UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

FIBRA PARA CÃES: EFEITOS SOBRE O PROCESSO DE EXTRUSÃO, DIGESTIBILIDADE, FERMENTAÇÃO MICROBIANA, TEMPO DE RETENÇÃO INTESTINAL E PALATABILIDADE DE RAÇÕES PARA CÃES

Mariana Monti
Médica Veterinária

2015

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Dissertação de Mestrado apresentado à Faculdade de Ciências Agrárias e Veterinárias –Unesp, Campus de Jaboticabal, como pré-requisito para a obtenção do título de Mestre em Medicina Veterinária (Clínica Médica Veterinária).

2015

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CAMPUS DE JABOTICABAL

FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS DE JABOTICABAL

CERTIFICADO DE APROVAÇÃO

TÍTULO: FIBRA PARA CÃES: EFEITOS SOBRE O PROCESSO DE EXTRUSÃO, DIGESTIBILIDADE, FERMENTAÇÃO MICROBIANA, TEMPO DE RETENÇÃO INTESTINAL E PALATABILIDADE DE RAÇÕES PARA CÃES

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DADOS CURRICULARES DO AUTOR

MARIANA MONTI – Nascida em 13 de Setembro de 1988, em Ribeirão Preto –SP, graduada em Medicina Veterinária pela Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Campus de Jaboticabal em Dezembro de 2012. Foi bolsista PET veterinária (2009-2010) nesta mesma instituição e bolsista de iniciação científica da Fundação de Amparo à Pesquisa do Estado de São Paulo (2011-2012) na área de nutrição de cães e gatos com ênfase em imunologia, nutrição e envelhecimento de cães Beagles sob a supervisão do Prof. Aulus Cavalieri Carciofi e da Prof[a]. Márcia de O.S. Gomes. Realizou Estágio Curricular na empresa de alimento para cães e gatos Selecta Pet Care Company e no Serviço de Nutrição Clínica da Universidade Autônoma de Barcelona, Espanha, supervisionado pela Profa. Cecília Villaverde. Fez mestrado na Faculdade de Ciências Agrárias e Veterinárias da UNESP, campus de Jaboticabal, Laboratório de Pesquisa em Nutrição e Doenças Nutricionais na UNESP, Campus Jaboticabal na área de nutrição de cães e gatos com ênfase em fontes de fibras para cães e processo de extrusão. Foi bolsista do CNPQ e teve orientação dos Professores Aulus C Carciofi e Cecília Villaverde. Atualmente é pesquisadora na empresa Premier Pet, traballhando na área de desenvolvimento de produtos.

Dedico

Àos meus pais Eliana Aparecida Varanda e Rubens Monti,

Àos meus pets: Neni, Nâna, Filho, Fio, Tita, Bel, Sara, Sansão e Totó

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SUMÁRIO

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"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 07895/14 do trabalho de pesquisa intitulado **"Efeito do tamanho de particulas de fibra de caule (cana-de-açúcar, *Sacharum L.*), semente (farelo de trigo, *Triticum L.*) e fruta (goiaba, *Psidium guajava*) sobre o processamento de extrusão e função gastrointestinal de cães"**, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 08 de maio de 2014.

Jaboticabal, 08 de maio de 2014.

Prof.ª Dr.ª Paola Castro Moraes
Coordenadora - CEUA

FIBRA PARA CÃES: EFEITOS SOBRE O PROCESSO DE EXTRUSÃO, DIGESTIBILIDADE, FERMENTAÇÃO MICROBIANA, TEMPO DE RETENÇÃO INTESTINAL E PALATABILIDADE DE RAÇÕES PARA CÃES

RESUMO-. Existe um grande interesse atual no desenvolvimento de alimentos para cães com alta concentração de nutrientes e baixa densidade energética. A adição de fibra em rações para *pet* é uma maneira de controlar as calorias do alimento propiciando benefícios, e ao mesmo tempo, promover a utilização mais eficiente das fontes de fibra que não são destinadas à alimentação humana. Desta forma, foram formuladas 8 dietas experimentais: Controle (CO), sem adição de ingredientes fibrosos; dietas com fibra de goiaba (GF3, GF6, GF12), com níveis de inclusão de 3%, 6%, e 12%; dietas com fibra de cana (SC), com 9% de inclusão e dois diferentes tamanho de partícula (grande - SC_L e pequeno- SC_S) e dietas com farelo de trigo (WB), com 32% de inclusão e dois diferentes tamanhos de partícula (grande- WB_L e pequeno- WB_S). Objetivou-se neste trabalho avaliar os efeitos de inclusões crescentes de fibra de goiaba, bem como o efeito da fibra de cana e do farelo de trigo moídos em diferentes tamanhos sobre o processo de extrusão, digestibilidade dos nutrientes, fermentação no intestino, tempo de retenção gastrointestinal (TRGI) e palatabilidade das dietas. Foi utilizado o programa SAS para análise estatística e as médias foram comparadas por contrastes polinomiais e ortogonais (P<0,05). No Capítulo 2, a adição da fibra de goiaba resultou em aumento linear da amperagem (P<0,001), temperatura (P<0,001) e pressão (P<0,001) na saída da extrusora. Níveis crescentes de goiaba trouxeram maior implemnto de energia mecânica específica (EME) (P<0,001), redução na expansão radial (ER) (P<0,001), aumento na densidade aparente (DA) e menor cozimento do amido. A fibra de cana, em comparação com a fibra de trigo reduziu a amperagem (P<0,001), EME (P=0,013) e DA, mas aumentou o comprimento específico (P<0,001). A inclusão de fibra em menor tamanho reduziu a amperagem (P<0,001), EME (P<0,001) e DA, mas aumentou a ER (P=0,008). No capítulo 3, as rações CO, GF3, GF6 e GF12 foram fornecidas a 24 Beagles adultos por 15 dias de adaptação e após esse período os animais foram alojados em gaiolas metabólicas para acessar a digestibilidade e fermentação. Os animais receberam por via oral uma pílula contendo 10 marcadores radiopacos para determinação do TRGI. O teste de palatabilidade foi realizado com 38 cães utilizando-se teste versus por comparação. A adição da fibra de goiaba não alterou a ingestão de nutrientes, exceto para fibra dietética total (FDT) (P<0,001). A inclusão de fibra resultou em menor digestibilidade para matéria seca (MS) (P<0,001), matéria orgânica (MO) (P<0,001), proteína (PB) (P<0,001), energia bruta (EB) (P<0,001) e energia metabolizável do alimento (EM) (P<0,001). A fibra de goiaba não alterou a concentração fecal de amônia, ácido láctico, pH fecal e ácidos graxos de cadeia ramificada (AGCR), porém, reduziu a concentração dos ácidos graxos de cadeia curta (AGCC) acético e propiônico (P=0,007 e P=0,006). A inclusão de 6% de goiaba não alterou o TRGI, mas 12% de inclusão resultou em menor TRGI (P=0,046) comparado com a dieta CO. No capítulo 4, as dietas CO, SC_L, SC_S, WB_L e WB_S foram fornecidas a 30 Beagles adultos seguindo as mesmas metodologias do estudo com fibra de goiaba. Em comparação com a dieta CO, as dietas com fibra de cana e trigo aumentaram a ingestão de todos os nutrientes (P<0,001) e diminuíram a digestibilidade da MS, MO e ME (P<0,001), sem diferenças para PB, gordura, FDT e amido. Cães alimentados com farelo de trigo, em relação aos alimentados com fibra de cana, obtiveram aumento na concentração de ácido láctico (P<0,001) e diminuição da amônia (P<0,001) e pH (P<0,001) fecal, ácido isovalérico e ACCR total (P<0,001). A inclusão de ambas as fontes de fibra reduziram o TRGI comparado com a dieta CO (P<0,001). Os cães apresentaram preferência alimentar pela dieta sem fibra (P<0,01) sem efeitos claros quanto ao tamanho de partícula. No geral, concluiu-se que a adição de fibras limita o cozimento do amido e aumenta o gasto de energia na extrusão; torna as dietas duras, densas e exigem mais cautela para serem recobertas. A fibra de goiaba, por sua vez não causa alterações na fermentação intestinal e no TRGI de cães até 12% de inclusão. A fibra de cana e trigo com pequenos tamanhos de partículas utilizado nesse estudo não resultaram em melhoras quanto à digestibilidade de dietas com alta fibra e não alteraram o TRGI; entretanto, interferiram na formação de AGCC e preferência alimentar dos animais.

Palavras-chave: fibras, nutrição de cães, preferência alimentar, resposta fermentativa, tamanho de partícula, tempo de retenção intestinal.

FIBER FOR DOGS: EFFECTS ON EXTRUSION PROCESSING, NUTRIENTS DIGESTIBILITY, MICROBIAL FERMENTATION, GASTROINTESTINAL RETENTION TIME AND DIET PALATABILITY

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ABSTRACT- There is a great interest in developing dog food with high nutrient concentration and low energy density. The addition of fibers to commercial pet food is one way to moderate the energy density of these foods, provide health benefits to dogs and cats and, at the same time, to promote a more efficient utilization of feed resources that are not used in human foods. For this, a total of 8 fiber diets were manufactured: Control (CO), with no fibrous ingredient addition; guava fiber diets (GF3, GF6, GF12), at the inclusion levels of 3%, 6%, and 12%; sugarcane fiber diets (SC), with 9% inclusion and two different particle size (large - SC_L and small - SC_S) and wheat bran fiber diets (WB), with 32% inclusion and two different particle size (large - WB_L and small - WB_S). This study aimed to evaluate the effect of growing inclusion of guava fiber, as the effect of sugarcane and wheat bran with different particle size on extrusion processing, nutrients digestibility, intestinal fermentation, gastrointestinal retention time (GIRT) and palatability. The software SAS was utilized to statistical analysis and the meanings were compared by polynomial and orthogonal contrasts ($P<0.05$). In chapter 2, guava fiber inclusion resulted in linear increase of the amperage ($P<0.001$), temperature ($P<0.001$) and pressure ($P<0.001$) at the extruder die was verified. A higher implementation of specific mechanical energy (SME) ($P<0.001$), a reduction in radial expansion ($P<0.001$), and a greater specific piece density was verified for guava fiber supplemented diets. The sugarcane fiber in comparison with wheat bran reduced amperage ($P<0.001$), SME ($P=0.013$) and piece density, but increased specific length ($P<0.001$). The inclusion of fibers with smaller size, reduced the engine amperage ($P<0.001$), SME ($P<0.001$) and extruded piece density, but increased the radial expansion ($P=0.008$). In chapter 3, the diets CO, GF3, GF6 and GF12 were fed to 24 adult Beagle dogs during 15 days of adaptation and afterwards the dogs were housed in cages for fecal collection to assess digestibility and fermentation end products. The dogs received an oral pill containing radiopaque markers to determine gastrointestinal retention time (GIRT). Diet palatability was evaluated by the two-pan test using 38 dogs. The addition of guava fiber did not change nutrient intake except for dietary fiber ($P<0.001$). Fiber inclusion resulted in lower total tract apparent digestibility for dry matter ($P<0.001$), organic matter ($P<0.001$), crude protein ($P<0.001$), crude energy ($P<0.001$) and food ME ($P<0.001$). Guava fiber addition did not change the fecal concentration of ammonia, lactic acid, fecal pH, and branched chain fatty acids but it decreased short chain fatty acid concentrations for acetic and propionic acids ($P=0.007$ and $P=0.006$). The inclusion of 6% guava fiber did not alter the gastrointestinal transit time, but 12% inclusion did result in a reduced GIRT ($P=0.046$) compared to the control diet. The chapter 4, the Diets CO, SC_L, SC_S, WB_L e WB_S were offered to 30 adult Beagles following the same methodologies used in guava fiber study. In comparison with the CO diet, the diets with sugarcarne and wheat bran fiber increased the nutrient intake ($P<0.001$) and decreased the coefficients of total tract apparent digestibility (CTTAD) of DM, OM and food ME ($P<0.001$) without differences for CP, fat, TDF and starch. Dogs fed WB diets, comparing fiber sources, had greater lactic acid concentration ($P<0.001$) and lower fecal pH and fecal ammonia ($P<0.001$), isovaleric and total branched chain fatty acids concentration ($P<0.001$). The inclusion of both fibers decreased the gastrointestinal retention time compared to CO diet ($P<0.001$). Palatability testing results indicated that the CO treatment was preferred over the fiber diets without clear effects about particle size. In general, fibers addition limited the starch cooking, increases processing cost, turn the kibbles hard, and could bring difficulties for coating. The guava fiber not occasioned alterations in the intestinal fermentation and in the GIRT until 12% inclusion. The sugarcane and wheat bran with small particles did not result in improved digestibility of a high fiber diet and GIRT; however, may have interfered on BCFA and palatability.

Keywords: fiber, fermentative response, food preference, gastrointestinal retention time, particle size.

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CAPÍTULO 1

CONSIDERAÇÕES GERAIS

CAPÍTULO 1- Considerações gerais

1. INTRODUÇÃO

A população de cães domésticos no Brasil em 2014 foi estimada em 58,4 milhões de animais, presentes em quase 50% dos lares brasileiros. Essa população impulsiona mercado especializado, formado por mais de 140 mil pontos de venda, segundo os dados da Associação Brasileira de Indústria de Produtos para Animais de Estimação (ABINPET, 2014). A estimativa para o ano de 2015 é de crescimento de 7% sobre 2014, o que significa R$17,9 bilhões de faturamento dos segmentos "Pet", o que representa em torno de 0,31% do PIB nacional (EUROMONITOR, 2013). Deste faturamento, 67,5% abrange as indústrias de "Petfood" (ABINPET, 2014). A indústria de alimentação animal brasileira, em 2014, produziu mais de 67 milhões de toneladas de ração, de acordo com Sindirações, tendo como principais fatores propulsores a ascensão das classes sociais, o aumento da renda dos brasileiros e a ampliação da posse responsável, com maior dedicação e cuidado aos animais.

