF inclusion enhanced the preference of the diet by dogs.

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CAPÍTULO 3 – Chickpea, dehulled oat, faba bean starch flour and pea as dietary starch sources for dog foods: effects on extrusion, digestibility, fermentation end-products in feces, palatability and postprandial glucose and insulin responses³

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Chickpea, dehulled oat, faba bean starch flour and pea as dietary starch sources for dog foods: effects on extrusion, digestibility, fermentation end-products in feces, palatability and postprandial glucose and insulin responses

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Abstract

The present study compared chickpea (CP), dehulled oat (DO), faba bean starch flour (FB) and pea (PE) incorporated to dog extruded food as the main carbohydrate sources on extrusion variables, kibble macrostructure, apparent nutrient digestibility, fecal characteristics, fermentation end-products, palatability, and postprandial responses of glucose and insulin. A control diet based on broken rice (BR) was formulated and used as a reference carbohydrate source, and eight test foods were obtained replacing 50% or 100% of the broken rice by the ingredients in study: DO50, DO100, PE50, PE100, CP50, CP100, FB50 and FB100. Foods were extruded in a single screw extruder. Coefficients of total tract apparent digestibility (CTTAD) were determined with 6 dogs per diet by total collection of feces, in a completely randomized block design. Fresh feces were used to evaluate pH and fermentation products. The diets with BR and 100% of each ingredient in study were evaluated in 9 dogs per diet to determine glucose and insulin postprandial responses. Data were submitted to ANOVA and means compared by Tukey test ($P < 0.05$). CTTAD of DM, OM and GE were similar among BR, DO50, CP50, FB50 and FB100 diets, but lower than BR for DO100, PE50, PE100, and CP100 treatments ($P < 0.05$). Dogs fed the CP100 diet had lower crude protein digestibility ($P < 0.05$). Animals fed DO100 showed lower fat digestibility than those fed BR ($P < 0.05$). Feces production and moisture were greater for dogs fed PE100 and CP100 than those fed BR ($P < 0.05$). Fecal pH was lower and fecal lactate higher for most of non-conventional starch sources compared to BR ($P < 0.05$). Dogs fed PE100 and CP100 had higher acetate, propionate, and total volatile fatty acids concentration on feces than dogs fed BR ($P < 0.05$). Dogs fed DO100 and CP100-based foods presented lower maximum insulin peak and lower total area under the curve (AUC) of insulin in comparison to BR diet ($P < 0.05$). In conclusion, the FB can replace BR, while DO and chickpea can replace up to 50% of BR without influencing digestibility of nutrients. The total substitution of BR by DO, CP and PE reduced digestibility with a potential use for low energy diets. PE and CP increased fermentation end-products formation and may be used in diets to support specific intestinal conditions. Diets based on DO and CP reduced the postprandial insulin secretion in dogs.

Keywords: carbohydrates, dietary fibre, grain-free, kibble macrostructure, starch metabolism

1. Introduction

In the current competitive environment, the pet food industry is always searching for new or alternative ingredients that allow to create new appealing's to costumers, promote health benefits for the animals. The marking focus on carbohydrate sources can be observed on the grain-free diets, based on legumes and tubers as starch sources, largely present nowadays on the pet food marketing (Alvarenga and Aldrich, 2020). In this regard, chickpea (CP; *Cicer arietinum* L.), dehulled oat (DO; *Avena sativa* L.), faba bean starch flour (FB; *Vicia faba* L.) and pea (PE; *Pisum sativum* L.) are potential alternative ingredients to the conventional cereal grains (e.g. maize and rice), but still little explored scientifically.

Although not an essential nutrient for dogs, starch is the major source of dietary energy in standard dry dog food formulations, may comprising from 30 to 60% of dry matter depending on formulation (Carciofi, et al., 2008). Starch in extruded diets is the nutrient that present the higher coefficient of total tract apparent digestibility (CTTAD) by dogs, being higher than 96% in puppy, adult and old animals (Murray et al., 1999; Carciofi et al., 2008; Domingues et a., 2019; Pezzali and Aldrich, 2019; Ribeiro et al., 2019). However, some factors such as carbohydrate source (Murray et al., 1999; Carciofi et al., 2008; Bazolli et al., 2015), starch-protein interactions (Dhital et al., 2017), physical granule form (Dhital et al., 2017; Svihus, Uhlen, & Harstad, 2005), starch type (Rooney and Pflugfelder, 1986; Svihus et al., 2005), particle size after processing (Bazolli et al., 2015) and processing conditions (Moore et al., 1980; Peixoto et al., 2018; Ribeiro et al., 2019) may change starch digestibility and metabolism by animals, which may impact their implications to gut and general health. Although these aspects have been studied for dog foods, most publications focused on the traditional cereal sources. Starch is also a nutrient implicated in altering and influencing postprandial glucose and insulin concentrations in dogs (Nguyen et al., 1998). It is believed that faster and more complete digestion and absorption of starch leads to greater postprandial insulin responses in humans (Wolever and Bolognesi, 1996) and

dogs (Murray et al., 1999; Carciofi et al., 2008). However, in situations of impaired carbohydrate metabolism such as obesity (Brunetto et al., 2011); diabetes mellitus (Teixeira et al., 2018) or old age (Ribeiro et al., 2019) slowly carbohydrates that minimizes insulin responses are advantageous.

In addition to present starch, carbohydrate sources also have fiber, such as the legume seeds that may present higher fibre content than cereals (Carciofi et al., 2008; Alvarenga et al., 2020). Dietary fibre comprises complex substances that is not digested by the digestive enzymes of the small intestine, reducing nutrient and energy digestibility (Carciofi et al., 2008; Kawauchi et al., 2011), becoming available as a substrate for colonic bacterial fermentation, leading to the production of short-chain fatty acids (Knudsen, 2015), and may altering glucose and insulin postprandial responses (Alminger and Eklund-Jonsson, 2008; Gemen et al., 2011). Due this, the general impact of a starch source depending not only on it starch amount and type, but also on its fiber content and fermentation profile.

Lastly, but not less importantly, starch is also a major contributor for a good extrusion processing (Carciofi et al., 2012; Gibson and Alavi, 2013; Pacheco et al., 2018). Starch facilitates the extrusion process and product texture, allowing an adequate expansion rate, cellular structure formation and crispness of the kibbles (Allen et al., 2007; Monti et al., 2016). Although is recognized the importance of the starch to proper kibble formation and appearance, few studies determined the amount required for an efficient extrusion and the influence of the ingredient type on extrusion characteristics and kibble formation (Monti et al., 2016).

Considering the differences on chemical composition and starch granule structure, its hypothesized that CP, DO, FB, and PE may alter the extrusion characteristics, as well as diet digestibility, fermentation in colon and postprandial insulin responses in dogs. The effects of these different sources may probably be largely dependent on its chemical composition, specially their dietary fiber content and characteristics. The knowledge of these implications would allow the industry to make conscious decisions about the specific utilization of these carbohydrates sources. Therefore, the objectives of this study were to evaluate the effects of CP, DO, FB, and PE included replacing broken rice on extrusion variables, kibble macrostructure,

CTTAD of nutrients and energy, fecal characteristics, fermentation end-products, palatability, and postprandial glucose and insulin responses in dogs.

2. Materials and methods

The present study was performed at the Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos “Prof. Dr. Flávio Prada” located at the Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias (UNESP/FCAV), Campus Jaboticabal, SP, Brazil. The experimental procedures and housing were approved by the Ethics Committee on the Use of Animals (protocol number 017360/17) of UNESP/FCAV and performed in accordance with the Brazilian College of Animal Experimentation.

2.1. Diets and experimental design

Five carbohydrate sources (Table 1) were studied: broken rice (BR), chickpea (CP), dehulled oat (DO), faba bean starch flour (FB) and pea (PE). A control diet based on polished broken rice (BR) were formulated following the nutritional guidelines of the European Pet Food Industry (FEDIAF, 2019) for adult dogs. Each carbohydrate source was evaluated in two inclusion amounts. To this, the test ingredient replaced 50% or 100% of the BR in the formula, remaining constant all the other ingredients (Table 2). The procedure resulted in nine experimental diets: control diet based on broken rice (BR), diet in which dehulled oat replaced 50% of the BR (DO50), diet with 100% BR was replaced by dehulled oat (DO100), diet with 50% BR replacement by faba bean starch flour (FB50), diet with 100% BR replacement by faba bean starch flour (FB100), diet with 50% BR replacement by pea (PE50), diet with 100% BR replacement by pea (PE100), diet with 50% BR replacement by chickpea (CP50) and diet with 100% BR replacement by chickpea (CP100).

The extrusion study followed a completely randomized design with nine treatments and four replications per treatment. The experimental replication was considered the data and sampling collection at each 10 to 15 min during a stable processing operation. The digestibility study followed a randomized block design with six blocks of nine dogs each, one dog per food in each block, totaling six replications per food. The blocking factor was period, as the laboratory facilities does not allow the

use of all dogs at the same time. In each block (period), the foods were fed for 15 d; a 10 d for food adaptation followed by a 5-d total feces collection for apparent digestibility calculation and fresh feces collection to determine pH and fermentation end-products. The postprandial responses of glucose and insulin study included only the BR and the diets with 100% replacement of carbohydrate source, totaling 5 diets. It followed a randomized block design with nine blocks of five dogs each, one dog per diet in each block, totaling nine dogs (replications) per food.