A busca de alimentação saudável para cães e gatos é frequente por parte dos proprietários, visando aumentar a longevidade e prevenir o desenvolvimento de doenças, como a obesidade (BONTEMPO, 2005). Em virtude do aumento desta enfermidade (GERMAN et al., 2012), atualmente, a inclusão de fibras na dieta tornou-se realidade por parte das indústrias de "*petfood"* (KAWAUCHI et al., 2011). Versões light, diet ou de redução de calorias estão presentes nos produtos de praticamente todas as indústrias. Fibra tem sido empregada para se reduzir o valor energético da dieta e favorecer o ajuste entre ingestão e gasto calórico, favorecendo a manutenção de condição corporal saudável (FISHER et al., 2012). Já é estabelecida a influência da fibra na digestibilidade da energia. Segundo o Nutrient Requirements of Dogs and Cats (NRC, 2006), para cães cada ponto percentual de fibra adicionada ao alimento ocasiona redução de 1,43% da digestibilidade da energia da ração. Adicionalmente, fibra é incluída devido à sua influência na manutenção da saúde do trato gastrintestinal (REINHART; SUNVOLD, 1996),

formação e consistência das fezes (BURKHALTER et al., 2001), diluição da energia do alimento (BISSOT et al., 2010), regulação do apetite e saciedade (BOSCH et al., 2009) e devido a alegações de melhorar o metabolismo de carboidratos (CARCIOFI et al., 2005).

Os estudos até o momento para cães limitaram-se a avaliar inclusões de fibra, da forma como estas foram obtidas industrialmente e seus efeitos nos animais, incluindo digestibilidade dos nutrientes, formação de fezes, tempo de trânsito intestinal, produtos de fermentação e respostas metabólicas (FAHEY et al., 1990a, 1990b, 1992; SUNVOLD et al., 1995a; SWANSON et al., 2001; SA et al., 2013; MONTI et al., 2015). Com exceção de uma única publicação com celuloses purificadas (WICHERT et al., 2002), estudos sobre o processamento das fontes de fibra em si, especificamente quanto à influência do tamanho geométrico de suas partículas sobre as respostas induzidas nos animais não estão disponíveis para cães. O processamento industrial da fibra, no entanto, muda suas características físico-químicas, podendo aumentar sua fermentabilidade, solubilidade e viscosidade, alterando assim os efeitos que esta ocasiona nos animais (ZHANG et al., 2009; REDGWELL et al., 2011; ROBIN et al., 2012).

Outro aspecto relevante da fibra é indução de importantes alterações no processo de extrusão. Por se tratar de material altamente polimerizado e estruturado, esta não é expansível e apresenta variável absorção de água, interferindo na geração de viscosidade, cozimento, fluxo da massa no interior do tubo de extrusão, formação da estrutura celular e taxa de expansão do extrusado (KARKLE, 2011). Verifica-se, assim, que a inclusão de fibra nas formulações, apesar de justificada pelo aspecto nutricional, traz importantes desafios de processamento, com aumento do consumo de energia elétrica e produção de extrusados poucos expandidos, densos e duros (KARKLE, 2011; MORARU et al., 2003; CAMIRE et al., 2007). Estas informações, infelizmente, somente estão disponíveis para extrusão de alimentos para o homem, produzidas em estudos com cereais matinais. Cereais matinais são basicamente formulados com cerais (arroz, milho, trigo, aveia) e açúcares (glicose, sacarose e frutose), com muito pouca proteína e gordura (BRENNAN et al., 2008). Sendo assim, as grandes diferenças de matérias primas e composição química dos cereais matinais em relação às formulações para cães e

gatos fazem com que não seja possível se extrapolar diretamente estes dados para os alimentos comerciais para estes animais, apesar dos equipamentos de extrusão serem similares.

Tendo em vista o exposto anteriormente, esta Dissertação teve por objetivos gerais avaliar os efeitos de inclusões crescentes de fibra de goiaba, bem como o efeito da fibra de cana e do farelo de trigo moídos em diferentes tamanhos sobre o processo de extrusão, digestibilidade dos nutrientes, fermentação no intestino, tempo de trânsito intestinal e palatabilidade de alimentos extrusados para cães.

Pretendeu-se, como objetivos específicos:

- Estudar o efeito da adição de teores crescentes de fibra de goiaba, em formulação para cães, sobre o processo de extrusão, formação e macroestrutura dos extrusados, digestibilidade dos nutrientes e da energia, tempo de retenção do alimento no trato digestório, formação de produtos de fermentação e palatabilidade das dietas.
- Avaliar o efeito de dois tamanhos geométricos de fibra de cana e do farelo de trigo sobre o processo de extrusão, formação e macroestrutura dos extrusados, digestibilidade dos nutrientes e da energia, tempo de retenção do alimento no trato digestório, formação de produtos de fermentação e palatabilidade de dietas contendo teores moderados de fibra dietética alimentar.

2. Revisão de literatura

Fibra para cães

A fibra alimentar é composta por partes comestíveis das plantas, carboidratos resistentes à digestão e absorção que podem ser solúveis ou insolúveis em água, fermentados ou não fermentados pela microbiota do intestino grosso (AACC, 2001). Características como solubilidade e fermentabilidade definem a funcionalidade da fibra para cães (NRC, 2006; CARCIOFI, 2008). A fermentabilidade diz respeito à velocidade e extensão de degradação bacteriana da fibra e correspondente produção de ácidos graxos de cadeia curta (AGCC). Estes últimos são benéficos ao trato gastrintestinal, mas em elevadas concentrações promovem aumento do

peristaltismo, do teor de água das fezes e diminuição da digestibilidade da proteína e gordura (SUNVOLD et al., 1995; CARCIOFI, 2008). Fibra de baixa fermentação leva à diminuição da digestibilidade da matéria seca, mas ocasiona baixa interferência na digestibilidade dos demais nutrientes, no teor de água e na qualidade das fezes produzidas (CARCIOFI, 2005).

Fibra solúvel pode ter elevada capacidade de retenção de água, formando géis que aumentam a viscosidade luminal interferindo na cinética de digestão e absorção. Esta, geralmente é mais rapidamente degradada pela microbiota intestinal, resultando em concentrações significativas de AGCC (SWANSON et al., 2001). No entanto, nem sempre isto ocorre, pois algumas fibras de elevada solubilidade, como goma arábica e de Psillium são pouco fermentáveis pela microbiota de cães (SUNVOLD, 1995b). Em contraste, a fibra insolúvel tem menor capacidade de retenção de água e é, geralmente, pouco fermentável pela microbiota do intestino. Isto, no entanto, também é bastante variável entre as fontes de fibra insolúvel e algumas apresentam considerável capacidade de serem fermentadas, de modo que este aspecto deve sempre ser estudado e considerado nas formulações (BOSH et al., 2008; CALABRO et al., 2013).

A forma de processamento, produção, extração e manufatura das fibras muda sua funcionalidade. Neste contexto, característica importante da fonte de fibra é o tamanho geométrico médio de suas partículas. No único estudo localizado para cães, celulose microcristalina e com fibras longas foram comparadas. Foi verificado que a qualidade das fezes melhorou com o aumento do tamanho da fibra, a celulose em menor tamanho piorou a qualidade das fezes (WICHERT et al., 2002). Verifica-se, assim, que mesmo fibra insolúvel não fermentável como a celulose tem sua funcionalidade dependente da forma como foi previamente processada antes de fornecida aos cães. O maior ou menor tamanho de partículas da fonte de fibra pode alterar não somente a formação de fezes, como demonstrado anteriormente, mas também a interação da fibra com a microbiota (levando em conta que fibras com pequeno tamanho geométrico têm maior área exposta para colonização e degradação microbiana), sua fermentabilidade e a digestibilidade dos nutrientes.

A digestibilidade dos nutrientes pode ser afetada de modo diferente em função das características físico-químicas e da quantidade de fibra adicionada à

dieta (NRC, 2006). SUNVOLD et al., (1995b) verificaram maior digestibilidade da matéria seca de rações para cães suplementadas com fibra solúvel fermentável, mas esta reduziu a digestibilidade da proteína bruta e da gordura em relação à fibra insolúvel não fermentável. Em estudo recente com felinos, FISCHER et al. (2012), verificaram que apesar do farelo de trigo e fibra de cana-de-açúcar serem constituídos por fibras insolúveis, a inclusão de farelo de trigo resultou em maior digestibilidade dos nutrientes, formação de fezes com maior umidade e maior produção de AGCC que a fibra de cana, que por sua vez reduziu a resposta glicêmica pós-prandial dos animais.

Dependendo de sua quantidade e fonte, a fibra pode acarretar diminuição da palatabilidade dos alimentos (CARCIOFI, 2005). Esta possibilidade vem extrapolada da nutrição humana, pois estudos demonstraram que cereais matinais extrusados enriquecidos com fibra tornaram-se mais laxativos, mais densos, menos crocantes, mais duros e compactos, além de diferenciar-se em textura, aparência e sabor, o que reduziu sua aceitação pelas pessoas (KTENIOUDAKI et al., 2012; ROBIN et al., 2012; FOSCHIA et al., 2013;). Além das características sensoriais odor e palatabilidade, alteradas pela inclusão da fonte de fibra, a profunda alteração por ela induzida na extrusão muda a macroestrutura dos kibbles, característica também relacionada à mastigação, palatabilidade e consumo do alimento (CHALLACOMBE, 2011). O aumento da densidade específica e redução da expansão dos kibbles induzidas pela fibra os torna mais duros, tornando necessário empregar-se mais força durante sua mastigação, aumentando o tempo de ingestão dos alimentos. Estas características, no entanto, necessitam ser convenientemente caracterizadas para cães pois não foram estudadas para estes animais.

O tempo de retenção da digesta no trato gastrintestinal é outro parâmetro influenciado pela ingestão de fibra. O tempo de retenção se refere ao período compreendido entre a ingestão alimentar e a eliminação das fezes (BURROW et al., 1982). Este não retrata o tempo de permanência da ingesta nos diferentes compartimentos do trato digestório, como estômago, intestino delgado e intestino grosso. Estudos com cães avaliaram o efeito da fibra no tempo de retenção (FAHEY Jr et al., 1990a; 1992; HERNOT et al., 2005). Dissertação de mestrado de nosso grupo de pesquisa utilizou o método de marcadores radiopacos, com sucesso,

demonstrando redução do tempo de retenção mediante inclusão de fibra de cana na dietas de cães (SILVA, 2013).

Fibra de cana-de-açúcar

Na safra de 2013/2014, o Brasil realizou a moagem de 653 mil toneladas de cana-de-açúcar, o que originou 37.713 mil toneladas de açúcar e 27.543 mil m^3 de Etanol. Isto posiciona o bagaço de cana como coproduto agrícola de maior volume nacional, segundo a União da Indústria de Cana-de-Açúcar (ÚNICA).

A cana-de-açúcar (*Saccharum officinarum L.)* é uma das espécies mais cultivadas no Brasil. Do bagaço da cana, co-produto da indústria sucroalcooleira, por meio de processos de lavagem, purificação e micromoagem extrai-se a fibra de cana. Esta apresenta 53,5% de celulose, 31,3% de hemicelulose, 6,4% de lignina, 2,6% de proteína bruta, 2,6% de matéria mineral e conteúdo inexpressivo de gordura (VELOSO, 2011). Com cerca de 90% de fibra alimentar, a fibra de cana possui praticamente 100% de fibra insolúvel (PINTO, 2007). O ingrediente praticamente não é fermentado pela microbiota de cães (CALABRO et al., 2013) e quando adicionado em rações extrusadas reduz a digestibilidade da energia e promove formação de fezes adequadas (SILVA, 2013). Este é ingrediente nacional acessível, muito mais barato que a celulose de madeira purificada, tradicionalmente incluída em formulações especiais para cães.

Farelo de trigo

O farelo de trigo é um dos principais co-produtos da alimentação humana, obtido da moagem do trigo para produção da farinha de trigo, com largo emprego na alimentação animal. Este é constituído basicamente pelo pericarpo da semente de trigo, onde se concentram a maior parte de sua fibra e minerais (BUTOLO, 2010). Apresenta entre 16% e 19% de proteína e 32% e 40% de fibra alimentar, sendo esta constituída por 97% de fibra insolúvel. Para cães, sua inclusão no alimento induz redução da digestibilidade dos nutrientes e da energia, aumento da produção de

AGCC e da umidade das fezes, embora as fezes produzidas sejam adequadas (SÁ et al., 2013).

Devido a seu baixo custo e teor relativo de proteínas, o ingrediente é largamente empregado em alimentos econômicos. É possível que o farelo de trigo, à semelhança do verificado para a fibra de cana, possa ser reprocessado industrialmente e tornar-se fonte de fibra mais interessante, a fibra de trigo, mediante conveniente redução do tamanho geométrico de suas partículas. A inclusão de farelo de trigo está associada à redução do cozimento na extrusora, aumento do consumo de energia elétrica no processamento e formação de extrusados rugosos e de aspecto grosseiro (CASE et al., 1992). Estas características indesejáveis poderiam ser revertidas, ou minimizadas, mediante adequado preparo e processamento desta fonte de fibra.

Fibra de Goiaba

O Brasil se destaca no cenário mundial como terceiro maior produtor de frutas, comercializadas tanto *in natura* como processadas na forma de sucos, concentrados, polpa e doces (MAPA, 2012). Apesar disso, nenhum dos potenciais co-produtos desta importante indústria é atualmente empregado na indústria de alimentos para animais de estimação. A goiabeira (*Psidium guajava*) é espécie pertencente à família *Myrtaceae* e originária das regiões tropicais americanas. O Brasil ocupa o lugar de segundo maior produtor mundial de goiabas, com cerca de 275.000 toneladas produzidas por ano. São Paulo é o estado que se destaca no cenário brasileiro, produzindo 65% desse total (POMMER; MURAKAMI, 2006).

Durante seu processamento industrial, até 47% da goiaba pode ser descartada (DURIGAN et al., 2002). Considerando-se as estimativas de processamento anual de 202 mil toneladas de goiaba no Brasil, tem-se dimensão do potencial e disponibilidade deste co-produto (SILVA et al., 2009). Esses resíduos de frutas, ao saírem da indústria, apresentam alto teor de umidade. A polpa de goiaba pode chegar a apresentar mais de 55% de água (SILVA et al., 2006), o que tem limitado seu emprego na alimentação animal (PRASSAD; AZEEMODDIN, 1994). Resíduos de indústrias alimentícias apresentam normalmente baixo custo, estão

disponíveis e são caracterizados por apresentar elevada fibra alimentar (SERENA; BACH-KNUDSEN, 2007). O uso destes co-produtos é tendência crescente, agregando valor em descartes de processos e reduzindo a geração de resíduos pelas indústrias (YAGCI; GOGUS, 2010).

De modo geral, a fibra das frutas apresenta elevada concentração de pectina e hemicelulose em relação ao teor de celulose presente, com baixa concentração de gordura e proteína (FISHER, 2009). A composição química e as características fermentativas de várias polpas de frutas foram estudadas para cães utilizando um modelo *in vitro* (SWANSON et al., 2001). De acordo com os autores, as fibras de frutas podem fornecer balanço na proporção solúvel e insolúvel da fibra, colaborando para a saúde do trato gastrointestinal.

Fibra e o processo de extrusão

A extrusão é tecnologia amplamente utilizada para produção de diversos tipos de alimentos para o homem e animais. Umidade, temperatura, pressão e cisalhamento são combinados proporcionando mistura, cozimento, plasticização, texturização e formatação dos alimentos em curso espaço de tempo (RIAZ, 2007). O uso generalizado da extrusão termoplástica na indústria *pet food* deve-se ao fato dela promover mudanças físicas e químicas nos ingredientes, alterando sua qualidade e propriedades físicas, aumentando seu valor nutricional com eficiência e baixo custo relativo (TRAN, 2008).

Dentre os carboidratos, o amido é o principal substrato para que a extrusão ocorra de forma apropriada (CRANE et al., 2000). Durante a extrusão, grânulos de amido são umedecidos e recebem calor, atrito mecânico, corte e pressão, sofrendo o fenômeno de gelatinização: incham, derretem e perdem sua estrutura cristalina (RATNAYAKE; JACKSON, 2009). A fibra, por outro lado, não apresenta funcionalidade na extrusão e sua inclusão resulta em aumento do custo e piora relativa do processamento (GIBSON; ALAVI, 2013)

Não foram encontradas publicações sobre os efeitos da fibra na extrusão de alimentos para cães. Para o homem, adição de frutas e legumes em salgadinhos extrusados tem sido estudada desde 1980 (MAGA; KIM, 1989). Desde então,

estudos com poupa, pó, bagaço, cascas e sementes foram publicados para produtos extrusados para o homem (KARKLE, 2011; ALTAN et al., 2008; UPADHYAY et al., 2010). De modo geral, a adição de fibra parece induzir aumento da resistência ao fluxo da massa no tubo extrusor, o que resulta em aumento do consumo de energia elétrica e da aplicação de energia mecânica específica. Este aumento da aplicação de energia não resulta em ganho de cozimento, ao contrário a fibra pode limitar o cozimento do amido e a qualidade de processamento, com possível influência no aproveitamento e digestibilidade da dieta.

Em relação à macroestrutura do extrusado, a fibra atrapalha a formação de adequada estrutura celular, pois conduz o vapor de água sem que ocorra formação de células. Isto gera aumento da expansão longitudinal e redução da expansão radial, aumento da densidade específica e aparente e formação de extrusados mais duros (ROBIN et al, 2012). Todos estes aspectos podem ter reflexo importante, tanto no custo de processamento como na digestibilidade e palatabilidade de alimentos para cães suplementados com fibra.

Moagem

A eficiência do processo de produção dos alimentos está diretamente relacionada à eficiência na moagem dos ingredientes. Estudos realizados em codornas, peixes, frangos, suínos e seres humanos (OWSLEY et al., 1981; HEALY et al., 1994; DAHLKE et al., 2001; LEANDRO et al., 2001; SOARES et al., 2003;) demonstraram a relação entre a moagem dos ingredientes e o aproveitamento dos nutrientes. Entretanto, não foram encontrados, na literatura científica, trabalhos que estudaram a influência de diferentes moagens de fontes de fibras para cães.

Em relação à moagem de cereais, Bazolli et al. (2015), utilizando-se de formulações para cães contendo milho, arroz ou sorgo, moídos em 3 diferentes tamanhos de partícula (aproximadamente 300, 450 e 600 µm) reportaram redução na digestibilidade dos nutrientes para as dietas contendo milho e sorgo com maiores tamanhos de partícula. Owsley et al. (1981) estudaram o efeito do tamanho das partículas de sorgo na digestibilidade dos nutrientes para suínos. Os resultados indicaram relação inversa entre o tamanho da partícula e a digestibilidade da matéria

seca, amido, energia bruta, proteína e aminoácidos. Healy et al. (1994) comparam a moagem de milho e sorgo em diferentes tamanhos de partícula, em ração peletizada para suínos. Dois dos parâmetros checados foram o custo de processamento e a digestibilidade dos nutrientes. Os autores verificaram aumento na digestibilidade dos nutrientes com a redução da granulometria do alimento para valores abaixo de 500 µm. Houve aumento no custo de produção da ração, mas este foi compensado pela melhora no ganho de peso dos animais.

O método utilizado nessa dissertação para a redução do tamanho das partículas dos ingredientes fibrosos foi a moagem com moinho de rolos. Esse moinho é mais utilizado na moagem em pequenas quantidades e fornece produto de textura mais uniforme. Dois ou mais cilindros pesados giram em direções contrárias, a velocidades iguais ou diferentes. A alimentação de material é feita na parte superior do moinho. As partículas são comprimidas entre os rolos, que têm ranhuras longitudinais que promovem o corte do material, submetido a forças de compressão. A distância entre os rolos é regulável, determinado o tamanho final das partículas produzidas (BELLAVER; NONES, 2000).

Força de Corte (avaliação da textura)

O Teste de Textura ou Força de Cisalhamento, segundo Silva (2013), reproduz condições similares às de mastigação pelos animais. Ainda não se tornou prática comum na avaliação de alimentos para animais de companhia, mas é muito utilizado para avaliação de carnes e alimentos para o homem. A análise de textura tem importância na indústria de alimentos, favorecendo tanto a padronização e controle do processos de fabricação, como o desenvolvimento de novos produtos. O teste torna possível se explorar a influência dos ingredientes e do processamento na força de mastigação e aceitação do produto final. A produção de alimentos com diferentes forças de cisalhamento implica em rações com diferentes resistências à mastigação (ISHIZAKI et al., 2006), o que abre oportunidades para se investigar seu efeito na cinética de consumo, tempo de mastigação, palatabilidade e até mesmo na saúde oral dos animais, pelo efeito mecânico em limpeza dos dentes.

3. Hipóteses estabelecidas na presente Dissertação

Considerando as informações disponíveis na literatura científica, levou-se em conta para esse estudo que a fibra de goiaba, proveniente da indústria de sucos para alimentação humana, convenientemente desidratada, moída e preparada, poderia ter valor como fonte de fibra para cães. As características organolépticas do ingrediente deveriam resultar em bom odor e aceitação do alimento. Considerando-se a presença de importante quantidade de hemicelulose, deveria apresentar fermentação moderada, agregando funcionalidade na geração de ácidos graxos voláteis, relacionados à saúde do intestino.

Foi considerado, também, que a moagem fina da fibra de cana de açúcar e do farelo de trigo seria capaz de alterar as propriedades funcionais destas matérias primas. A utilização de fibras de menor tamanho geométrico geraria menor interferência no processo de extrusão e iria favorecer o cozimento adequado, menor consumo de energia elétrica durante o processamento e a formação de extrusados com melhor macroestrutura. A melhor macroestrutura dos extrusados poderia, por sua vez, favorecer a aceitação e palatabilidade do alimento. Esta redução do tamanho de partículas poderia alterar a degradação microbiana dos produtos, aumentando sua fermentabilidade e alterando sua influência na digestibilidade, tempo de retenção gastrointestinal, formação e qualidade das fezes.

Diante destas hipóteses, a presente Dissertação de mestrado foi dividida em 4 capítulos: O primeiro contém as considerações gerais, aqui apresentadas. Os capítulos de 2 a 4 foram desenvolvidos em formato de artigo, em língua inglesa, seguindo as normas dos periódicos escolhidos para publicação. O capítulo 2 intitulado "*Fibra, macroestrutura e processamento de rações extrusadas para cães", foi* escrito de acordo com as normas da revista ***"Animal Feed Science and Technology"*** e avaliou o efeito das fibras nos parâmetros do processo de extrusão; o capítulo 3 intitulado "*Fibra de goiaba como um novo ingrediente alimentar: caracterização e efeitos na digestibilidade, fermentação, tempo de retenção e palatabilidade em cães"* seguiu as normas da revista ***"Journal of Animal Physiology and Animal Nutrition"*** e pretendeu-se avaliar e caracterizar a fibra de goiaba; o capítulo 4 intitulado "*Efeito da fibra de cana e do farelo de trigo em diferentes tamanhos de partícula na digestibilidade, produtos da fermentação, preferência alimentar e tempo de retenção gastrointestinal de cães Beagles adultos"* foi escrito de acordo com as normas da revista ***"Journal of Animal Science"*** e estudou-se o efeito de diferentes tamanho de partículas da fibra de cana e farelo de trigo para cães.

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CAPÍTULO 2

FIBRA, MACROESTRUTURA E PROCESSAMENTO DE RAÇÕES EXTRUSADAS PARA CÃES[1]

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Running head: Fiber and extrusion of dog foods.

Fiber influence on macrostructure and processing traits of extruded diets for dogs

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Abbreviations: AAFCO, American association of feed control officials; NRC, National research council; SAS, Statistical Analysis Systems; SME, Specific mechanical energy; STE, Specific Thermal Energy; TSE, Total Specific Energy; WB_L and $SC_{L,}$ wheat bran and sugarcane fiber large particles; WB_S and SC_S; wheat bran and sugarcane fiber small particles

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Abstract

Fiber is currently used in dog food formulations due to its nutritional properties. However, few studies have evaluated the influence of fiber on the extrusion traits and kibble formation. The present study evaluated the effect of fiber type and particle size on extrusion processing parameters and kibble macrostructure of dog foods. In experiment 1, guava fiber was added to a control diet (CO) at different inclusion levels: 3% (GF3), 6% (GF6), and 12% (GF12). In experiment 2, two fiber types (sugarcane and wheat bran) and two sizes were compared to a control (CO) diet. Diets were manufactured using a single screw extruder. Each food was processed in two separated days and samples were collected four times per day, for a total of eight replications per diet. The processing conditions were not changed for any treatment. Data were analyzed via analysis of variance, and the α level of significance was set at 0.05. Guava fiber inclusion resulted in a linear increase on temperature, pressure, and specific mechanical energy (SME) implementation ($P<0.001$) during extrusion, whereas starch cooking and longitudinal expansion decreased linearly ($P<0.001$). Piece density and kibble cutting force increased linearly ($P<0.001$) with guava fiber inclusion. On experiment 2, fiber addition also increased SME implementation ($P<0.001$). Specific length and cutting force was higher ($P<0.001$), and radial expansion lower ($P=0.008$) for fiber supplemented diets. Starch gelatinization was lower for the large particle size fiber diets ($P<0.05$). Sugarcane fiber induced higher longitudinal expansion, with less dense and harder kibbles than wheat bran ($P<0.001$). The finely grind fibers lead to formation of kibbles with lower piece density ($P=0.018$). Fiber source addition increased resistance of the dough to flow inside the extruder, explaining the increased SME. Kibble macrostructure was less acceptable on fiber-supplemented diets, showing negative effects of fiber on kibble formation and macrostructure. In conclusion, fiber inclusion in dog diets increased the electric energy required to extrude and may reduce starch cooking and kibble expansion, leading to the production of denser and harder kibbles.

Keywords: extrusion, fiber sources, guava, particle size, processing, sugarcane

1. Introduction

During the extrusion process to make dog food a combination of moisture, shear, temperature, and pressure are applied to the mix of ingredients, creating a continuous and short process forcing the material through a specifically designed opening (Riaz, 2000). The process induce changes on food ingredients, resulting in extensive cooking and in a plasticized food dough (Altan et al., 2008; Yağci and Göğüş, 2008). These modifications are directly linked and depend of on the total energy transferred to the dough, composed by the implemented specific mechanical energy (SME) and the specific thermal energy (STE). The combination of these two types of energy promotes starch gelatinization, protein denaturation, lipid modification, inactivation of enzymes, and microbe viability reduction. At the end of the extruder barrel, the plasticized dough expands in contact with atmosphere, creating a particular kibble macrostructure that affects shape and texture (Griffin, 2003; Challacombe et al., 2011).

Fiber supplemented extruded diets are produced nowadays by most pet food companies. Fiber is used to dilute energy density and promote specific benefits on gut and general health (Kawauchi, et al., 2011; Fischer et al., 2012). The inclusion of fiber sources, however, influences the processing parameters and SME implementation, potentially altering the final product characteristics (Mendonça et al., 2000). Fiber is a highly structured not expandable material with variable water absorption capacity; it can affect viscosity, mass flow inside the barrel, and the cellular arrangement formation of the extrudates (Karkle et al., 2012). Due to this, kibble expansion ratio and important texture characteristics as hardness, and crispness may be altered by fiber (Karkle et al., 2012), thus changing food sensory attributes (Koppel et al., 2015).

Unfortunately, detailed information about the impact of fiber on extrusion traits and kibble macrostructure is only available for human foods (Brennan et al., 2008; Baik et al., 2004; Karkle, et al., 2012b). The characteristics of the specific fibrous material included in the recipe have also an impact on extrusion traits, and thus fiber effects cannot be generalized. Fibrous fruit and vegetable processing by-products has been studied in human extruded foods (Upadhyay et al., 2010; Karkle et al., 2012), as a way to add value to these foods and to minimize the environmental impact of these residues (Altan et al., 2008). Besides fiber type, fiber particle size is also important. It is possible to change the material dynamics into the extruder changing the fiber particle length. Understanding these effects could be important to achieve better cooking, reduce extrusion cost, and overcome some negative effects of fiber on extrudate macrostructure. However, there is no data regarding the effect of fiber particle size in the extrusion of canine diets.

The present study aimed to evaluate the influence of the addition of increasing amounts of guava fiber (experiment 1) and the addition of two different particle sized sugarcane and wheat bran fiber on the extrusion traits and kibble macrostructure of canine diets.

2. Material and Methods

2.1. Fiber ingredients and diet formulation

Two separate experiments were conducted, the first experiment was designed to study the inclusion level of guava fiber (*Psidium guajava*) and the second one to assess the impact of sugarcane fiber and wheat bran particle sizes. The company Dilumix (Leme, Sao Paulo,

Brazil) provided the fiber ingredients: guava fiber (68.9% of insoluble fiber and 1.4% of soluble fiber), sugarcane fiber (87.0% of insoluble fiber and 0.4% of soluble fiber), and wheat bran (37.8% of insoluble fiber and 1.7% of soluble fiber).

A control diet (CO) containing maize and poultry by-product meal was formulated for adult dogs, according to the European Pet Food Industry Federation nutritional guidelines (FEDIAF, 2013). The fiber sources were added to the control diet, replacing maize to create four formulations in experiment 1, the control diet plus three addition amounts of guava fiber (GF): 3% (GF3), 6% (GF6), and 12% (GF12). The guava fiber used had a mean particle size of 213 μm. On experiment 2 the same control formulation was used, and sugarcane fiber or wheat bran was added (Table 1), for a total of 5 dietary treatments. The same lot of raw fiber material was ground to obtain two different particle sizes: large sugarcane fiber (SF_L), small sugarcane fiber (SF_S), large wheat bran (WB_L), and small wheat bran (WB_S); with a diameter of 395μm, 197μm, 345 μm, and 143μm respectively. The particle sizes were determined using laser diffraction particle size analysis (Boac et al., 2009).