Table 1. Analyzed chemical composition (% , DM basis) of the carbohydrate sources used in the study.

Item	Carbohydrate sources				
	BR	DO	PE	CP	FB
Moisture	12.07	11.13	11.39	10.29	11.11
Ash	0.82	1.38	3.12	3.14	2.41
Crude fat	2.30	5.91	2.04	5.91	1.59
Crude fiber	0.64	1.11	7.08	2.23	1.50
Crude protein	9.40	14.84	24.46	21.61	20.98
TDF	1.93	11.88	25.81	13.00	8.83
IDF	1.93	3.60	23.95	10.98	5.95
SDF	0.00	8.28	1.86	2.01	2.88
Starch	72.45	60.53	34.15	40.16	56.05
GE (kJ/g)	17.95	19.08	18.33	19.20	18.16

BR, broken rice; DO, dehulled oat; PE, pea; CP, chickpea; FB, faba bean starch flour. TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; GE, gross energy.

Table 2. Ingredients (% as-fed) composition of dog food formulations with alternative carbohydrate sources.

Item	Broken	Dehulled oat		Pea		Chickpea		Faba bean	
	rice	50 %	100 %	50 %	100 %	50 %	100 %	50 %	100 %
Polished broken rice	46.38	23.18	-	23.18	-	23.18	-	23.18	-
Dehulled oat	-	23.20	46.38	-	-	-	-	-	-
Pea	-	-	-	23.20	46.38	-	-	-	-
Chickpea	-	-	-	-	-	23.20	46.38	-	-
Faba bean starch flour	-	-	-	-	-	-	-	23.20	46.38
Poultry by-product meal	36.50	36.50	36.50	36.50	36.50	36.50	36.50	36.50	36.50
Poultry fat	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50
Beet pulp	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palatant enhancer	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit-min premix *	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Potassium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Choline chloride	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Mold inhibitor [†]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant [‡]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Mean geometric diameter (µm)	236	232	287	269	273	275	325	320	384

Geometric standard device	1.42	1.49	1.46	1.40	1.38	1.38	1.46	1.45	1.48
(μm)									

* Rovimix, DSM Produtos Nutricionais Brasil S.A., Jaguaré, Brazil. Added per kg of food: Vitamin A, 18,750 IU; Vitamin D3, 1,500 IU; Vitamin E, 125 IU; Vitamin K3, 1,5 mg; Vitamin B1, 5 mg; Vitamin B2, 16.25 mg; Pantothenic Acid, 37.5 mg; Vitamin B6, 7.5 mg; Vitamin B12, 45 mcg; Vitamin C, 0,125 g; Nicotinic Acid, 0.0625; Folic Acid, 0.75 mg; Biotin, 0.315 mg; Choline, 0.625 g; Iron, 0.1 g; Copper, 9.25 mg; Manganese, 6.25 mg; Zinc, 0.15 g; Iodine, 1.875 mg; Selenium, 0.135 mg.

† Mold-Zap Citrus, Alltech do Brasil Agroindustrial Ltda., Araucária, Brazil.

‡ Banox, Alltech do Brasil Agroindustrial Ltda., Araucária, Brazil.

2.2. Diets processing

The ingredients of each treatment were mixed and ground in a hammer mill fitted with a 0.8 mm screen sieve size (Sistema Tigre de Mistura e Moagem, Tigre, Sao Paulo, Brazil). During food production, the DO100 diet did not flow through the mill, been retained in the milling chamber. Due this, only for this treatment was necessary to use a 1.4 mm screen sieve size on the hammer mill. After particle size reduction, extrusion process of diets was carried out on a single-screw extruder (MEX-250, Manzoni Industrial Ltda, Campinas, Brazil) with a 250 kg/h production capacity, equipped with a differential diameter cylinder (DDC) preconditioner. The extruder screw profile had five sections: first – single flight screw and no steam lock; second – single flight screw and small steam lock; third – double flight uncut screw and small steam lock; fourth – double flight uncut screw and medium steam lock; fifth – double flight cut cone screw. A circular die with one opening of 7.0 mm in diameter was used. During production, the DO100 diet did not extrude adequately using this configuration, the kibble shown practically no expansion (bulk density after extruder > 500 g/l). Consequently, for this treatment a circular die with one opening of 4.5 mm in diameter was used, the extruder screw profile was altered on the third section using a double flight cut screw, and a large steam lock was used after the fourth screw element. After extrusion, the diets were dried in a double-deck horizontal air dryer for 20 min at 110°C and coated with poultry fat and palatants.

The target extrusion processing conditions was established for the control BR diet. After the stabilization, the software conditions were kept constant and were not changed for any diet, except the hardware configurations to the DO100 diet. Due to this all alterations measured was attributed to an influence of the carbohydrate source. For each treatment, after processing stabilization a minimum of 30 min was awaited, and extrusion parameters were recorded every 10 to 15 min, as detailed previously (Pacheco et al., 2018). Kibble bulk density (g/l) after extrusion was recorded at each sampling time (measured as the weight of food corresponding to a 1 L volume). The mass flow rate from the extruder was measured directly in a bucket at each sampling time. At each observation time, food samples were collected from the preconditioner, from the extruder and from the dryer, and stored at -20°C for further analysis.

The specific mechanical energy (SME; kW-h/ton) was calculated using the following equation:

$$\text{SME} = ((\sqrt{3} \times \text{Voltage} \times (\text{At} - \text{Av}) \times \cos\text{Fi}) \div 1000) \times 1000 / \text{M}$$

Where: Voltage = 220 V; At = torque load working amperage (A); Av = no torque load working amperage (A); cosFi = power factor; M = mass flow rate from extruder (kg/h).

The specific thermal energy (STE; kW-h/ton) in the preconditioner and extruder was calculated by mass and energy balance equations according to Riaz (2000) as described in more detail by Pacheco et al. (2018). The feed, water and steam total input and output mass amounts were determined. These mass values and the corresponding specific heat values from each component of the system were used to calculate the total amount of heat produced. The total specific energy (TSE; kW-h/ton) was obtained by the summation of SME and STE.

2.3. Particle size distribution and kibble characteristics

The mean geometric diameter (MGD) and geometric standard deviation (GSD) of the raw materials mixture after grinding were performed according to the methodology described by Zanotto & Bellaver (1996) using 100 g of sample in an analytical sieve shaker with a horizontal circular motion and a vertical tapping motion (ABRT 820, Bronzinox, Sao Paulo, Brazil). An agitation time of 10 min and 12 screen sieves sizes were used: 0.841 mm, 0.595 mm, 0.500 mm, 0.420 mm, 0.354 mm, 0.297 mm, 0.250 mm, 0.210 mm, 0.177 mm, 0.149 mm, 0.105 mm, 0.74 mm, 0.53 mm plus plate. The MGD and GSD were calculated using Granucalc software (Embrapa Suínos e Aves, Concordia, Brazil).

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

starch gelatinization (Sá et al., 2013) and the in vitro digestibility of organic matter (Hervera et al., 2007) were measured.

2.4. Digestibility protocol

A total of 54 healthy adult Beagle dogs with a mean of 3.4 ± 0.8 years and 11.8 ± 1.2 kg were used. The digestibility protocol was carried out through of total collection of feces without urine collection method considering the FEDIAF (2019) recommendations. During the adaptation period (10 d), dogs were housed in 1.5×4.0 m kennels with a solarium and were released daily into a collective playground for exercise and socialization. During the feces collection period (5 d), dogs were individually housed in $1.0 \times 1.0 \times 1.0$ m stainless steel metabolic cages. Animals were fed twice daily (09:00 and 16:00 h) in sufficient amounts to cover their metabolizable energy requirements ($ME, \text{kcal/d} = 110 \times \text{Body Weight}^{0.75}$), as recommended by the National Research Council (NRC, 2009). Water was offered ad libitum. Fecal output was quantitatively collected and weighed at each feeding time, and immediately stored at -20°C until further processing. At the end of the collection period, feces were pooled by dog, thawed, homogenized, and dried using a forced-air oven (MA035, Marconi, Piracicaba, Brazil) at 55°C for 72 h for chemical analysis. The fecal consistence was scored on a 0 to 5 scale (Carciofi et al., 2008): 0 = watery liquid that can be poured; 1 = soft, unformed; 2 = soft, poorly formed stool that assumes the shape of its container; 3 = soft, formed, and moist stool that retains its shape; 4 = well-formed and consistent stool that does not adhere to the floor; and 5 = hard, dry pellets, which are small and hard masses.