The total dietary fiber content of all ingredients was determined before diet manufacture and the diets containing sugarcane fiber and wheat bran were formulated to provide approximately 16% of total dietary fiber.

2.2. Diet preparation

The ingredients, with the exception of fiber sources, were weighed, mixed, and ground using a hammer mill fitted with a screen sieve size of 0.8 mm (Sistema Tigre de Mistura e Moagem, Tigre, Sao Paulo, Brazil). The fiber source was then added to the ingredients and mixed again, compounding the final diet.

Diets were extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with a processing capacity of 250 kg/h, screw diameter of 15.9 mm, L/D ratio of 29.3 and die open area of 15.9 mm^2. The extruded screw have five sections: initial - single flight and no steam lock; second – single flight and small steam lock; third – double flight uncut and small steam lock; fourth - double flight uncut and medium steam lock; fifth - double flight cut cone. The preconditioner shaft speed was set to obtain an average residence time of 3.5 minutes. Thermal energy was implemented on the dough at preconditioner by direct steam infusion. For all treatments, the extruder screw speed was set to 465 rpm.

Each food was processed separately on two different days for replicates. Each day the production was started with a high fiber diet to stabilize the equipment and establish the basal processing conditions. After the stabilization of the equipment (minimum of 45 min), the processing was kept constant and no alterations was done on any software conditions (feed rate, extruded screw speed, water and steam injection, and cutting knife speed). After this moment, production parameters were registered at each 15 minutes, with at least four measurements per diet and day, totaling eight observations per diet. The parameters registered were: preconditioner temperature, engine amperage, dough temperature before die, dough temperature out extruder, dough pressure before die, extruder output mass, water infusion, bulk density after extruder, and bulk density after dryer. Other parameters registered included ambient temperature, working water temperature, mash feed temperature, and steam pressure. At each observation time, samples of food were collected from the preconditioner, the extruder and the dryer and stored at -20ºC for further analysis. After extrusion, the kibbles were dried in a forced air dryer at 105°C for 20 minutes, and coated with fat and liquid palatability agents.

Table 1. Ingredients and chemical composition of the experimental diets used on experiment 1 and experiment 2.

Item	Diets [a]					
	CO	GF3	GF6	GF12	SC	WB
Ingredients, (g/kg, as-fed basis)						
Maize	578.2	546.8	516.4	449.4	474.6	304.4
Poultry by product meal	318.0	318.6	318.2	325.2	325.6	261.2
Poultry Fat	64.4	65.2	66.0	66.0	73.2	76.4
Guava Fiber [b]	-	30.0	60.0	120.0	-	-
Sugarcane Fiber [c]	-	-	-	-	90.0	-
Wheat Bran [d]	-	-	-	-	-	320.0
Fish oil	1.5	1.5	1.5	1.5	1.5	1.5
Palatant [e]	20	20	20	20	20	20
NaCl	5	5	5	5	5	5
KCl	5	5	5	5	5	5
Vitamin and Mineral mix [f]	3	3	3	3	3	3
Choline Chloride	2	2	2	2	2	2
Antioxidant [g]	0.4	0.4	0.4	0.4	0.4	0.4
Mold inhibitor [h]	1	1	1	1	0.1	0.1
Analyzed chemical composition of the final product (g/kg, DM-basis)						
Moisture	59.0	68.0	60.0	73.0	58.0	58.6
Ash	60.0	55.7	66.2	61.0	56.8	61.0
Crude Protein	252.1	257.7	259.5	251.7	254.8	252.1
Crude Fat	153.0	159.0	152.0	146.1	148.7	150.2
Total dietary fiber	107.5	131.3	166.3	183.6	166.1	180.0
Insoluble fiber	107.5	128.5	163.7	176.9	166.1	171.7
Soluble fiber	0.00	2.8	2.7	6.7	0.0	8.4
Starch	412.0	387.0	357.0	357.0	379.1	344.0

[a] CO-Control diet, without added fiber source, GF3 – addition of 3% guava fiber, GF6 – addition of 6% guava fiber, GF12 – addition of 12% guava fiber, SC – addition of sugarcane fiber, WB – addition of wheat bran.

[b] Dilufiber Guava, Dilumix, Leme, SP, Brazil.

[c] Vit2be Fiber, SPF do Brazil, Descalvado, Brazil

[d] Wheat bran fiber, Dilumix, Leme, SP, Brazil.

[e] Liquid Palatant, SPF do Brazil, Descalvado, Brazil.

[f] Added per kg of diet: iron, 120 mg; copper, 15 mg; magnesium, 75 mg; zinc, 150 mg; iodine, 2 mg; selenium, 0.3 mg; vitamin A, 18,000 IU; vitamin D3, 1,000 IU; vitamin E, 100 IU; vitamin K, 2 mg; biotin, 0.6 mg; thiamine. 20 mg; riboflavin, 10 mg; pantothenic acid, 50 mg; niacin, 75 mg; vitamin B6, 6 mg; folic acid, 4 mg; vitamin B12, 0.1 mg.

[g] Banox: BHA-butylated hydroxyanisole, BHT-butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda.

[h] Mould Zap: Ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brasil Agroindustrial Ltda.

2.3. Laboratory Analyses

Food samples were grounded in a cutting mill (Mod MA-350, Marconi, Piracicaba, Brazil) fitted with a 1mm screen. The samples were analysed by oven-drying of the sample for dry matter (DM) (method 934.01), by muffle furnace incineration for ash content (method 942.05), by the Kjeldahl method for crude protein (method 954.01), and with a Soxhlet apparatus extraction for acid hydrolysed ether extract (method 954.02), following the methods described by the Association of Official Analytical Chemists (AOAC, 1995). Organic matter (OM) of the samples was calculated as DM minus ash. Dietary fibre (total, soluble, and insoluble) was measured by using a combination of enzymatic and gravimetric procedures (method 991.43, AOAC, 1995), the total amount of starch was measured according to described by Hendrix (1995), and the degree of gelatinization of the starch was determined by the amyloglucosidase method as described by Sá et al (2013).

2.4. Kibble traits and macrostructure

The extrudate cutting force was measured in 20 kibbles per diet. Kibbles were first dried to achieve the same moisture in an oven (ETS Modelo 532, Systens Eletro-Tech, Inc., Glenside, PA, EUA) at 55°C during 24 hours. Individual kibbles were weighted and the cutting force determined with a texturometer (Texture Analyser TAX/T2I - Stable Micro Systems Ltda, Godalming, UK) equipped with a load cell of 50kg.f. The samples were compressed with a 10mm penetration distance and speed of 2.0mm/s, using a Warner Bratzler Knife. To ensure that the samples were representative, the diameter of scanned kibble should correspond to the treatment average diameter.

Electronical micrographs of the kibbles were obtained in a scanning field emission scanning electron microscope (JEOL, JSM- 7500F; Miaka, Tokyo, Japan), adjusted to 20 kV. Images were evaluated by a trained examiner, blinded to the experimental groups. The analyses were performed on the Chemistry Institute, UNESP, campus of Araraquara. All image analysis was qualitative, not submitted to statistical evaluation. Images of the external and internal surface were conducted. For the internal evaluation, kibbles were cut along the medial direction. Pictures of the internal surface were processed with standardized filter sets using the software Adobe Photoshop CC 2015 (Adobe Systems, California, USA) and the total area of inner cells measured. The results was expressed as the percentage of cell areas in relation to mean kibble diameter area, with the following formula:

$$\text{Percentage of cell areas} = \frac{\text{Total area of inner cells measured}}{\text{mean kibble diameter area}}$$

For each treatment, the length (le), diameter (de) and mass (me) of 20 extrudates were measured and used to obtain the radial expansion ratio (RE), specific length (l_{sp}) and piece density (ρ), as described below.

$$RE = \frac{d_e^{\,2}}{d_d^{\,2}}$$

$$l_{sp} = \frac{le}{me} \quad (mm/g)$$

$$\rho = \frac{4\,me}{\pi \times \frac{de^2}{2} \times le} \quad (kg/m^3)$$

were: d_d= die open area.

2.5. Specific mechanical energy and Specific thermal energy calculations

The SME was calculated for each treatment in accordance with Riaz (2007), with the following formula:

$$SME=\frac{(\sqrt{3}\times Voltage\times(WA-EA)\times(cosFi\div 1000))\times 1000}{Production}$$

Where;

Voltage = (220V)

WA = working amperage, the motor amperage during processing

EA = empty amperage, the motor no load amperage

The STE is the sum of the thermal energy contributions of raw materials, water and process water vapor divided by the feed rate (kg/h). This was calculated after the mass balance according to Riaz (2000):

-Preconditioner energy balance:

$$Q_R + Q_W + Q_S = Q_{Pc} + Q_{SL} + Q_{HL} + \sum\Delta h$$

- Extruder energy balance:

$$Q_P + Q_{W+} Qsme + Qbarrel = Qex + Q_{SL} + \sum\Delta h$$

Where;

Q_R = Raw material heat capacity

Q_{HL} = Preconditioner heat loss by convection

Q_{Barrel} = Extruder heat loss by convection

Q_W = Water input heat capacity;

Qsme = Mechanical energy amount

Q_S = Steam input heat capacity

Qte = Thermal energy amount

Q_p = Preconditioner Product heat capacity

Qex = Extruder Product heat capacity

Q_{SL} = Steam loss heat capacity

$\sum \Delta h$ = Reaction energy

The amount of heat (Q) was obtained from the formula: Q = m.c.T

Where:

m = mass;

c = specific heat capacity

T = temperature

- Preconditioner mass balance equation (preconditioner output):

Mraw + Mw + MS = Msl + Mpc

- Extruder balance mass equation:

Mpc + Mw = Msl + Mf

Where:

Mraw = raw material mass

Mw = water mass;

Ms = steam mass;

Msl = steam loss mass

Mpc = preconditioner mass;

Mf = final mass

The Total Specific Energy (TSE) was obtained by the sum of the SME and STE.

2.6. Statistical analysis

Each experiment was analyzed separately. Both studies follow a randomized block design, with two blocks (days of extrusion) and four repetitions per block (each 15 min interval of sampling), totaling eight repetitions per treatment. The experimental unit was considered the time sampling, except for kibble macrostructure and deforming force, when the experimental unit was one kibble (with 20 repetitions per treatment). Data were submitted to analysis of variance, model sums of squares were separated into treatment (diet) and block (days of extrusion) effects. When differences were found on F test, on experiment 1 polynomial contrasts were used to evaluate the inclusion levels of guava fiber, and on experiment 2 orthogonal contrasts were used to detect differences between: control and fiber supplemented foods (CO *versus* SF_L + SF_S + WB_L + WB_S); sugarcane and wheat bran fibers (SF_L + SF_S *versus* WB_L + WB_S); fiber particle sizes (SF_L + WB_L *versus* SF_S + WB_S). The mixed procedure of SAS statistical software (version 9.1; SAS Institute, Cary, NC, USA) was used to perform the analysis. All data were found to comply with ANOVA assumptions. Values of $P<0.05$ was considered significant. The α level of significance was set at 0.05.

3. Results

3.1. Experiment 1

During extrusion the preconditioner temperature ($P<0.001$) and output moisture ($P=0.005$) decreased linearly as guava fiber inclusion increased (Table 2). The mass flow in the extruder fluctuated randomly. Starch gelatinization after preconditioner was similar

among diets, with a mean value of approximately 33% (P>0.05). The motor load (engine amperage), and die temperature and pressure increased linearly with guava fiber inclusion (P<0.001). Extruder output mass varied according to the preconditioner mass flow (P=0.003). Regarding energy balance, the SME implementation increased approximately 84% when comparing CO with GF12 (P=0.047). The STE implementation increased linearly (P=0.015), which together resulted in linear increase on TSE (P=0.001). Because the SME presented greater proportional increase than STE, the STE:SME ratio decreased quadractically (P=0.002). The addition of guava fiber reduced linearly the kibble radial expansion rate and starch gelatinization (P<0.001), and increased the piece density (P<0.001). Fiber inclusion resulted in a quadratic reduction on kibble specific length (P<0.001), whereas it resulted in an increased kibble hardness, as verified by the linear increase on cutting force (P<0.005). Figure 1 illustrates internal and external pictures of the kibbles produced on experiment 1. It is possible to note that the cellular structure in the CO diet is composed by larger cells compared to the GF12 diet. The percentage of cell area in relation to total kibble diameter area was 15.3% for CO, and decreased to 13.4% for GF3, 13.0% for GF6, and 12.6% for GF12. It is possible to see bigger pores on the kibble surface of the CO diet compared to GF3 and GF6 diets, whereas the kibbles of the GF12 diet showed an uneven and rough surface.

3.2. Experiment 2

The parameters measured at preconditioner did not change on experiment 2, with the exception of a lower output mass for fiber supplemented diets in comparison with the CO diet (P=0.007; Table 3). The engine amperage was lower for sugarcane fiber than wheat bran diets, and lower for the small particle size than large particle size fibers (P<0.001). Pressure at

die was lower for fiber supplemented diets than for CO diet (P=0.046). Extruded output mass varied accordingly to the preconditioner output, with a higher value for the CO diet (P=0.046) compared to fiber diets. The SME implementation was higher for fiber diets compared to CO diet, also higher for wheat bran compared to sugarcane fiber diets and for large particles compared to small particle fibers (P<0.01). Fiber supplemented diets presented higher STE implementation than CO (P<0.001), and so did the sugarcane fiber compared to the wheat bran diets (P=0.013). These variations resulted in higher TSE addition and lower STE:SME ratio for the fiber supplemented diets than for the CO diet (P<0.01), and higher STE:SME ratio to sugarcane than wheat bran supplemented diets (P<0.001). Regarding kibble traits, diets with small particle size fiber had higher starch gelatinization than large particle size fiber diets (P=0.051). Piece density was higher for the wheat bran than for sugarcane fiber diets, and for large fiber particle than for small fiber particle diets (P<0.02). Fiber inclusion reduced the kibble radial expansion compared to the CO diet (P=0.008). Longitudinal expansion (specific length) was increased after fiber addition to diets (P<0.001), and was higher for sugarcane in comparison with wheat bran diets (P<0.001). Fiber inclusion resulted in a significant increase on cutting force (P<0.001) compared to CO diet, and this was higher for the sugarcane than for wheat bran diets (P<0.001). The internal and external pictures of the kibbles produced on experiment 2 are shown in Figure 2. The cellular structure of the CO and the diets produced with small particle fibers (SF_S and WB_S) are composed by bigger cells compared to large particle fiber diets (SF_L and WB_L). The percentage of cell area in relation to total kibble diameter area was 15.6% for CO, 13.2% for SF_L, 14.6% for SF_S, 17.0% for WB_L, and 16.1% for WB_S. There were no differences on pore formation of the kibble surface among treatments.

Table 2. Processing parameters and kibble macrostructure traits of extruded dog diets with different inclusions of guava fiber. Experiment 1.

Item	Diets [a]				S.E.M.[b]	Contrast [c]	
	CO	GF3	GF6	GF12		Linear	Quadratic
Preconditioner							
Temperature (°C)	85.6	84.4	85.7	82.2	0.59	<0.001	0.301
Output moisture (%)	27.7	26.1	25.7	25.2	0.01	0.005	0.577
Output mass (as-fed, kg/h)	181.9	170.2	162.8	190.8	2.31	0.004	0.005
Starch gelatinization (%)	35.6	30.3	33.6	33.5	1.28	0.987	0.756
Extruder							
Engine amperage (A)	42.1	42.80	46.6	49.18	0.70	<0.001	0.160
Die temperature (°C)	125.8	130.3	141.3	134.3	1.46	<0.001	0.137
Die pressure (bar)	61.7	61.7	70.3	70.6	0.88	<0.001	0.909
Output mass (as-fed, kg/h)	170.4	161.2	149.6	153.2	3.32	0.043	0.003
Energy balance (kW-h/ton)							
SME[d]	15.3	19.5	25.1	28.1	0.81	<0.001	0.047
STE[e]	74.7	74.4	84.6	87.2	2.27	0.015	0.682
TSE[f]	90.0	93.9	109.6	115.3	3.52	<0.001	0.815
STE:SME	4.88	3.81	3.37	3.10	0.21	<0.001	0.002
Kibble traits (After Dryer)							
Starch gelatinization (%)	92.8	91.1	90.5	88.3	0.93	<0.001	0.823
Piece density (kg/m^3)	0.37	0.40	0.42	0.43	0.01	<0.001	0.190
Radial expansion rate	4.1	3.9	3.5	3.2	0.05	<0.001	0.263
Specific length (mm/g)	47.4	40.8	41.6	46.3	0.37	0.097	<0.001
Cutting force (kg.f) [g]	2.4	2.9	3.1	3.4	0.06	<0.005	0.263

[a] CO= control diet, without added fiber source, GF3= addition of 3% guava fiber, GF6= addition of 6% guava fiber, GF12= addition of 12% guava fiber. [b] S.E.M= standard error of the mean (n= 32). [c] Linear and quadratic effect of guava fiber additions. [d] SME= specific mechanical energy. [e] STE= specific thermal energy. [f] TSE= total specific energy. [g] n=20 kibbles per diet.

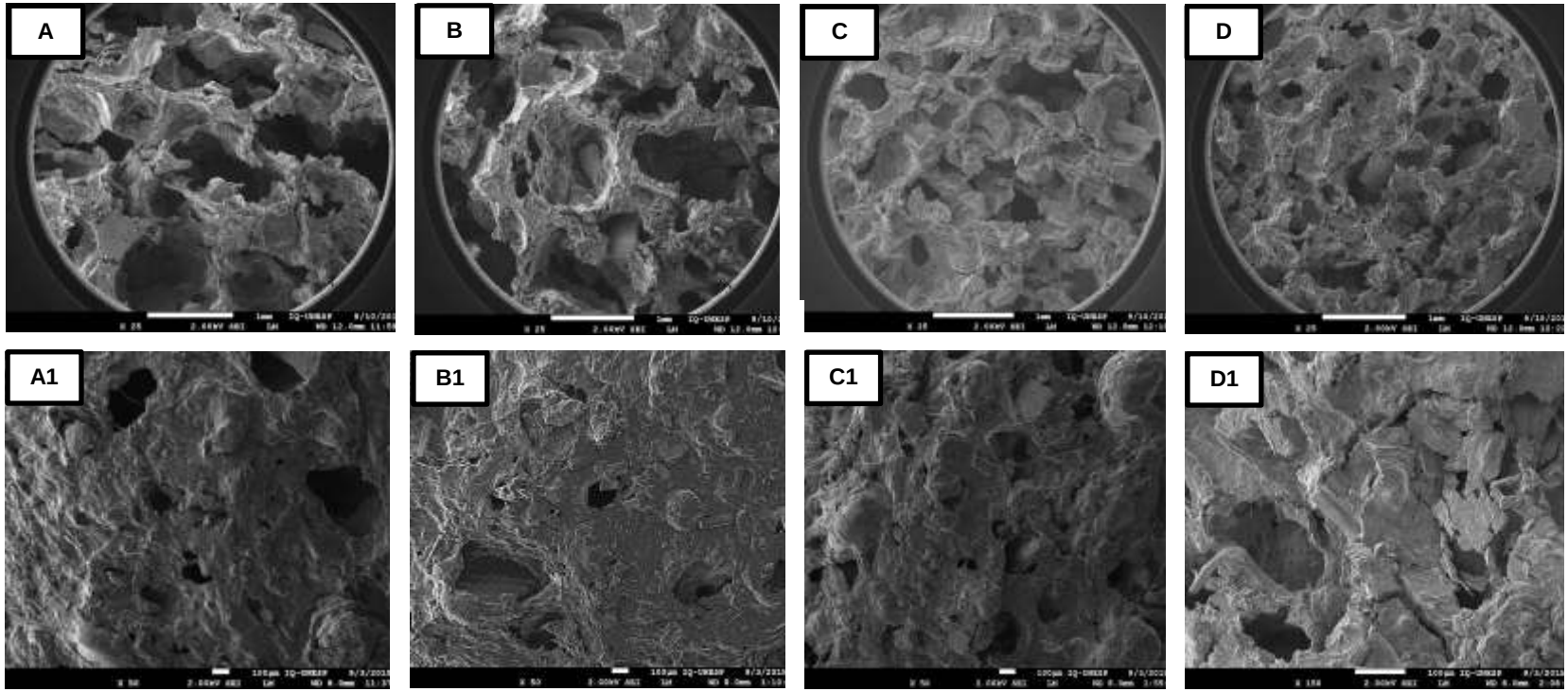

Figure 1. Field scanning electron micrograph of diets with guava fiber inclusion. A, B, C and D – Internal area (increased 25x) of CO, GF3, GF6 and GF12 diets, respectively. A1, B1, C1 and D1- External area (increased 50x) of CO, GF3, GF6, and GF12 diets, respectively. CO= control diet, without added fiber source, GF3= addition of 3% guava fiber, GF6= addition of 6% guava fiber, GF12= addition of 12% guava fiber.

Table 3. Processing parameters and kibble macrostructure traits of extruded dog diets with different types and size of fiber. Experiment 2.

Item	Diets [a]					S.E.M. [b]	Contrast		
	CO	SF_L	SF_S	WB_L	WB_S		SC x WB[c]	Large x Small[d]	CO X Fiber[e]
Preconditioner									
Temperature, (°C)	85.6	84.1	84.1	82.9	84.3	0.48	0.616	0.522	0.1879
Output moisture, (%)	27.7	26.7	27.9	27.0	28.3	0.004	0.675	0.102	0.8124
Output mass (as-fed, kg/h)	181.9	168.86	154.34	166.09	164.61	3.01	0.465	0.136	0.007
Starch gelatinization, (%)	35.6	30.5	31.6	29.9	33.9	1.07	0.572	0.124	0.431
Extruder									
Engine amperage (A)	42.1	41.0	41.0	45.9	40.9	0.49	<0.001	<0.001	0.936
Die temperature (°C)	125.8	126.3	118.7	123.9	124.9	1.16	0.443	0.170	0.314
Die pressure (bar)	61.7	53.4	52.5	56.2	60.2	1.57	0.110	0.569	0.047
Output mass (as-fed, kg/h)	170.4	152.0	141.3	150.0	152.0	4.03	0.479	0.237	0.046
Energy balance (kW-h/ton)									
SME [f]	15.3	16.7	16.9	24.0	17.07	0.81	0.014	<0.001	<0.001
STE [g]	74.7	110.3	120.1	108.4	90.6	0.68	0.013	0.209	<0.001
TSE [h]	90.0	127.0	136.1	132.4	108.3	5.06	0.058	0.071	<0.001
STE:SME	4.88	6.60	7.06	4.51	5.12	0.28	<0.001	0.104	0.007
Kibble traits (After Dryer)									
Starch gelatinization (%)	92.8	93.8	94.6	91.9	94.0	0.48	0.673	0.051	0.545
Piece density (kg/m^3)	0.37	0.38	0.36	0.45	0.41	0.008	<0.001	0.018	0.103
Radial expansion rate	4.1	2.9	3.0	2.8	3.1	0.04	0.578	0.701	0.008
Specific length (mm/g)	47.4	57.0	58.7	51.4	51.7	0.69	<0.001	0.317	<0.001
Cutting force (kg.f) [i]	2.4	4.0	4.1	3.4	3.1	0.06	<0.001	0.2122	<0.001

[a] CO= control diet, without added fiber source, SF_L= sugarcane fiber, large particle; SF_S= sugarcane fiber, small particles; WB_L= wheat bran, large particles; WB_S= wheat bran, small particles. [b] S.E.M.= standard error of the mean (n= 40). [c] Sugarcane fiber (SF_L + SF_S) *versus* wheat bran (WB_L+WB_S). [d] Large fiber particles (SF_L + WB_L) *versus* small fiber particles (SF_S + WB_S). [e] Control (CO) *versus* fiber supplemented diets (SF_L + SF_S + WB_L + WB_S) [f] SME= specific mechanical energy. [g] STE= specific thermal energy. [h] TSE= total specific energy. [i] n=40 kibbles per diet

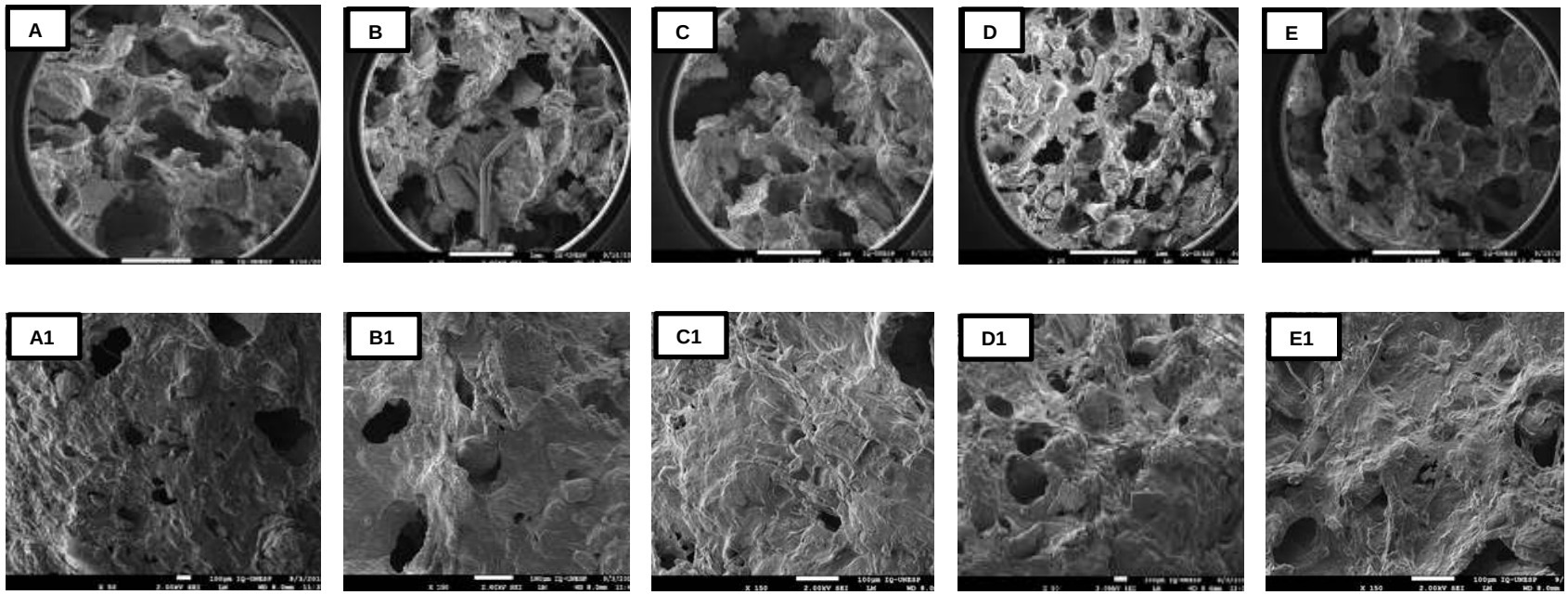

Figure 2. Field scanning electron micrograph of diets with sugarcane and wheat bran fiber. A, B, C, D, and E – Internal area (increased 25x) of CO, SC_L, SC_S, WB_L, WB_S, diets, respectively. A1, B1, C1 and D1- External area (increased 25x) of CO, SC_L, SC_S, WB_L, WB_S, diets, respectively. CO= control diet, without added fiber source, SF_L= sugarcane fiber, large particle; SF_S= sugarcane fiber, small particles; WB_L= wheat bran, large particles; WB_S= wheat bran, small particles.

4. Discussion

This study evaluated the effect of the type and particle size of fiber sources on extrusion parameters in diets for dogs. Although processing conditions were kept constant, some fluctuation on preconditioner temperature, output moisture, and output mass were noted on experiment 1. The reductions in moisture and temperature could be related to a reduced capacity of steam absorption of the dough after guava fiber inclusion. It is possible that the fiber conducted the steam, reducing its condensation on the dough, as discussed by Robin et al. (2012) about kibble expansion. On experiment 2 we observed a tendency for lower preconditioner moisture for the large particle size fibers, reinforcing this hypothesis and suggesting that the fiber structure might play an important role for this effect.