2.5. Chemical analyses

Experimental ingredients, diets and dried feces were ground in a cutting mill (MA680, Marconi, Piracicaba, Brazil) fitted with a 1.0 mm screen sieve and analyzed according to official methods (AOAC, 1995) for dry matter (method 934.01); crude fat was determined by acid hydrolysis (method 954.02); ash content, by muffle furnace incineration (method 942.05); crude fiber (method 962.09); crude protein (method 990.03) using a LECO nitrogen/protein analyzer (FP-528, LECO Corporation, Saint Joseph, USA); and total and insoluble dietary fiber by the enzymatic-gravimetric

method (method 991.43). Soluble dietary fiber was calculated as total fiber minus insoluble fiber. Organic matter (OM) was calculated as DM minus ash. Gross energy (GE) was determined in a bomb calorimeter (IKA C2000 Basic, IKA-Werke GmbH & Co., KG, Staufen, Germany). The total starch content was determined using an enzymatic method (Hendrix, 1993). All analyses were carried out in duplicate and repeated when the coefficient of variation was higher than 5 %.

2.6. Fecal pH and fermentation end-products

Fresh fecal samples were taken before 15 min of defecation on three consecutive days to pH, short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), lactate and ammonia determination. Fecal pH was measured using a pHmeter (DM20, Digimed Analítica Ltda., São Paulo, Brazil) immediately after collection in 2 g of fresh feces diluted in 6 ml of ultrapure water. For SCFA and BCFA analyses, 10 g of fresh feces were placed in collector containers and immediately stored at -20°C until further processing. At the end of collection, each sample taking was mixed in 30 ml of formic acid solution at 4.2 N, pooled by animal and the supernatant was centrifuged three times (5,000 G at 4°C for 15 min). The analysis was performed by gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan) according to Erwin, Marco, & Emery (1961). Lactic acid was measured according to Pryce (1969) by mixing 3 g of fresh feces with 9 ml of ultrapure water and subsequent evaluation with a colorimetric method (Spectrophotometer Quick-Lab, Drake, Sao José do Rio Preto, Brazil). The ammonia concentration was assessed in the extracts prepared for SCFA and BCFA analysis according to Vieira (1980). For this, 2 ml of extracts were thawed at room temperature, diluted in 13 ml of ultrapure water and in 5 ml of potassium hydroxide solution at 2 N. The solution was distilled in a nitrogen system (Tecnal TE-036/1, Tecnal, Piracicaba, Brazil) and recovered in a beaker containing 10 ml boric acid solution. Ammonia was titrated using hydrochloric acid at 0.005 N and then calculated.

2.7. Postprandial glucose and insulin responses

The glucose and insulin postprandial responses of dogs were determined only in the control diet (BR) and the formulations with 100% replacement of BR by the tested

ingredients, totaling five diets (BR, DO100, CP100, PE100 and FB100). A total of 45 healthy adult Beagle dogs, males or females, with a mean of 3.2 ± 1.0 years, 12.1 ± 1.2 kg and 5.6 ± 1.6 body condition score (BCS) on a nine-unit scale (Laflamme, 1997) were used. Postprandial response profiles were evaluated after the digestibility trials following the procedure described by Carciofi et al. (2008), with modifications to blood collection times: 0 (zero before the meal), 15, 30, 60, 120, 180, 240, 300, 420, 540 and 720 min after the end of the meal. Dogs were conditioned to ingest all food within 10 min, in one single meal per day. Thereby the animals were tested 24 h after the last meal. The time counting to establish blood sampling was started immediately after the end of the meal. On the day of blood collection, each dog was aseptically catheterized using a peripheral intravenous catheter inserted into the cephalic vein (Angiocath 20 GA x 1.16 in., Becton-Dickinson, Franklin Lakes, NJ, USA). In each collected time, 3.0 ml of blood were collected and divided in two containers. For glucose analysis, 1.0 ml of blood was deposited into sodium fluoride and EDTA tube (NaF/Na₂EDTA, BD Vacutainer), centrifuged at 2000 g for 10 min at 24°C, and the plasma separated into a polypropylene tube. For insulin test, 2.0 ml were deposited in the container with anticoagulant (Tube serum, BD Vacutainer), centrifuged at 2000 g for 10 min at 24°C and the serum separated into a polypropylene tube. Glucose and insulin samples were stored at -80°C until analysis. Before collecting the samples, 0.3 ml of blood was withdrawn and discarded to avoid any dilution of the analytes of interest by saline in the catheter.

The plasma glucose concentrations were determined with the glucose oxidase test (GOD-ANA, Labtest Diagnóstica S.A., Lagoa Santa, Brazil) using a semi-automated glucose analyzer (Labquest model BIO-2000, Labtest Diagnóstica S.A., Lagoa Santa, Brazil). All the analysis was conducted in duplicate and repeated when the coefficient of variation was greater than 5%. The serum insulin concentration was performed using a kit Quantikine ELISA (human/canine/porcine insulin immunoassay, R&D Systems, Minneapolis, USA), following the recommendations of the manufacturer. The reading was performed in microplate reader (Biochrom Asys Expert Plus, Biochrom, Cambourne, Cambridge, UK), using a 450 nm filter. The insulin analysis inter-assay coefficient of variation was 6.5% and the intra-assay coefficient of variation was 6.3%. The responses profiles were compared computing the basal,

minimum, mean, and maximum concentrations, and the time to peak of the absolute and incremental values (absolute minus basal metabolite value of the animal). The integrated areas under the postprandial glucose and insulin response curves were calculated by numerical integration by the trapezoidal method using software GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

2.8. Palatability testing

Palatability comparisons was conducted at Affinity Petcare (Campinas, São Paulo, Brazil) using the two-pan test (Griffin, 2003). All test foods were compared against to the BR, totaling eight comparisons. A panel of 20 adult dogs of different breeds, sex and body weight, trained and validated to perform palatability comparisons were used. For each test, dogs were individually offered two identical bowls, one with each diet, placed side-by-side. At the end of the 30 min test period, the amount of each food consumed was measured. The protocol was performed on two consecutive days, changing the bowls position on second day to avoid side bias. The average food intake of the two meals was used for statistical comparisons. The food chosen first was also recorded. Due to the different body weight of the dogs, food preference was calculated based on the intake ratio.

2.9. Statistical analysis

The results of the extrusion processing were analyzed in a complete randomized design. The experimental unit was considered the sampling and data collection at each 10 to 15 min during a stable production, with four repetitions per treatment. For extrudate macrostructure comparison, the experimental unit was one kibble with 20 repetitions per treatment. Data on digestibility and fecal characteristics and fermentation end-products were analyzed in a randomized block design, with six blocks (period) and one repetition (dog) per block, totaling six dogs per food. The experimental unit was one dog. Response profiles were analyzed for each postprandial period using a mixed model analysis to allow for repeated measures over time (diet and time). Fecal score and body condition score were evaluated by the Kruskal-Wallis test. All data were submitted to analysis of variance by the MIXED procedure of the SAS Software, when differences were detected at F test, means were compared by

the Tukey test. Significant differences were set up at the 5% and tendency at 10% level. Palatability test was analyzed in a completely randomized design and results of food intake ratio compared by the Student's t-test at 5% probability level.

3. Results

3.1. Carbohydrate sources and diets

The carbohydrate sources presented different nutrient composition (Table 1). Although the moisture contents were similar among ingredients, crude protein content was higher in PE (24.46%), CP (21.61%) and FB (20.98%), while BR and DO were, respectively, 9.40% and 14.89%. Ash content was similar among carbohydrate sources. CP and DO had higher fat content than other sources. Total dietary fibre (TDF) content was higher in PE (25.81%) followed by DO (11.88%), CP (13.00%), FB (8.83%) and BR (1.93%). DO presented the highest soluble fibre fraction content (8.28%) than other sources. On the other hand, BR had higher (72.45%) and PE had lower (34.15%) starch content.

The difference in ingredient nutrient composition led to a difference in food nutritional composition once diets were not formulated to be iso-nutrient (Table 3). Moisture, ash, and gross energy contents were similar among diets. Crude protein levels were above of 31% for all non-conventional ingredient diets compared to BR diet (28.90%), while starch content was lower for all diets compared to BR diet. The PE100 and CP100 had lowest values for starch content, below 20%. Total dietary fibre content increased as non-conventional carbohydrate sources were added to the diets, being higher on PE100 (11.85%) and CP100 (10.61%) diets, and lower on BR (3.28%) diet.

Table 3. Analyzed chemical composition of dog food formulations with alternative carbohydrate sources.