An increased resistance of the dough to the flow inside the extruder could explain the differences in extruder amperage, temperature and pressure shown on experiment 1. Guava fiber addition resulted in an increased motor work, electric energy consumption, and shear, increasing the temperature, pressure, and SME implementation. On experiment 2 this effect of fiber was not so evident, probably due the higher feed rate (preconditioner output mass) of the CO diet, interfering on the outcomes. However, when the mass production was corrected, the SME implementation was also higher for the fiber supplemented diets compared to the CO diet. Altan et al. (2008), Upadhyay et al. (2010), and Karkle et al. (2012) already demonstrated this effect of fiber for human extruded foods. At least partially, this can be related to a reduction in the available water during the extrusion when fiber is added to the recipe, thus reducing dough fluidity (Hill, 2003). In experiment 1, the treatments with higher guava fiber inclusion also showed less in-barrel moisture (preconditioner output moisture). This was an unexpected outcome, and could have contributed to the increase in dough flow

resistance. Nevertheless, the differences in moisture are small, less than 10% between CO and GF12. For experiment 2, in-barrel moisture did not vary between diets, and the water holding effect of fiber was more evident increasing dough resistance. An increase in shear might explain the higher STE, and TSE implemented after fiber addition, effect shown in both experiments. The reduction on STE:SME ratio happened because the mechanical energy increased more than the thermal energy addition.

Wheat bran and sugarcane fiber differ regarding their influence on processing traits. Wheat bran inclusion resulted in higher SME implemented, suggesting a higher resistance to flow in comparison with sugarcane fiber diets. An effect of fiber particle size was also observed, but only the WB_L resulted in increased SME, and no differences were seen between SF_L and SF_S. These differences are likely related to particular physical characteristics and influences of each ingredient on the flow dynamic inside the extruder.

The increased mechanical energy consumption has implications on the production cost of the food. The utilization of electric power increased 85% after 12% addition of guava fiber in experiment 1, and wheat bran and sugarcane inclusion resulted in a mean elevation of 22% in experiment 2. The increase was particularly important for the WB_L, with a value 57% higher than for CO. These increases in electric power add cost to the production of fiber supplemented diets, and alternatives to a more rational processing condition should be studied.

Kibble macrostructure was worse on fiber-supplemented diets on both experiments, showing the negative effects of fiber on kibble formation and macrostructure. A reduction in starch cooking was observed in experiment 1 with guava fiber addition, and in experiment 2with large particle size fibers. The starch fraction works as a thermoplastic polymer during extrusion. When water, energy, and time are sufficient in the processing, the starch granule

loses its crystallinity, swells, and disrupts, forming an amorphous mass that binds all food components forming a continuous structure (Ding et al., 2005). Energy implementation was higher for fiber supplemented diets, and does not explain the lower starch gelatinization and worse kibble formation on fiber supplemented diets in both experiments. A possible explanation could be a limitation on available water for starch cooking with fiber inclusion (Hill, 2003; Nelson, 2001). It is possible that the added fiber retained the water (Santos et al., 2008; Khanna and Tester, 2006), that then became less available for hydration, dough lubrication, and starch gelatinization.

High extrudate density with a shift toward longitudinal instead of radial expansion were verified on kibbles of both experiments after fiber inclusion. This effect was already demonstrated after cellulose inclusion on human food (Chinnaswamy and Hanna, 1991). Usually these are the result of the formation of kibbles with small size and thicker cell walls (Robin et al, 2012). Imaging from the diets from our study suggest that this is the case with high guava fiber and large particle size fiber inclusion, although this was only qualitatively evaluated. These structural changes could be responsible for the increased of kibble hardness (cutting force) with fiber addition. The higher cutting force is explained by the smaller cells with thicker walls, and by the strengthen effect of the fiber particles against the rupture of the molten starch mass. Fiber effect in expansion and cell formation has been also attributed to its water binding capability (Moraru and Kokini, 2003; Jin et al., 1994; Camire and King, 1991). In addition, due to its structure, fiber can conduct the water vapor out of the kibble, reducing the flash off effect on cell formation (Lue et al., 1991; Robin et al., 2012), directly affecting expantion. We also noted differences between the effect of wheat bran and sugarcane on kibble formation, with sugarcane inclusion resulting in lower piece density and higher specific length and cutting force. This means that sugarcane fiber induce higher longitudinal

expansion, with lighter and harder kibbles than wheat bran. This diverse structure forming traits should be explored in specific diets when differences in density or specific influences on chewing dynamics are of interest. Regarding particle size, the finely grind fibers favoring formation of kibbles with lower piece density, signaling that fiber particle reduction might be a means of increase kibble expansion.

Several implications for this interference of fiber addition on expansion, cell structure formation and increase in hardness should be considered. Absorption of fat added by surface coating could be less efficient in fiber enriched diets. The lesser internal open space and external porous may impede the internal migration of fat, which will stay on surface. Higher residence time on coating equipment, or even special coating systems with vacuum may be required for high fat addition. The diets from the present experiment were evaluated by an expert human panel to describe their sensory traits (Koppel et al., 2014). Fracturability, and initial crispness were physical attributes that differentiated the CO with the fiber supplemented diets. This could affect acceptability of the kibble by the dogs, although there are very few studies that evaluate the influence of the physical attributes of the kibble on canine diet palatability. It is possible that these physical alterations change the mastication kinetics or sensation, altering the acceptability of the food by the dog. The interference of fiber supplementation of chewing sensation, reducing food acceptance For is well described in human beings (Karkle et al., 2012a, Martin et al., 2013).

5. Conclusions

The addition of fiber sources to dog food formulations increases electric energy required to extrude, and may reduce starch cooking and kibble expansion, leading to the production of denser and harder kibbles.

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CAPÍTULO 3

FIBRA DE GOIABA: CARACTERIZAÇÃO E EFEITOS NA DIGESTIBILIDADE, FERMENTAÇÃO, TEMPO DE RETENÇÃO E PALATABILIDADE EM CÃES.

Escrito de acordo com as normas da revista Journal of Animal Physiology and Animal Nutrition

Running Head: Guava fibre in dog foods formulation

Guava fibre: material characterization and effect on digestibility, fermentation, gastrointestinal retention time and palatability in dogs*

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Abbreviations: AAFCO, American association of feed control officials; BCFA, branched chain fatty acid; CTTAD, coefficients of total-tract apparent digestibility; CE, crude energy; CP, crude protein; DM, dry matter; GE, gross energy; ME, metabolizable energy; NRC, National research council; OM, organic matter; SAS, Statistical Analysis Systems; SCFA, short chain fatty acid; TGA, thermogravimetric analysis; VFAs, volatile fatty acids.

Guava fibre: material characterization and effect on digestibility, fermentation, gastrointestinal transit time and palatability in dogs

SUMMARY - The inclusion of fibre sources is common in extruded pet foods and the use of fruit fibres as a way of reusing industrial waste is a trend in the industrial environment. This study aimed to characterize guava fibre as a fibrous ingredient and to evaluate the effects of its addition to extruded diets for dogs on nutrient digestibility, faecal traits, fermentation products, gastrointestinal retention time, and palatability. Four diets were formulated: CO (control, no fibrous ingredients added), GF3 (3% guava fibre), GF6 (6% of guava fibre), and GF12 (12% guava fibre). The diets were fed to 24 adult Beagle dogs for 15 days of adaptation and afterwards the dogs were housed in cages for faecal collection to assess digestibility and fermentation end products. The dogs received a radiopaque pill to determine GIRT. Diet palatability was evaluated by the two-pan test. The results showed that guava fibre did not change nutrient intake except for TDF ($P<0.001$). Fibre inclusion resulted in lower CTTAD for DM ($P<0.001$), OM ($P<0.001$), CP ($P<0.001$), CE ($P<0.001$) and food ME ($P<0.001$). Guava fiber did not change the faecal concentration of ammonia, lactic acid, faecal pH, and branched chain fatty acids but it decreased short chain fatty acid concentrations for acetic and propionic acids ($P=0.007$ and $P=0.006$). Inclusion of 6% of guava fibre did not alter the gastrointestinal transit time, but 12% inclusion did result in a faster transit time ($P=0.046$) compared to the control diet. Therefore, guava fibre was characterize as a novel insoluble ingredient that could be safety used in levels up to 12.0% as a dietary fibre source for canine extruded diet.

Keywords: canine, diet, food intake, fruit fibre, intestinal microbiota.

Introduction

Currently, the inclusion of fibre in diets is a reality in the pet food industry. Light or reduced calorie diets with several fibres types and amounts are part of the product range of most pet food companies. Fibre is used to decrease the energy density of the diet and thus to facilitate the adjustment between energy consumption and expenditure, helping maintain a healthy body weight. Fibre has also been studied in several disease states in pets, such as diabetes mellitus and gastrointestinal problems (Fahey et al., 1990[b]). Fibre could have different effects on nutrient digestibility, faecal formation, intestinal microbiota, food preference and transit time of dogs, depending on the fibre source and its inclusion level (de Godoy et al., 2013). Common sources of dietary fibres used for pet foods formulations include beet pulp and cellulose. Beet pulp, according to the same authors contains a variable ratio of insoluble: soluble fibre of 1.9-5.3:1. On the other hand, cellulose is composed of mainly insoluble and poorly fermentable fibre, with a varying ratio of insoluble: soluble fibre of 27.5 – 42.2 :1).

Currently, due to the perception of the beneficial effects of dietary fibres in promoting health, as well as the popularity of functional foods and holistic and natural diets, the use of alternative and novel carbohydrates (here defined as fibre sources not typically used in diet matrixes) has become extensive in human and pet food. Consequently, poorly digestible carbohydrates and cereal grains with a low-glycaemic index or rich in fermentable and soluble fibres, such as soluble corn, fruit fibres, and whole grains, have received attention by the pet food industry.

Brazil stands out in the world as the third biggest producer of fruits, sold as processed juices, pulp, or candy (MAPA, 2012). The use of by-products obtained

from fruits and vegetables is described as a growing trend in the literature and one of the reasons for this is the addition of value to by-products and the reduction of waste from industries (Yagci and Gogus, 2010). Despite this, the traditional market of fruits in Brazil is not being used currently in the manufacturing of dog food.

Guava (*Psidium guajava)* belongs to the *Myrtaceae* family and it originates from the American tropics. Brazil is the second largest producer of guava in the world, with a production of about 275.000 ton/year, showing a big potential of this by-product for the pet food industry (Silva et al., 2006). São Paulo State produces 65% of this amount (Pommer and Murakami, 2006). The described organoleptic properties of guava may result in good flavour and acceptance when included in dog food.

During the juice production process up to 47% of the guava can be discarded (Durigan et al., 2002). This fruit waste has an initial high moisture content, presenting more than 55% of water, which has limited its use in animal feed (Prassad and Azeemoddin, 1994). Regarding its chemical composition, fruit fibre sources generally have a high concentration of pectin and hemicellulose relative to cellulose content, with low fat and protein concentrations (Fisher, 2009). One study assessing the chemical composition and fermentation characteristics of various fruit pulps using an *in vitro* model of dogs (Swanson et al., 2001) found that fruit fibres could provide the gut with a good balance of soluble and insoluble fibre that may enhance gastrointestinal healthy. Apple, carrot pomace, and flaxseed fibres showed the greatest fermentability whereas fruit blend, grape pomace, and pistachio showed the lowest fermentability. Pea hulls and tomato pomace presented intermediate fementability values. In addition, fruit and vegetable fibre sources contain bioactive compounds, such as carotenoids, flavonoids, and polyphenols, which have been

proposed to aid as an adjuvant therapy in some clinical conditions (Zaine et al., 2014).

Considering their nutritional potential and their appeal to dog owners, studies regarding the use of fruit fibres are of interest within industrial ecology. This ingredient, provided that it is processed, conveniently dried, milled, and prepared, could have value as a source of fibre for dogs. Due to the presence of a significant amount of hemicellulose, guava fibre could provide moderate colonic fermentation, adding functionality to generate short chain fatty acids (SCFA), of importance to gut health.

Thus, this study was designed to nutritionally characterize guava fibre and investigate the effects of its consumption on nutrient and energy digestibility, formation of fermentation products, faecal traits, gastrointestinal mean retention time, and diet palatability in adult dogs fed dry diets.

Materials and methods

Animals and experimental design

The study was conducted in the Laboratory of Research on Nutrition and Nutritional Diseases of Dogs and Cats of the School of Agrarian and Veterinary Sciences, UNESP- Univ Estadual Paulista, Campus de Jaboticabal, São Paulo, Brazil, and all procedures received ethical approval from the Ethics Committee for Animal Well-Being (protocol number 07895/14).

Twenty-four adult clinically healthy Beagle dogs were were randomly allotted to the four experimental treatments. They had an average body weight of 12.4±1.43 kg, a mean age of 7±2.2 years, and a mean body condition score of 5.0±0.20 on a nine-point scale (Laflamme, 1997). During the adaptation, the dogs were housed in pairs in 1.5 x 4.0 m^2 kennels with a solarium and were exercised daily in a 200 m^2 grassy area. During the collection period, the animals were kept in individual stainless steel metabolic cages (90 cm x 90 cm x 100 cm) equipped with a system to separate faeces and urine for collection.

The energy requirement of each dog was individually calculated in accordance with the published Nutrient Requirements of Dogs and Cats (NRC, 2006) using a standard equation for maintenance energy requirements of adult dogs (ME, $kJ \cdot day^{-1} = 523 \times kg^{0.75}$). The amount of food offered was calculated dividing the energy requirements by the metabolizable energy content of the experimental foods, estimated from their chemical composition. The total amount was divided into two equal daily meals and offered at 10a.m. and 4p.m. in stainless steel bowls. Fresh water was available ad libitum and the food consumption was recorded daily. The dogs were weighed every week and the food allowance was individually adjusted to promote maintenance of body weight.

The study followed a completely randomized block design with four dietary treatments, two blocks of 12 dogs and 3 dogs per diet and block (for a total of six dogs per dietary treatment). Dogs were fed the experimental diets during 15 days for adaptation; in the following 6 days, the dogs were kept into individual metabolic cages for total collection of faeces to measure digestibility. After a 3 day period of rest with no access to grass, the dogs were relocated in the individual cages for 6

additional days to assess the fermentation end products in faeces and to determine gastrointestinal retention time of food. Each block lasted a total of 30 days.

Diets

The diets were formulated to meet the nutrient requirements for adult canine maintenance according to the European Pet Food Industry Federation (FEDIAF, 2013). A basal diet (CO) was formulated whose main ingredients were maize and poultry by-product meal and no fibre addition. Guava fibre was added at different inclusion levels to this basal diet to create three additional dietary treatments: 3% (GF3), 6% (GF6), and 12% (GF12). The fibre in the experimental diets was added by substituting maize in the CO diet, with small adjustments to the amount of poultry fat. Total dietary fibre (TDF) was analysed in all ingredients before the diets were manufactured. The diet with 12% guava fibre (GF12) was formulated to provide 16% of TDF, which is a level typically used in commercial high-fibre canine diets (de Oliveira et al., 2011). The formulation and ingredients of the experimental diets are shown in Table 1.

The ingredients, with the exception of the fibre source, were weighed, mixed, and ground before extrusion using a hammer mill (Model 4, D'Andrea, Limeira, Brazil) fitted with a 0.8mm screen. The fruit fibre was provided already ground by the supplier (Dilumix, Leme, SP, Brazil).

The diets were extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with a processing capacity of 250 kg/h. Water, steam, screw speed and ration flux were adjusted according to diet formulation. A pre-conditioner was used to treat the diets with steam and water prior to extrusion. After extrusion

the kibbles were dried in a forced air dryer at 105°C for 30 minutes and coated with fats and palatability enhancers.

Table 1. Ingredient list, chemical composition, and starch gelatinization of the experimental diets with different additions of guava fibre.

Item	Experimental Diets*			
	CO	GF3	GF6	GF12
Ingredients, % (as-fed basis)				
Maize	57.89	54.72	51.70	44.97
Poultry by-product meal	31.80	31.86	31.82	32.52
Guava fibre †	-	3.00	6.00	12.00
Poultry fat	6.44	6.52	6.60	6.60
Palatability enhancer‡	2.00	2.00	2.00	2.00
Sodium chloride	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20
Potassium chloride	0.65	0.65	0.65	0.65
Vitamin and mineral premix§	0.30	0.30	0.30	0.30
Fish oil	0.15	0.15	0.15	0.15
Mould inhibitor¶	0.10	0.10	0.10	0.10
Antioxidant**	0.04	0.04	0.04	0.04
Chemical composition, % (as-fed basis) ††				
Moisture	5.90	6.80	6.00	7.30
Ash	6.00	5.57	6.62	6.10
Crude Protein	25.21	25.77	25.95	25.17
Crude Fat	15.30	15.90	15.20	14.61
Total dietary fibre	10.75	13.13	16.63	18.36
Insoluble fibre	10.75	12.85	16.37	17.69
Soluble fibre	0.00	0.28	0.27	0.67
Starch	41.20	38.70	35.70	36.08
Starch gelatinization degree (%)	92.82	91.04	90.50	88.33

* CO: control diet, without fibre; GF3: supplemented with 3% of guava fibre; GF6, supplemented with 6% of guava fibre; GF12, supplemented with of 12% of guava fibre.
†Dilufibre Guava, Dilumix, Leme, SP, Brasil.
‡ Liquid palatant, SPF do Brazil, Descalvado, Brazil.
§ Supplied per kilogram of diet: vitamin A, 18,000 IU; vitamin D, 1,200 IU; vitamin E, 200 IU; thiamin, 6 mg; riboflavin, 10 mg; pantothenic acid, 40 mg; niacin, 60 mg; pyridoxine, 6 mg; folic acid, 0.30 mg; vitamin B12, 0.1 mg; iron, 100 mg; copper, 10 mg; magnesium, 10 mg; zinc, 150 mg; iodine, 2 mg; selenium, 0.3 mg;
¶ MoldZap: ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brasil Agroindustrial Ltda, Curitiba, Brazil;
** Banox: butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda, Curitiba, Brazil.
†† Analysed in duplicate.

Material characterization

- Particle Size

The particle size of guava was determined using wet laser diffraction, according to Boac (2009) in the United States Department of Agriculture, Grain Marketing and Production Research Centre (Manhattan, KS, USA). The fibre samples also were analysed by the Laboratory of Scanning Electron Microscopy of São Paulo State University (Jaboticabal, São Paulo, Brazil) to obtain high-resolution imaging of surfaces.

- Thermogravimetric analysis (TGA)

For TGA, 5g of sample were heated to 800°C in an aluminium crucible under a nitrogen (N_2) atmosphere. The carbonization behaviour of the samples was monitored by TGA (TA Instrument, Delaware, USA, SDT/Q600) and the test was performed at a constant heating rate of 10°Cmin^{-1} under an air flow of 70cm^3min^{-1}. This analysis was performed in the Laboratory of Physical Chemistry of Materials, Chemistry Institute of Araraquara, UNESP- Univ Estadual Paulista.

Digestibility trial and chemical composition

On the first day of faecal collection, all faeces were removed from the cages and discarded before 8 am and total faecal output for each dog was collected from

this point onwards for the next 5 days. Faeces were collected twice per day and pooled per dog. Diet samples were also collected during the digestibility trial. Faecal samples were weighed and frozen (−15°C). Afterwards, the faeces were dried in a forced-air oven (Fanem, São Paulo, Brazil) at 55ºC for 72h. Dried faecal and diet samples were ground in a cutting mill (Mod MA-350, Marconi, Piracicaba, Brazil) fitted with a 1mm screen.

Dietary and faecal samples, as well as guava fibre samples, were analysed for dry matter (DM) by oven-drying of the sample (method 934.01), for ash content by muffle furnace incineration (method 942.05), for crude protein (CP) by the Kjeldahl method (method 954.01), and for acid hydrolysed ether extract by Soxhlet apparatus extraction (method 954.02). All these methods were performed according to the Association of Official Analytical Chemists (AOAC, 1995). Organic matter (OM) of the samples was calculated as DM minus ash. Dietary fibre (total, soluble, and insoluble) was measured by using a combination of enzymatic and gravimetric procedures (method 991.43, AOAC, 1995).

In the food samples, we also measured the total amount of starch according to the method described by Hendrix (1993). Moreover, the degree of gelatinization of the starch was determined by the amyloglucosidase method described by Sá et al, (2013).

In the guava fibre samples, the neutral detergente fibre (NDF) was determined using α- amylase and without the addition of sodium sulphite, following Van Soest et al, (1991). Acid detergent fibre (ADF) was determined using the method described by Goering & Van Soest, (1970) and acid detergent lignin was determined by solubilization of cellulose with sulphuric acid, according to Van Soest & Robertson

(1985). The cellulose content were calculated subtracting ADF less lignin and the hemicellulose content were calculated subtracting NDF less ADF.

The analyses, with the exception of TDF in food samples, were carried out in duplicate and repeated when the coefficient of variation among the duplicates was higher than 5%.

The coefficients of total tract apparent digestibility (CTTAD) of nutrients were calculated according to the quantitative collection of faeces protocol and calculation procedures described by the Association of American Feed Control Officials (AAFCO, 2008). The metabolizable energy (ME) content of the experimental diets was calculated from the obtained digestible energy and digestible protein value the diets (NRC, 2006).

Fermentation end products and faecal quality

Faecal samples were scored twice a day at the time of collection according to the following system by Carciofi et al. (2008): 1 = watery, liquid that can be poured; 2 = soft and unformed, stool assumes shape of container; 3 = soft, formed, and moist, softer stool that retains shape; 4 = hard, formed, and dry stool, remains firm and soft; 5 = hard, and dry pellets, small, hard mass.

Fresh faecal samples were collected immediately after elimination to measure volatile fatty acids (SCFA and branched chain fatty acids, BCFA), lactic acid, and ammonia. Approximately 10 g of fresh faeces were mixed with 30 mL of a 16% (v/v) formic acid solution, precipitated at 4°C for 72 h, and the supernatant was centrifuged (5804R, Eppendorf, Hamburgo, Brazil) three times at 4500 G at 15°C for 15 minutes.

The concentration of SCFA (acetic, propionic, and butyric acids) and BCFA (isobutyric, isovaleric, and valeric acids) were analysed by gas chromatography (model 9001, Finnigan, San Jose, USA) according to Erwin et al, (1961), using a glass column 2 m in length and 3.17 mm in width, covered with 80/120 Carbopack B-DA / 4 % Carbowax 20 M. Nitrogen was the carrier gas with a flow rate of 25mL/min. Working temperatures were 220°C at injection, 210°C in the column, and 250°C in the flame ionization detector

Lactic acid was measured separately according to the Pryce method, (1969) with some modifications, using phosphoric acid 85% (Molar mass 98) as precipitating reagent, dissolving 23.29 mL in distilled water to complete 1000 mL. The optical density was measured with l-cm cuvettes, at a wavelength of 565nm using a colorimetric method (Spectrophotometer Quick – Lab, Drake, São José do Rio Preto, Brazil).

The concentration of ammonia was determined in the same extracts used for SCFA. The extracts were thawed at room temperature, diluted into distilled water (2:13 v/v) and ammoniac nitrogen was distilled using potassium hydroxide 0.2 N and boric acid 0.9 N in a nitrogen system (TE Tecnal – 036/1, Piracicaba, Brazil). Hydrochloric acid 0.005 N was used for titration according to Vieira, (1980).

Faecal pH was determined by mixing 9 mL of distilled water with 4 g of fresh faeces (v/w) and immediately measuring the pH in the final solution using a calibrated pH meter (DM20, Digicrom Analítica Ltda, São Paulo, Brazil).

Digesta mean retention time

Digesta retention time in the gastrointestinal tract was evaluated according to an adaptation of the method described by Burrows et al, (1982). For the assay, dogs were restricted to their cages and the time of each defecation was recorded. The animals were fed once a day at 10a.m. and gelatine capsules containing 10 radiopaque markers (Sitzmarks, Konsyl Pharmaceuticals Inc., Fort Worth, Texas USA) were mixed with the food. The markers were 4.5 mm in diameter and had densities of 1.25 g/ml. On each day a different marker format was utilized, allowing three consecutive observations of the retention time.

The time of the marker administration was registered, and the animals and their depositions were observed at 2h intervals until the last marker was recovered in the faeces. All faeces were collected, weighed and the time of sampling was recorded. When the exact faecal elimination time was not observed within those time windows, the average time between samplings was considered for the purposes of this experiment. All faeces were radiographed and the markers counted.

The digesta mean retention time was computed as the time interval (in hours) between the food intake plus capsule administration and the time of excretion of the faeces containing the last marker recovered. The mean food retention time was the average for the three days of observation. In addition, the marker recovery rate was computed as equation 1:

$$\text{Marker recovery rate } (\%) = \frac{\text{number of markers recoved in faeces}}{\text{total of markers orally dosed}} \times 100 \qquad (1)$$

To validate the observation a minimum recovery rate of 90% of the markers was established. The number of defecations per day and the weight of each defecation were also recorded.

Palatability testing procedure

The palatability procedure follows the methodology described by Griffin et al. (2003) and currently is the most common test used to quantify the relative dietary preference in dogs and cats is the two-pan method, which was applied in this study (Smith et al., 1984; Mc Arthur et al., 1993; Hutton, 2002; Greg et al., 2015). The tests were performed in Panelis, Diana Group (Descalvado, São Paulo, Brazil). Palatability was assessed for two of the experimental diets (GF6, GF12) in comparison to the CO diet (CO x GF6 and CO x GF12), using the two-pan method on two meals in one day. Each test was carried out with two different groups of 38 dogs per group in individual kennels. In the morning after a 12h fast the dogs received two pans, each containing one of the foods, and were allowed to eat for 30 minutes. The position of the food pans was switched at the evening meal.

The amount of food offered in each pan surpassed the consumption capacity of the animal to ensure there would be left overs to measure. After 30 minutes the pans were removed, the remains weighed and consumption rate was calculated (Equation 2). Due to the large differences in body weights the results were calculated as relative consumption of each diet, and the mean intake of the two meals for each dog per day was compared.

$$\text{Relative intake of (\%)} = \frac{\text{Food A intake}}{\text{Food A intake + Food B intake}} \times 100 \qquad (2)$$

Statistical analysis

The data analysis was performed using the Statistical Analysis System (SAS) software (version 9.1; SAS Institute, Cary, NC, USA). An analysis of variance was performed, considering the effects of block (period) and dietary treatment (diet), with two blocks of 12 dogs and six animals per treatment. When treatment differences for digestibility, faecal traits, digest mean retention time and palatability were detected, polynomial contrasts were performed to evaluate the effect of guava fibre inclusion level. Tukey's multiple comparison tests were used for nutrient intake and digestibility to determine which means amongst the treatments differs. Data is presented as average plus minus standard error and the alpha level of significance was set at 0.05.

Results and Discussion

Material Characterization

- *Guava fibre - Chemical composition*

Guava fibre is a by-product of the guava processing industry and consists of dried and ground peels and core. The guava fibre source used in the present study had a particle size of 213 ± 82.7 µm and had a chemical composition of 9.3% ± 0.01 moisture, 1.9% ± 0.07 ash, 3.2% ± 0.10 CP, 6.8% ± 2.64 starch, 2.3 %± 0.10 ether extract, 70.3 % ± 1.88, TDF (68.90% insoluble, 1.36% soluble), 36% ± 2.6 cellulose, 17.3% ± 1.80 hemicellulose, and 11.3% ± 0.91 lignin. Figure 1 shows images of guava fibre particles.

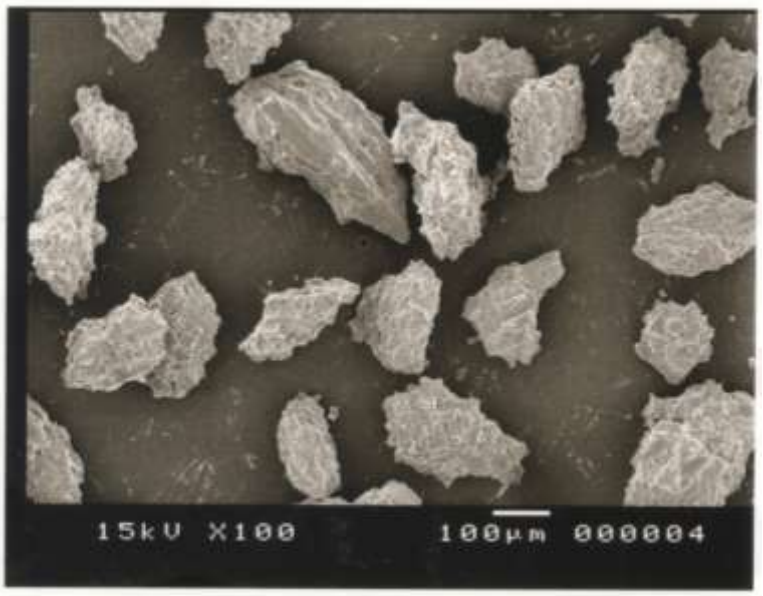
- *Thermogravimetric analysis*

The TGA technique is used for characterization of materials, specifically to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated. The measurement is normally carried out in air or in an inert atmosphere, such as helium or argon, and the weight is recorded as a function of increasing temperature.