Item	Broken	Dehulled oat		Pea		Chickpea		Faba bean	
	rice	50%	100%	50%	100%	50%	100%	50%	100%
Moisture	6.44	6.66	5.55	7.63	6.60	6.41	5.63	6.08	6.10
Ash	9.14	9.19	9.83	9.18	10.49	9.20	10.51	9.11	9.35
Crude fibre	1.47	1.82	2.06	2.67	4.76	2.18	3.01	1.88	1.80
Crude protein	28.90	31.08	32.75	33.31	35.46	33.45	34.88	32.40	33.90
Crude fat	15.92	18.08	18.66	17.67	17.31	15.65	19.31	16.34	18.76
Starch	37.62	31.45	25.91	28.48	19.77	29.77	19.25	31.38	26.35
Total dietary fibre	3.28	5.38	7.22	8.56	11.85	7.58	10.61	4.01	6.09
Insoluble dietary fibre	2.64	3.45	4.44	7.33	8.63	5.10	7.61	2.63	3.94
Soluble dietary fibre	0.64	1.93	2.79	1.23	3.22	2.48	3.00	1.39	2.15
Gross energy (kJ/g)	20.38	21.09	21.05	20.50	20.29	20.46	20.38	20.42	20.79

3.2. Extrusion processing and kibble macrostructure

The results of the extrusion variables are described in Table 4. The first important result of our study is about the manufacturing of the diet with 100% of dehulled oat, since it was not possible to grind the DO100 diet in the same screen size of the others (0.8 mm) and the extrusion conditions had to be changed to better shape the product. In general, few differences were observed among diets after the replacement of broken rice by the ingredients used in this study. The main variation was observed for the diet with 100% dehulled oat (DO100), however, this cannot be solely attributed to the ingredient, but to the more restrictive extruder configuration changes as it was mentioned before. The extruder open area was approximately 250 mm²/ton/h for the other diets (original configuration), while the more restrictive die resulted in 120 mm²/ton/h for processing DO100. This higher restriction together with the use of a cut screw on third zone and a large steam lock on the fourth zone increased the motor amperage, SME transference, and mass pressure before the die ($P < 0.05$). Thermal energy application was also higher for the DO100 diet, a greater infusion of steam resulted in higher preconditioner temperature ($P < 0.05$). All this higher energy input allowed a more adequate product formation and a bulk density inside the range of the other diets, which assured the DO100 intake by the dogs.

Although the ingredients induced few changes on software extrusion variables, the implications to the kibble macrostructure were relevant, as all test diets presented higher bulk density than the BR diet ($P < 0.05$). Additionally, comparing 50% and 100% replacement in carbohydrate sources, in general higher bulk densities was observed for 100% replacement ($P < 0.05$). This was not observed for dehulled oat, but this need to be interpreted considering the higher SME application for the 100% replacement, that induced a reduction on bulk density. For FB, the bulk density also did not increase for the 100% replacement. All diets were well extruded presenting high degree of gelatinization, which was lower for CP100 (94.09 %). The alternative carbohydrate sources also reduce the *in vitro* digestibility of organic matter ($P < 0.05$), being higher for BR and lower for PE100.

Table 4. Extrusion processing parameters and kibble macrostructure of extruded dog food with alternative carbohydrate sources.

Item	Broken	Dehulled oat		Pea		Chickpea		Faba bean		SEM	P value
	rice	50%	100%*	50%	100%	50%	100%	50%	100%		
Preconditioner											
Temperature (°C)	85.8 ^b	87.8 ^{ab}	92.8 ^a	87.8 ^{ab}	87.3 ^b	84.8 ^b	87.5 ^b	86.8 ^b	86.3 ^b	0.49	<0.001
Extruder											
Amperage (A)	37.8 ^b	37.2 ^b	39.7 ^a	36.9 ^b	37.1 ^b	38.0 ^b	37.3 ^b	36.5 ^b	36.9 ^b	0.18	<0.001
Pressure (Bars)	16.4 ^b	18.0 ^b	25.8 ^a	17.0 ^b	18.3 ^b	17.0 ^b	17.0 ^b	18.2 ^b	18.6 ^b	0.56	<0.001
Temperature (°C)	118.0 ^c	123.3 ^{ab}	110.5 ^c	119.8 ^{bc}	123.8 ^{ab}	118.5 ^c	126.0 ^a	120.3 ^{bc}	120.3 ^{bc}	0.77	<0.001
Throughput (kg/h)	185.1 ^a	181.0 ^a	134.5 ^b	185.3 ^a	179.0 ^a	173.3 ^a	177.5 ^a	175.3 ^a	171.8 ^a	2.72	<0.001
Bulk density (g/l)	321.0 ^e	403.3 ^{bc}	407.0 ^{bc}	365.5 ^d	428.5 ^b	390.8 ^{cd}	470.8 ^a	367.3 ^d	389.0 ^{cd}	7.04	<0.001
In-barrel moisture (%)	29.6 ^a	29.1 ^a	27.4 ^{ab}	28.2 ^{ab}	28.0 ^{ab}	26.0 ^{bc}	23.4 ^c	28.6 ^{ab}	29.0 ^{ab}	0.37	<0.001
Energy Balance (kW-h/ton)											
SME	11.0 ^b	10.1 ^b	17.5 ^a	9.5 ^b	10.1 ^b	12.2 ^b	10.5 ^b	9.2 ^b	10.1 ^b	0.48	<0.001
STE	91.9 ^a	71.4 ^{abc}	61.6 ^{bcd}	75.3 ^{abc}	79.6 ^{ab}	48.8 ^{cd}	41.7 ^d	37.1 ^d	86.8 ^{ab}	3.67	<0.001
TSE	102.9 ^a	81.5 ^{ab}	79.1 ^{abc}	84.8 ^{ab}	89.7 ^a	61.0 ^{bcd}	52.1 ^{cd}	46.3 ^d	96.9 ^a	3.66	<0.001
STE:SME ratio	8.5 ^a	7.1 ^{ab}	3.6 ^c	7.9 ^a	7.9 ^a	4.0 ^{bc}	4.1 ^{bc}	4.0 ^{bc}	8.6 ^a	0.41	<0.001
EOA (mm ² /ton/h)	207.9 ^a	212.8 ^a	119.0 ^b	207.8 ^a	215.3 ^a	222.1 ^a	217.4 ^a	219.6 ^a	224.4 ^a	5.45	<0.001
Kibble macrostructure											

Expansion ratio	3.28 ^c	3.01 ^e	1.73 ^g	3.39 ^{bc}	2.86 ^f	3.15 ^d	2.75 ^f	3.42 ^b	3.57 ^a	0.04	<0.001
Hardness (N)	42.52 ^{cd}	27.01 ^g	38.87 ^{ed}	51.21 ^{ab}	52.56 ^a	34.47 ^{ef}	47.43 ^{abc}	32.22 ^{fg}	45.43 ^{bcd}	0.79	<0.001
Piece density (g/cm ³)	0.41 ^e	0.47 ^{cd}	0.48 ^{cd}	0.46 ^d	0.51 ^b	0.46 ^{cd}	0.58 ^a	0.41 ^e	0.48 ^{bc}	0.004	<0.001
Specific length (cm/g)	1.93 ^b	1.84 ^c	3.13 ^a	1.65 ^d	1.77 ^c	1.77 ^c	1.63 ^d	1.82 ^c	1.49 ^e	0.03	<0.001
SG (%)	98.12 ^a	99.26 ^a	97.04 ^a	97.15 ^a	95.96 ^a	96.43 ^a	94.09 ^b	98.16 ^a	96.56 ^a	0.42	0.004
<i>In vitro</i> DOM (%)	86.98 ^a	84.97 ^b	83.78 ^d	82.62 ^e	78.05 ^g	82.67 ^e	79.43 ^f	84.93 ^{bc}	84.24 ^{cd}	0.46	<0.001

SEM, standard error of mean (n = 4 replications per treatment); SME, specific mechanical energy; STE, specific thermal energy; TSE, total specific energy; EOA, extruder open area; SG, starch gelatinization; DOM, digestibility of organic matter.

a, b, c, d, e, f, g Mean values in the same row not sharing a common superscript letter differ (P < 0.05).

* More restrictive configuration (extruder open area of 119 mm²/ton/h).

3.3. Nutrient intake and digestibility

No difference was observed for DM and OM intake among diets ($P > 0.05$; Table 5). Due the differences on crude protein (28.90 to 35.46%), crude fat (15.65 to 19.31%), starch (19.25 to 37.62%) and total dietary fibre (3.28 to 10.66%) content of the diets, these nutrients intake differed among dogs ($P < 0.05$). Dogs fed BR diet had lower intake of protein, and higher intake of starch. Crude protein intake was similar among diets, except for BR and CP50 that had lower intake by dogs. Fat intake was lower for CP50, BR, and FB50, and did not differ among other diets. Starch intake was lower for PE100 and CP100, while the total dietary fibre was higher for those diets. The FB50 and FB100 diets induced lower total dietary fibre intake by dogs.

The total tract apparent digestibility (TTAD) of nutrients and energy differ among diets ($P < 0.05$; Table 5). The TTAD of DM and GE were similar for BR, DO50, CP50, FB50 and FB100 diets, while the PE100 and CP100 diets presented the lowest digestibility. The OM digestibility was higher for BR, DO50 and FB50, but lower for PE100. The diets presented similar TTAD of protein, except CP100 diet that had lower digestibility for this nutrient. Fat digestibility was lower for DO100 diet, while the other diets had similar TTAD. Despite starch digestibility differ statically among diets, all carbohydrate sources tested proved to be highly digestible by dogs, higher than 99.4%.

Table 5. Nutrient intake and total tract apparent digestibility of extruded dog foods with alternative carbohydrate sources.

Item	Broken	Dehulled oat		Pea		Chickpea		Faba bean		SEM	P value
	rice	50%	100%	50%	100%	50%	100%	50%	100%		
Nutrient intake (g/dog/d)											
Dry matter	181.68	172.81	178.48	170.76	181.32	174.65	178.56	176.08	179.76	2.01	0.932
Organic matter	176.43	168.13	170.36	167.89	173.77	169.44	171.14	170.40	173.54	1.92	0.985
Crude protein	56.11 ^c	57.57 ^{bc}	61.78 ^{abc}	61.56 ^{abc}	68.91 ^a	62.51 ^{abc}	66.74 ^{ab}	60.74 ^{abc}	64.89 ^{bac}	0.87	0.004
Fat	30.91 ^{bc}	34.12 ^{abc}	35.25 ^{ab}	32.66 ^{abc}	33.60 ^{abc}	29.20 ^c	36.92 ^a	30.63 ^{bc}	35.91 ^{ab}	0.50	<0.001
Starch	73.04 ^a	58.22 ^b	48.95 ^c	52.64 ^{bc}	38.38 ^d	55.55 ^{bc}	36.81 ^d	58.83 ^b	50.44 ^{bc}	1.55	<0.001
Total dietary fiber	6.37 ^f	9.96 ^e	13.64 ^{cd}	15.82 ^c	23.00 ^a	14.15 ^c	20.29 ^b	7.52 ^f	11.66 ^{de}	0.74	<0.001
Total tract apparent digestibility (%)											
Dry matter	85.76 ^a	84.95 ^a	81.49 ^{bc}	81.30 ^{bc}	77.47 ^d	82.81 ^{abc}	79.61 ^{cd}	84.41 ^{ab}	83.25 ^{ab}	0.41	<0.001
Organic matter	91.38 ^a	90.43 ^{ab}	87.88 ^{cd}	87.12 ^{de}	82.87 ^f	88.79 ^{bcd}	85.27 ^e	90.20 ^{abc}	88.78 ^{bcd}	0.39	<0.001
Crude protein	86.23 ^a	86.38 ^a	83.91 ^{ab}	84.47 ^{ab}	83.60 ^{ab}	85.31 ^{ab}	82.27 ^b	86.31 ^a	85.52 ^{ab}	0.29	<0.001
Fat	94.94 ^{ab}	94.70 ^{ab}	93.10 ^b	93.82 ^{ab}	93.50 ^{ab}	93.57 ^{ab}	94.56 ^{ab}	94.92 ^{ab}	95.27 ^a	0.16	<0.001
Starch	99.84 ^{abc}	99.89 ^{ab}	99.98 ^a	99.67 ^{bcd}	99.50 ^{def}	99.64 ^{cde}	99.42 ^{ef}	99.75 ^{abc}	99.39 ^f	0.03	<0.001
Total dietary fiber	25.00 ^{de}	37.52 ^{bc}	47.99 ^{ab}	42.90 ^{abc}	36.03 ^{cd}	45.52 ^{abc}	51.25 ^a	23.89 ^e	38.43 ^{bc}	1.50	<0.001
Gross energy	91.44 ^a	90.78 ^a	88.10 ^{bcd}	87.81 ^{cd}	84.40 ^e	89.11 ^{abc}	85.96 ^{de}	90.43 ^{ab}	89.39 ^{abc}	0.34	<0.001

SEM, standard error of mean (n = 6 dogs per food).

a, b, c, d, e, f, g Mean values in the same row not sharing a common superscript differ (P < 0.05).

3.4. Fecal characteristics and fermentation products

Feces production were affected by carbohydrate sources ($P < 0.05$; Table 6), being greater by dogs fed CP100 and PE100, intermediate by dogs fed DO100 and PE50, and lower by dogs fed BR, DO50, FB50 and FB100. In comparison with the BR diet, dogs fed PE100, CP100 and FB100 produced lower fecal DM ($P < 0.05$). Despite this difference, fecal consistence did not differ ($P > 0.05$) and remained on the adequate or ideal score for dogs. Fecal pH was higher by dogs that received BR, but it did not differ from dogs fed DO50 and FB50, while PE100 and CP100 had the lowest values ($P < 0.05$).

Regarding fermentation end-products (Table 6), CP100 and PE100 diets induced higher production of acetate and propionate in feces ($P < 0.05$). In comparison to BR diet, butyrate concentration showed greater in feces of dogs fed FB100 ($P < 0.05$), but similar those other diets. Valeric acid production was higher by dogs fed CP100 compared to the BR diet ($P < 0.05$), that induced similar production than other diets. Fecal ammonia content was unaffected by the carbohydrate source ($P > 0.05$). However, lactate concentration was greater than BR for practically all tested diets ($P < 0.05$), showing higher for PE50 and FB100, intermediate for DO50, DO100 and CP100, and lower for BR, CP50 and FB50.

Table 6. Fecal characteristics and fermentation end-products in feces of dogs fed diets formulated with alternative carbohydrate sources.

Item	Broken	Dehulled oat		Pea		Chickpea		Faba bean		SEM	P value
	rice	50 %	100 %	50 %	100 %	50 %	100 %	50 %	100 %		
Fecal characteristics											
Output as-is (g/d)	60.6 ^c	64.9 ^c	80.8 ^{bc}	81.4 ^{bc}	108.4 ^a	81.4 ^c	104.9 ^{ab}	66.4 ^c	79.8 ^{bc}	2.80	<0.001
Output DM (g/d)	25.9 ^c	25.9 ^c	33.0 ^{bc}	32.1 ^{bc}	40.9 ^a	30.1 ^{bc}	36.4 ^{ab}	27.4 ^c	30.1 ^{bc}	0.83	<0.001
DM (%)	43.3 ^a	42.0 ^{ab}	40.8 ^{ab}	39.8 ^{abc}	38.0 ^{bc}	40.4 ^{ab}	35.0 ^c	41.5 ^{ab}	37.8 ^{bc}	0.55	<0.001
Score	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	0.01	0.621
pH	6.8 ^a	6.6 ^{ab}	6.3 ^{bcd}	6.4 ^{bc}	6.0 ^d	6.4 ^{bc}	6.0 ^d	6.6 ^{abc}	6.3 ^{cd}	0.05	<0.001
Fermentation end-products (mmol/g of DM)											
Acetic acid	254.1 ^{cd}	250.0 ^d	318.8 ^{bcd}	336.8 ^{abcd}	411.2 ^{ab}	311.4 ^{bcd}	432.2 ^a	278.9 ^{cd}	362.5 ^{abc}	11.31	<0.001
Propionic acid	99.9 ^b	113.5 ^b	150.9 ^b	169.9 ^b	260.1 ^a	169.0 ^b	284.0 ^a	128.0 ^b	178.1 ^b	9.37	<0.001
Butyric acid	54.7 ^b	66.2 ^{ab}	74.0 ^{ab}	70.8 ^{ab}	63.7 ^{ab}	59.8 ^{ab}	68.1 ^{ab}	69.5 ^{ab}	80.3 ^a	1.90	0.012
Total SCFA	408.7 ^d	429.8 ^d	573.3 ^{bcd}	577.5 ^{bcd}	734.9 ^{ab}	540.3 ^{cd}	784.3 ^a	466.8 ^{cd}	619.0 ^{abc}	20.93	<0.001
Isobutyric acid	8.5	7.9	9.7	8.8	8.7	7.7	9.2	18.6	10.8	1.09	0.363
Isovaleric acid	13.2	12.7	15.1	14.4	13.2	12.7	13.9	14.9	17.3	0.49	0.335
Valeric acid	3.9 ^b	5.7 ^{ab}	5.6 ^{ab}	5.1 ^{ab}	6.7 ^{ab}	5.3 ^{ab}	6.9 ^a	4.7 ^{ab}	5.5 ^{ab}	0.22	0.007
Total BCFA	25.7	26.3	30.6	28.3	28.5	25.6	29.9	38.2	33.7	1.36	0.398
Total VFA	434.4 ^d	456.0 ^d	603.9 ^{bcd}	605.8 ^{bcd}	763.4 ^{ab}	565.9 ^{cd}	814.3 ^a	505.0 ^{cd}	652.7 ^{abc}	21.17	<0.001

Ammonia *	189.3	183.9	201.5	171.3	160.2	148.6	166.8	186.8	199.5	4.97	0.143
Lactate *	1.9 ^c	3.4 ^b	3.2 ^b	3.5 ^{ab}	3.3 ^b	2.9 ^{bc}	3.2 ^b	2.8 ^{bc}	4.6 ^a	0.13	<0.001

SEM, standard error of mean (n = 6 dogs per food); DM, dry matter; SCFA, short-chain fatty acids; BCFA, branched-chain fatty acids; VFA, volatile fatty acids.

a, b, c, d Mean values in the same row not sharing a common superscript differ (P < 0.05).

* Ammonia and lactate are expressed in mmol/kg of DM.

3.5. Glucose and insulin postprandial responses

The body weight and body condition score of dogs did not differ among treatments ($P > 0.05$; Table 7). In the postprandial test day, dogs consumed similar amounts of dry matter and organic matter ($P > 0.05$; Table 6). However, fat, crude protein, starch and TDF intakes differed ($P < 0.05$). Dogs fed diets based on chickpea, faba bean and dehulled oat had higher fat intake than those fed broken rice and pea diets. Protein intake was lower by dogs fed broken rice, while it did not change among other diets. Starch intake was higher for broken rice, intermediate for dehulled oat and faba bean, and lower for pea and chickpea diets.

Basal, minimum, mean and time to peak glucose did not differ among the treatments ($P > 0.05$; Table 8). However, a difference was verified for the maximum glucose postprandial response ($P = 0.016$). Dogs fed rice-based diet presented higher values (104.53 mg/dL) for maximum glucose response, while dogs fed the diet based on dehulled oat showed lower concentrations (93.10 mg/dL). The AUC of glucose did not differ among treatments ($P > 0.05$; Table 7), but a tendency was observed from 120 to 720 min ($P = 0.056$), and over 720 min ($P = 0.094$). Figure 1 shows the mean glucose postprandial response from basal concentration over 720 min, which was affected by carbohydrate sources among time ($P < 0.05$).

Table 7. Body weight (BW), body condition score (BCS) and nutrient intake of dogs fed diets formulated with alternative carbohydrate sources during the postprandial glucose and insulin tests.

Item	Diets					SEM	P value
	Broken rice	Dehulled oat	Pea	Chickpea	Faba bean		
BW (kg)	12.06	11.90	12.22	12.05	12.19	0.19	0.985
BCS	5.6	5.3	5.2	5.8	5.8	0.25	0.779
Nutrient intake (g/dog/d)							
Dry matter	180.43	182.12	183.14	183.11	183.61	2.04	0.989
Organic matter	175.22	173.87	175.52	175.50	177.25	1.96	0.990
Fat	30.66 ^c	36.05 ^{ab}	32.94 ^{bc}	38.43 ^a	36.76 ^a	0.58	<0.001
Protein	55.73 ^b	63.05 ^a	69.61 ^a	68.44 ^a	66.28 ^a	1.06	<0.001
Starch	72.54 ^a	49.95 ^b	38.76 ^c	37.75 ^c	51.52 ^b	1.99	<0.001
Total dietary fibre	6.33 ^e	13.87 ^c	23.24 ^a	20.55 ^b	11.90 ^d	0.98	<0.001

SEM, standard error of mean (n = 9 dogs per food).

^{a, b, c} Mean values in the same row not sharing a common superscript differ (P < 0.05).

Table 8. Basal, minimum, mean, maximum, time to peak, and area under the curve (AUC) of glucose of dogs fed diets formulated with alternative carbohydrate sources.

Item	Diets					SEM	P value
	Broken rice	Dehulled oat	Pea	Chickpea	Faba bean		
Basal glucose (mg/dl)	77.8	78.3	78.0	79.7	77.9	0.76	0.929
Minimum glucose (mg/dl)	74.3	72.9	71.5	74.8	74.6	0.72	0.542
Mean glucose (mg/dl)	88.7	82.6	81.9	84.3	85.9	0.94	0.112
Maximum glucose (mg/dl)	104.5 ^a	93.1 ^b	93.5 ^{ab}	94.2 ^{ab}	99.1 ^{ab}	1.38	0.016
Time to peak (min)	261.8	224.3	360.0	314.3	286.7	20.4	0.206
AUC (mg/dl/h)							
0-120'	168.4	158.7	156.6	160.3	164.3	1.70	0.161
120-420'	482.3	434.7	434.2	441.5	458.0	6.73	0.056
420-720'	450.5	419.5	415.8	438.9	436.9	5.43	0.213
0-720'	1101.4	1012.9	1006.6	1040.8	1059.3	13.11	0.094

SEM, standard error of mean (n = 9 dogs per food).

^{a, b} Mean values in the same row not sharing a common superscript differ (P < 0.05).

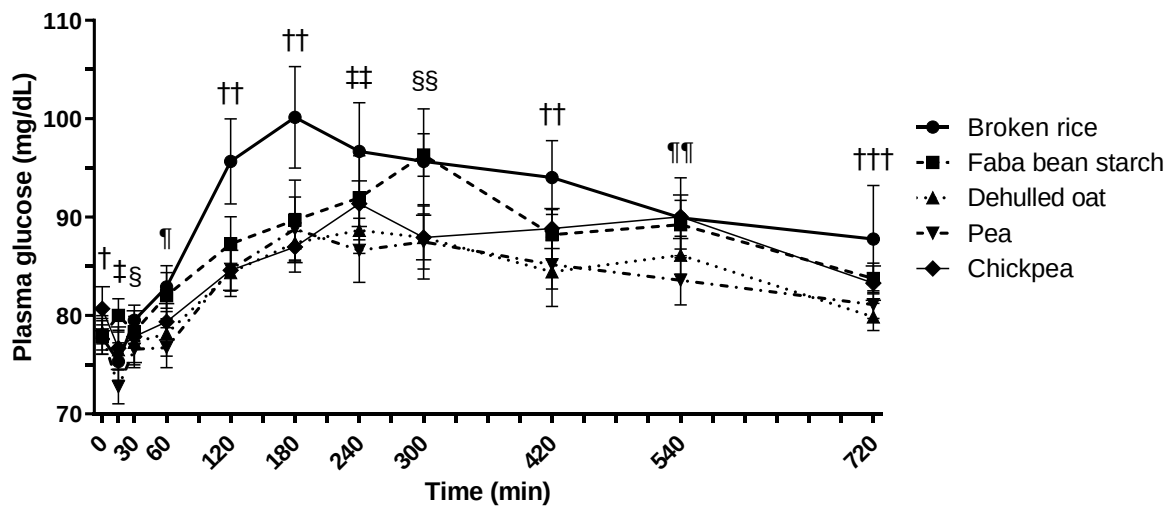


Figure 1. Postprandial responses of plasma glucose (mg/dL) according to the time of adult dogs fed the different carbohydrate sources.

† Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets ($P < 0.05$).

‡ Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets; faba bean starch diet different from pea diet ($P < 0.05$).

§ Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets; broken rice diet different from dehulled oat diet ($P < 0.05$).

¶ Chickpea diet different from broken rice, faba bean starch and pea diets; faba bean diet different from pea diet ($P < 0.05$).

†† Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets; broken rice diet different from dehulled oat and pea diets ($P < 0.05$).

‡‡ Chickpea diet different from broken rice, faba bean starch and pea diets; broken rice diet different from dehulled oat and pea diets; faba bean starch diet different from pea diet ($P < 0.05$).

§§ Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets; pea diet different from broken rice and faba bean diets ($P < 0.05$).

¶¶ Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets; faba bean starch diet different from pea diet ($P < 0.05$).

††† Chickpea diet different from broken rice, dehulled oat, faba bean starch and pea diets; dehulled oat diet different from broken rice, faba bean starch and pea diets; faba bean starch diet different from broken rice and pea diets ($P < 0.05$).

Regarding insulin parameters, basal, minimum, mean and time to peak did not differ among treatments ($P > 0.05$; Table 9), although has been observed a tendency for mean glucose ($P = 0.084$). Maximum insulin secretion response was influenced by carbohydrate sources ($P < 0.05$), being higher in dogs fed rice-based diet (338.64 pmol/L) and lower for diets based on dehulled oat (162.07 pmol/L) and chickpea (179.96 pmol/L). In relation to the AUC, there was only an effect of diet on the AUC of insulin from 120 to 420 min ($P = 0.025$), which was higher in dogs fed rice diet and lower in dogs fed dehulled oat diet. The mean insulin postprandial response of dogs is shown in Figure 2. Insulin secretion after 180 min was higher in dogs fed diets based on rice (265.79 pmol/L) than faba bean starch flour (158.76 pmol/L), pea (143.31 pmol/L), dehulled oat (123.87 pmol/L) and chickpea (103.22 pmol/L) diets. At 240 min, dogs fed rice based diet had higher insulin concentration (201.25 pmol/L) compared to dehulled oat (110.62 pmol/L), and did not differ from dogs fed faba bean (185.81 pmol/L), pea (160.05 pmol/L) and chickpea (139.75 pmol/L). Fava bean differ from dehulled oat. At 300 min, rice-based diet induced higher insulin secretion (256.25 pmol/L) compared to faba bean (180.33 pmol/L), chickpea (140.22 pmol/L) and dehulled oat (129.04 pmol/L). Rice did not differ from pea (197.81 pmol/L), which was higher than dehulled oat. After 420 min, insulin secretion was similar among rice (184.48 pmol/L), faba bean (139.52 pmol/L) and chickpea (118.33 pmol/L), but higher than pea (100.55 pmol/L) and dehulled oat (108.26 pmol/L).

Table 9. Basal, minimum, mean, maximum, time to peak, and area under the curve (AUC) of insulin of dogs fed diets formulated with alternative carbohydrate sources.

Item	Diets					SEM	P value
	Broken rice	Dehulled oat	Pea	Chickpea	Faba bean		
Basal insulin (pmol/l)	44.0	36.6	42.0	42.3	40.7	2.23	0.825
Minimum insulin (pmol/l)	34.4	33.4	37.2	35.4	38.1	1.85	0.921
Mean insulin (pmol/l)	145.9	86.9	112.5	95.1	108.9	7.39	0.084
Maximum insulin (pmol/l)	338.6 ^a	162.1 ^b	231.8 ^{ab}	179.9 ^b	232.8 ^{ab}	17.64	0.005
Time to peak (min)	243.7	345.0	293.3	330.0	262.5	22.89	0.609
AUC (pmol/l/h)							
0-120'	201.6	129.3	188.1	150.9	185.8	12.27	0.320
120-420'	1107.7 ^a	578.1 ^b	755.2 ^{ab}	619.3 ^{ab}	802.6 ^{ab}	59.99	0.025
420-720'	713.6	499.7	548.5	548.4	470.4	47.66	0.559
0-720'	2020.6	1204.9	1491.8	1316.3	1456.7	105.94	0.111

SEM, standard error of mean (n = 9 dogs per food).

^{a, b} Mean values in the same row not sharing a common superscript differ (P < 0.05).

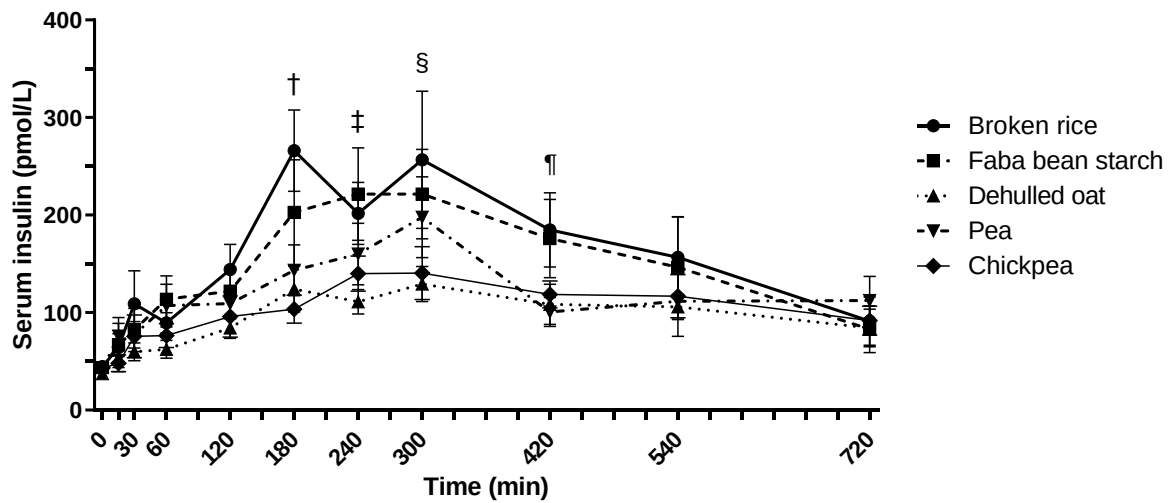


Figure 2. Postprandial responses of serum insulin (pmol/L) according to the time of adult dogs fed the different carbohydrate sources.

† Control diet different from chickpea, dehulled oat, faba bean starch and pea diets ($P < 0.05$).

‡ Broken rice diet different from dehulled oat diet ($P < 0.05$).

§ Broken rice diet different from chickpea, dehulled oat and faba bean starch diets ($P < 0.05$).

¶ Broken rice diet different from faba bean starch and pea diets ($P < 0.05$).

3.6. Palatability study

Before palatability study, all diets were corrected to achieve same moisture content. In addition, the diets were coated with 6.0% of poultry fat and 1.0% of standard palatant enhancer. Food preference was calculated using a consumption ratio of the amount of tested food consumed (A or B) by total food consumed (A + B). The results indicated that BR was preferred by dogs over DO50, DO100, CP50, FB50 and FB100 diets ($P < 0.05$; Table 9). However, PE50, PE100 and CP100 had similar intake ratio than BR ($P > 0.05$), indicating no food preference among these carbohydrate sources by dogs.

Table 9. Palatability tests by dogs fed diets formulated with alternative carbohydrate sources.

Diet A vs. B *	First choice (%)		Intake ratio (IR) †		P-value
	Diet A	Diet B	Diet A	Diet B	
BR vs. DO50	65.0	33.0	0.65 ^a	0.35 ^b	0.008
BR vs. DO100	67.5	27.5	0.69 ^a	0.29 ^b	0.001
BR vs. PE50	45.0	55.0	0.47	0.53	0.611
BR vs. PE100	72.5	27.5	0.57	0.43	0.140
BR vs. CP50	62.5	37.5	0.61 ^a	0.39 ^b	0.042
BR vs. CP100	70.0	30.0	0.58	0.42	0.143
BR vs. FB50	58.0	40.0	0.59 ^a	0.38 ^b	0.018
BR vs. FB100	67.5	32.5	0.68 ^a	0.32 ^b	0.001

^{a, b} Mean values in the same row not sharing a common superscript differ ($P < 0.05$).

* Moisture content of diets: BR 8.8%; DO50 7.7%; DO100 7.8%; PE50 7.4%; PE100 7.4%; CP50 7.8%; CP100 7.7%; FB50 7.5%; FB100 7.8%.

† Intake ratio A = [ingestion diet A / (ingestion diets A + B)]; Intake ratio B = [ingestion diet B / (ingestion diets A + B)].

4. Discussion

Commercial dry pet foods are formulated with a mixture of carbohydrate sources, which can be categorized in three classes: cereals (maize, rice, and

sorghum), legumes or pulses (bean, chickpea, lentil, and pea) and tubers (potato, sweet potato, and cassava/tapioca). Grain-free diets are becoming increasingly popular in the pet food market with claims for health and functional properties in comparison to the traditional cereal grains. However, there are few studies in literature evaluating the inclusion of these carbohydrate sources in pet nutrition (Carciofi et al., 2008; Domingues et al., 2018; Pezalli and Aldrich, 2019; Alvarenga et al., 2020). This study is the first that evaluated the inclusion of chickpea, dehulled oat faba bean starch flour as the main starch source or in a combination with rice in dog diets, while pea has been studied in dogs already (Carciofi et al., 2008).

The inclusion of starch sources studied did not alter the extrusion processing variables, maintaining similar SME input to produce expanded and cooked food. Higher motor amperage (or torque), SME and pressure values for DO100 diet were obtained by altering extruder screw and die configurations. The extruder screw and die configuration changes can be made along with a formulation adjustment to change the final product. These alterations in processing variables were necessary to improve the final product quality, compensating some variations in the properties of the raw material. The dehulled oat used in the current study presented 5.91% fat content, which in terms may be the reason for the change in extrusion configuration of DO100 diet. Fat or lipid content is a critical parameter for single-screw extruder because it acts as a lubricant during the processing material, which difficult to transfer SME necessary for properly cooking, affecting expansion rate and bulk density (Ilo et al., 2000). In addition, the combination of screw and die configuration, and higher steam addition into the preconditioner contributed to improve extrusion process and extruded characteristics. The change in extrusion variables such as screw configuration (Onwulata et al., 2001), feed moisture, screw speed and barrel temperature (Geetha et al., 2014; Meng et al., 2010; Wang et al., 2019a) and preconditioning step (Pacheco et al., 2018) have been studied to enhance extrusion performance and product quality. Higher mass temperature observed for DO50, PE100 and CP100 may be attributed to higher fibre content presented in those diets that may increase the shear inside the barrel (Monti et al., 2016).

The effects of dietary fiber content in the final product quality have been extensively studied in human products, but it is still little explored in pet food. Anyway,

increasing fiber content in the dog food formulation resulted in increased bulk density, piece density and kibble hardness, and decreased expansion rate (Monti et al., 2016). Starch is the main nutrient responsible for product expansion, it is attributed to bubbles formation during flash-off of moisture and vapor as product leaves extruder (Moraru and Kokini, 2003; Arhaliass et al., 2009). The flash evaporation extension configure the expansion degree which has been related with the fibre inclusion and air cells size; as fibre content increase in the recipe, the size of air cells and expansion decreased (Lue et al., 1991), and hardness increased (Chassagne-Berces et al., 2011). Similarly, an increase in bulk density, piece density and hardness, and a decreased in expansion ratio has been reported when oat (Liu et al., 2006) and chickpea (Shevkani et al., 2019) were incorporated in the food matrix. Increasing levels of pea into rice-based products also have been reported to reduce expansion ratio (Singh et al., 2007). Recently, the effects of faba bean inclusion in dog formulations was evaluated on extrusion processing and kibble macrostructure (Alvarenga and Aldrich, 2019). The authors reported a linear reduction in expansion ratio and a linear increased in piece density and hardness increasing levels of faba beans (0, 10, 20, and 30%). Products based on chickpea demonstrated less expansion, and higher piece density and hardness. Chickpea has been reported to be less expansion, increasing bulk density and hardness than cereals (maize and sorghum) when processed at same extrusion condition (Wang et al., 2019b), probably due the their higher protein and fibre contents compared to the cereals grain. Although fiber inclusion had been related to reduce starch gelatinization (Monti et al., 2016), the diets in the present study were well processed (ground and extruded) and showed high cooking degree. On the other hand, *in vitro* digestibility of organic matter was lower for diets supplemented with 50 or 100% carbohydrate tested compared to broken rice.

Due the difference on chemical composition of ingredients sources tested, the nutritional composition varied among diets, leading to difference in the intake of protein, fat, starch and total fibre by dogs, as expected. Although no difference was observed for DM and OM intakes, broken rice-based diet showed higher value for DM digestibility in agreement with previously studies that compared rice to other cereals or legumes (Walker et al., 1994; Kempe et al., 2004; Carciofi et al., 2008; Alvarenga et al., 2020). Diets based in a combination of rice and legumes (chickpea, pea, and fava

bean) or dehulled oat promoted similar nutrient digestibility. In contrast, diets based on 100% pea and chickpea decreased nutrient digestibility. Diets based on pea and chickpea had lowest and similar digestibility of DM. In addition, pea-based diet had lower digestibility for OM and GE, while chickpea had lower crude protein digestibility. The reduction on nutrient digestibility adding carbohydrate sources rich in dietary fiber was previously reported and are expected (Carciofi et al., 2008). Diet based on dehulled oat had lowest fat digestibility. All diets presented higher starch digestibility (above 99.39 %) despite statistical difference among diets. The properly diet processing, that include grinding and extrusion cooking promoted higher starch gelatinization for all diets, which may be attributed to this higher digestibility in accordance with other studies (Carciofi et al., 2008; Bazolli et al., 2015; Pezzali and Aldrich, 2019). There is no information about the starch gelatinization or cooking degree needed to promote adequate starch digestibility, although studies have showed that diets processed with a low cooking degree are well digestible by dogs (Pacheco et al., 2018; Ribeiro et al., 2019). In the present study, all diets presented similar particle size distribution, even diet based on 100% dehulled oat, that was ground in a 1.7 mm sieve size. The grinding process is also an important parameter for pet food industry, since each starch source can behave differently when ground at the same sieve size, influencing food quality and digestibility (Bazolli et al., 2015).

Although grain-free diets have increasing in pet food market, there are few studies in literature. Furthermore, there is no published studies evaluating chickpea, dehulled oat and fava bean as main starch sources in dog foods. Recently, a study compared two diets for dogs formulated with a combination of cereal grains (spelt, millet and sorghum) or grain-free (potato, pea, and tapioca starch), and no difference was observed for digestibility of nutrients and energy, except for TDF that was higher for dogs fed grain-free diets (Pezzali and Aldrich, 2019). In the present study, both diets based on faba bean showed a good starch source, which can be replaced by rice with no effect on nutrient and energy digestibility. Different varieties of beans has been evaluated in dog nutrition (Foster et al., 2012; Alvarenga et al., 2020). The inclusion of navy and fava bean in pet foods did not affect nutrient digestibility in comparison with a diet formulated based on common cereal grains (corn and/or rice).

The fecal quality is also an important parameter to evaluate food quality. In the present study, dogs fed diets formulated with 100% of pea or chickpea produced higher fecal bulk, and lower fecal DM and pH. This was expected because these both diets presented higher amount of total dietary fibre (TDF) content. Similar results have been reported by dogs fed diets containing pea (Carciofi et al., 2008) or a combination of pea, potato, and tapioca (Pezzali and Aldrich, 2019). Dietary fibre intake, especially insoluble fibre, is responsible to increased fecal output because this fibre fraction is poorly or non-fermented in large intestine. Despite lower fecal DM, fecal consistency score was kept ideal among diets. In addition, most soluble fibers do not contribute to fecal bulk, but are fermented by colonic bacteria in the colon, inducing lower colonic pH that inhibits the growing of pathogenic bacteria. Pea have been reported to induced lower fecal pH in dogs in comparison to rice (Carciofi et al., 2008), however, some authors did not observe any difference in fecal pH of dogs fed diets with 15% of chickpea or pea incorporated in a commercial extruded diet based on rice (Sandri et al., 2019).

In present study, SCFA increased as alternative starch sources was added to replace rice. Fermentation of dietary fibers by colonic microbiota in the colon results in the production of SCFA (acetate, propionate, and butyrate), gases (H₂, ammonia, and methane) and lactate (Holscher, 2017). This alteration in fermentative profiles was expected, once the carbohydrate sources used in this study had higher dietary fibre content than broken rice. Additionally, diets formulated with 100% of pea, chickpea and fava bean induced greatest SCFA production and lactate. In a study with diets supplemented with 15% of chickpea or pea did not found any difference on SCFA production in dogs (Sandri et al., 2019). Probably these low levels of inclusion it was not sufficient to increase these organic acids, and then decreased fecal pH. Dietary fiber and their metabolites play an important role in the metabolism and health of animals, especially butyrate, considered the major energy source preferred for the colonic cells (Topping and Clifton, 2001). Increased levels of butyrate are associated with anti-inflammatory effects and enhanced colonic immune response (Inan et al., 2000; Peng et al., 2009; Jiminez et al., 2017). Production of SCFAs also reduces pH, creating an environment that prevents the growth of harmful bacteria and enhanced beneficial bacteria (Maria et al., 2017).

Nowadays, there is a great interest in understanding the relationship between different types of dietary carbohydrate and glycemic and insulinemic responses as one possible way to solve the rise of obesity and insulin resistance, considered the major important canine health problems worldwide. However, few studies have evaluated glucose and insulin responses in front of different carbohydrate sources in dogs (Kempe et al., 2004; Carciofi et al., 2008; Adolphe et al., 2012; Adolphe et al., 2015; Rankovic et al., 2020). In the postprandial test of this study, the diet based on dehulled oat significantly lowered postprandial glucose and insulin concentrations compared to broken rice diet, followed by pea, chickpea and faba bean. This indicates that oat can have an overall positive metabolic effect. The delayed glucose absorption has been previously shown due to the high viscosity of β -glucans (soluble fiber fraction) present in the oat (Hallfrisch and Behall, 2000; Wood, 2007; Regand et al., 2011). A lower glycemic and insulinemic values was also observed in dogs fed diets based on pea, chickpea and faba bean in comparison to broken rice, despite no difference in the statistical analysis. Previously, dogs fed oat groats present similar postprandial glucose and insulin curve than barley, corn, wheat, and rice (Kempe et al., 2004). Dogs fed pea-based diet showed lower maximum incremental glucose than other starch sources like cassava flour, corn, sorghum, brewer's rice, and lentil (Carciofi et al., 2008). Further, the use of pea in dog foods has been reported to have a lower glycemic and insulinemic responses than rice (Adolphe et al., 2012), although the results did not differ statistically.

Starch is considerate the main nutrient responsible to alters glucose and insulin responses in dogs (Nguyen et al., 1998). Additionally, the digestion and absorption of starch sources are influenced by several intrinsic (e.g. starch properties and amylose to amylopectin ratio) and extrinsic factors (e.g. processing and amount ingested) (Toutounji et al., 2019). The amount of starch consumed have been showed to be more important to rise glucose profiles in dogs than protein, fat, and dietary fiber (Nguyen et al., 1998; Monti et al., 2016). A study demonstrated that dogs fed low starch diet (9.5%) had lowest glucose and insulin responses in than moderate starch (23.0%) and high starch (31.7%) diets (Hewson-Hughes et al., 2011). However, those diets also differed on their nutritional composition, as starch content increased, protein and fat decreased. The low-rise glucose concentration observed in dogs fed diets formulated with non-

conventional starch sources may also be due the amylose to amylopectin ratios among the sources, as the ratio of these two polysaccharides varies according to the botanical origin of the starch (Tester et al., 2004). In general, C-type starches like legumes presented higher amylose content than A-type starches mainly found in cereals. Amylose content of the legumes is reported to vary in the range of 17 to 52% (Wani et al., 2016). The amylose content in chickpea (31 – 34%), pea (38 – 43%), and faba bean (22 – 49%) varying according to the cultivars (He and Wei, 2017), while oat ranged from 27.5 to 29.8% (Tester and Karkalas, 1996). Starches with a higher amount of amylose in relation to amylopectin forms a more compact structure that is less susceptible to enzymatic hydrolyzation (Hu et al., 2004), inducing lower glycemic responses (Denardin et al., 2012).

Palatability is an important way to determine the acceptance of food by dogs. It is also one major factor that may limit the use of some ingredients in pet food formulation. In the current study, dogs preferred diet formulated with rice as starch source than diets based on both concentrations of dehulled oat and faba bean or with 50% chickpea. In contrast, no difference on food preference was detected between rice and the diets based on pea or chickpea at concentration of 100%. Unfortunately, there are few studies comparing the effects of non-conventional carbohydrate sources over traditional grains in dog palatability, which limited the comparison of the present results. A comparison between traditional grains-based diet and legumes and tuber-based diet did not observed any difference on food preference by dogs (Pezalli and Aldrich, 2019). Faba bean also was recently evaluated in dog foods with an inclusion of 10, 20 or 30 % replaced rice to the control diet (Alvarenga and Aldrich, 2020). In that study, dogs preferred diet based on rice than diets formulated with an inclusion of 10 or 30 % faba bean. However, 20% faba bean inclusion showed similar effect on first choice and food intake compared to the control diet.

5. Conclusion

In conclusion, the alternative carbohydrate sources had small impact on extrusion processing. Except the dehulled oat, which was fixed changing screw and die profiles. Faba bean it is a good source that can replace broken rice without influencing digestibility of nutrients, while dehulled oat and chickpea can replace up to

50%. The total substitution of broken rice by dehulled oat, chickpea and pea reduced digestibility with a potential use for low energy diets. In addition, pea and chickpea increased fermentation end-products formation and may be used in diets to support specific intestinal conditions. Dehulled oat and chickpea are alternative sources of carbohydrate which may be used in pet foods to reduce the postprandial insulin secretion.

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