Thermal analysis curves (TG and dTG) represented in Figure 2 shows three general regions of weight loss: a first degradation stage (<10% loss) between 40 and 110°C related to moisture evaporation followed by an additional sharp peak between 110 and 150°C attributed to the loss of trapped water; a double stage of mass drop can be distinguished in the second main decomposition region (~44% weight loss between 150 and 400°C), related to a possible more complex volatilization of matter: the first one, occurring at temperatures above 220°C, can be attributed to depolymerisation (demethoxylation, dihydroxylation and decarboxylation) of pectin chains , the main component of dehydrated guava (Einhorn-Stoll et al., 2007; Ghaffari et al., 2007; Shi and Gunasekaran, 2008), and the second, at temperatures higher than 300°C, related to high amylose starch decomposition (Massicote et al., 2008), since it prevails in samples containing a higher proportion of this polymer. The third region (>350°C) shows a more stable degradation stage, after volatilization of water and other compounds, in which about 30% of residual mass is lost. These results could help us to understand the guava behaviour in the extrusion and other processing involving heat. The curve suggests

that guava fibre is a degradable material with structural and electrochemical characteristics with lower thermal stability.

(A)



(B)

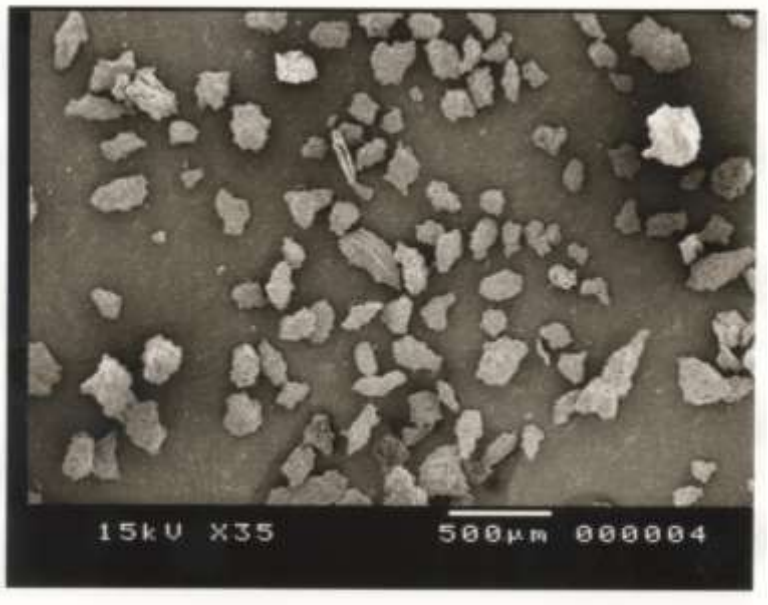


Figure 1. Scanning electron micrograph of guava fibre used in the study. A - Increased 100x; B – Increased 35x.

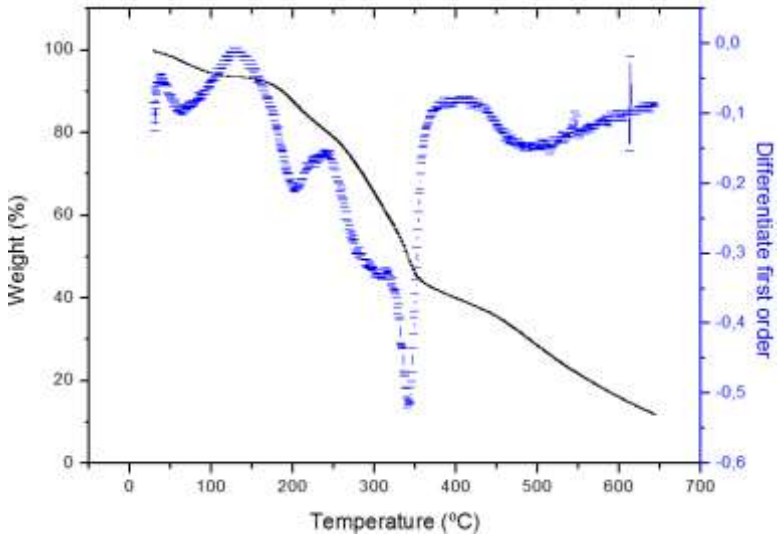


Figure 2. Thermogravimetric (TG/dTG) curves profiles of powders obtained by dehydration of guava. For thermogravimetric decomposition the heating rate was of 10°C /min, between 30 and 650°C.

Body weight and energy intake

As expected, body weight was kept stable during the experimental days (Table 2). During the study, animals were fed according to their energy requirement. Because body weight remained stable throughout the feeding period, no actions were taken to adjust food intake. There were no variations in food DM and ME intake ($P > 0.05$; Table 2).

The dogs consumed all experimental diets adequately and there were no adverse reactions noted in any of the dogs at any time during the experimental period.

Chemical composition of the diets and apparent digestibility

The ingredient and chemical composition of the diets fed to dogs is presented in table 1. The TDF content varied from 13.13 (GF3) to 18.36% (GF12) on a DM basis. All diets contained approximately 94% DM, 25% CP, 15% ether extract and 6.0% ash, but varied in their TDF and starch content with increasing fibre addition. Starch gelatinization decreased linearly as the fibre inclusion level increased.

Nutrient intake and digestibility data are presented in table 3. The TDF intake increased with guava fibre addition ($P<0.001$), as expected. In comparison with CO, the inclusion of guava fibre did not alter the CP, fat and starch intake. Regarding digestibility, fibre addition resulted in quadratic reductions ($P<0.001$) of protein digestibility, except for starch and fat. This reduction in digestibility resulted in a decrease of the ME of diets with increasing fibre inclusion ($P<0.001$). Looking at the results in more detail, the CO and GF3 diets showed similar nutrient CTTAD, and digestibility decreased linearly ($P<0.001$) from 6% inclusion level onwards, showing that a small amount of guava fibre (3%) did not reduce the overall nutrient apparent digestibility. There was no difference in starch digestibility among the treatments, indicating that the cooking of the starch was sufficient for this nutrient to be almost fully absorbed, despite the decrease in starch gelatinization noted with increasing fibre addition. Thus, guava fibre, independently of its inclusion level, resulted in adequate starch cooking, gelatinization, and digestion of the experimental diets.

Differences in nutrient intake can affect digestibility. An increased nutrient intake will dilute the nutrient endogenous losses, thus overestimating the final result.

However, during the digestibility assay, despite differences in fibre intake (P<0.01), there were no differences in the intake of DM or other nutrients for the duration of the experiment, even for the dogs fed the high fibre diet (GF12), which has a lower experimental ME.

According to some authors, fibre could interfere negatively with digestibility of nutrients (Burrows et al., 1982; Fahey et al., 1990[ab]; Sa et al., 2013). In 1981, Allen et al, already found that DM digestibility in dogs decreased linearly with increasing levels of beet pulp supplementation (6 and 12% as fed), but no significant differences in energy or CP digestibility were noted among treatments, as opposed to our results. However, Fahey et al. (1990[b]), demonstrated that the inclusion of different fibre sources (such as beet pulp, tomato pomace, peanut hulls, wheat bran and wheat straw) into meat-based, extruded dog diets to provide 12.5% of TDF in DM basis resulted in slightly lower nutrient digestibility coefficients, including those for CP and energy.

Moreover, Fahey et al, (1992) studying oat fibre (OF) inclusions at 2.0, 5.0 and 7.5% DM noted a linear decrease in the percentage of GE as the concentration of OF increased. Earle et al, (1998) using a database with 27 prepared dog foods reported the apparent digestibility of OM and of GE correlated negatively with the content of those fibre fractions (% dry matter).Inclusion of tomato pomace in extruded diets for dogs (between 6-12% TDF DM) resulted in reductions in DM, energy and CP digestibility coefficients, according to Swanson et al, (2004). Carciofi et al, (2008), using different starch sources in extruded diets for dogs (cassava flour, brewer's rice, corn, sorghum, peas or lentils), showed a reduction in the DM, OM, and GE digestibility in the diets with more TDF (14.13 % DM). Burkhalter et al. (2001)

investigated the effects of soybean hull (SH) inclusion in dog diets. They found that the CTTAD of nutrients were not affected by varying the ratio of insoluble to soluble fibre (I:S) among the SH-containing diets, but increasing TDF resulted in negative effects upon nutrient digestibility. The diets with higher TDF content (6.6 to 8.6% on as-is basis) had a modest negative effect on total-tract DM, OM, fat and GE digestibility compared to the control diet. This is in agreement with our study, where the diets with a TDF content of 13.13 – 18.36% DM had moderate effects in the apparent digestibility of same nutrients, including CP, concluding that increasing levels of TDF result in decreased CTTAD of DM, energy and CP in adult dogs.

Regarding fat digestibility, fibre inclusion might act as a physical barrier and can thus decrease reabsorption of bile and bile acids in the ileum. This would result in bile acids remaining in the intestinal lumen for longer and this would increase their faecal losses and thus alter fat digestibility (Kritchevsky, 1974; Eastwood et al., 1986). In this study we did not observe a reduction in fat digestibility. A possible explanation for this is that lignin is the fibre component with the highest ability to bind bile acids (Kay, 1982) and the guava fibre used in our study had a moderate lignin content of 11.34% ± 0.91. As a comparison, beet pulp, a common source of fibre in pet food, contains a small quantity of lignin <2% (Okojie; Sargent, 1990).

Table 2. Body weight, dry matter and metabolizable energy intake of dogs fed experimental diets with different additions of guava fibre.

Item	Experimental diets*				S.M.E†	P value	Contrasts‡	
	CO	GF3	GF6	GF12			Linear	Quadratic
Body weight (kg)								
Initial	13.3	12.7	12.1	11.7	0.30	0.059	ns§	ns
Final	13.2	12.5	12.3	11.8	0.29	0.058	ns	ns
P value ¶	0.999	0.975	0.969	0.913	-	-	-	-
Food intake (Mean of 30 days of study)								
Dry matter ($g/kg^{0.75}$ day^{-1})	28.6	29.6	30.5	31.9	0.61	0.245	ns	ns
Metabolizable energy, ($kJ/kg^{0.75}day^{-1}$) **	464.4	481.8	487.5	469.9	8.91	0.2334	ns	ns

* CO: control, without fibre; GF3: supplemented with 3% of guava fibre; GF6, supplemented with 6% of guava fibre; GF12, supplemented with of 12% of guava fibre.
† S.E.M. = standard error of the mean (n = 6 dogs per treatment).
‡ Linear and quadratic effect of guava fibre addition.
§ ns = not significant (P>0.05).
** P value by F test
¶ Calculated with the food metabolizable energy values determined *in vivo*.

Table 3. Nutrient intake, coefficient of total tract apparent digestibility (CTTAD), and metabolizable energy content of experimental diets for dogs with different additions of guava fibre.

Item	Experimental diets*				S.E.M†	P value	Contrasts‡	
	CO	GF3	GF6	GF12			Linear	Quadratic
Nutrient intake during the digestibility study (g/kg$^{0.75}$ day^{-1})								
Dry matter	28.6	28.9	30.5	31.6	0.65	0.084	ns§	ns
Organic matter	26.8	27.3	28.4	29.7	0.59	0.092	ns	ns
Crude protein	8.34	8.32	8.68	9.02	0.18	0.132	ns	ns
Acid-hydrolysed fat	4.39	4.59	4.64	4.62	0.09	0.216	ns	ns
Total dietary fibre	3.08^{a}	3.79^{a}	5.08^{b}	5.81^{b}	0.24	<0.001	<0.001	ns
Starch	12.0	11.3	11.5	11.8	0.23	0.229	ns	ns
CTTAD (%)								
Dry matter	80.8^{a}	82.6 ^{a}	77.7^{b}	74.5^{c}	0.72	<0.001	<0.001	0.003
Organic matter	84.0 ^{a}	85.6 ^{a}	81.0 ^{b}	77.0 ^{c}	0.73	<0.001	<0.001	0.001
Crude protein	82.5 ^{a}	83.8 ^{a}	82.0 ^{a}	79.0 ^{b}	0.49	<0.001	0.001	0.005
Acid-hydrolysed fat	89.5	92.6	88.5	88.7	0.73	0.013	ns	ns
Total dietary fibre	23.0 ^{a}	38.7 ^{b}	38.2 ^{b}	24.5 ^{a}	2.09	<0.001	ns	<0.001
Starch	99.7	99.7	99.7	99.7	0.02	0.996	ns	ns
Gross energy	84.5 ^{a}	85.8 ^{a}	82.3 ^{b}	77.8 ^{c}	0.71	<0.001	<0.001	<0.001
Food ME (ME, kJ/g as-fed basis)	16.23^{ab}	16.67^{a}	15.95^{b}	14,91^{c}	0.15	<0.001	<0.001	<0.001

* CO: control, without fibre; GF3: supplemented with 3% of guava fibre; GF6, supplemented with 6% of guava fibre; GF12, supplemented with of 12% of guava fibre.
† S.E.M. = standard error of the mean (n = 6 dogs per treatment).
‡ Linear and quadratic effect of guava fibre addition.
§ ns = not significant (P>0.05).

Fermentation end products

Table 4 shows the concentration of fermentation products determined in canine fresh faeces. Guava fibre inclusion did not change the faecal pH, lactic acid, and ammonia concentrations when compared to the control diet.

However, there was a linear decrease in the concentration of acetic and propionic acids ($P<0.05$) with increasing fibre content. The magnitude of this reduction was not enough to alter the molar proportion of SCFA to BCFA ($P>0.05$), which was kept constant among treatments.

Gut bacteria produce SCFA in the large intestine and are obtained mainly from the fermentation of non-starch polysaccharides (fibres). The products of this digestion are hydrogen, methane, carbon dioxide, and SCFA; mainly acetate, propionate and butyrate (Kritchevsky, 1988). According to the same author, SCFA are absorbed from the colonic lumen and contribute to normal intestinal function through their actions and on the colonic musculature and vasculature and through their metabolism by colonocytes. Butyrate, in particular, is an important energy source for the colonic cells (Roediger, 1982), provides proper absorption of ions, and promotes intestinal blood flow and peristalsis (Roediger, 1982; Campbell et al., 1997). The microorganisms found in the colon of dogs are capable of degrading different types of fibre (Reinhart; Sunvold, 1996; Case et al., 2010), causing a range of effects on the composition of the intestinal microbiota (den Besten et al., 2013) depending of the amount and type of fibre source consumed. These SCFA from fiber lower intestinal pH by changing the composition and metabolic activity of the intestinal microbiota. Other organic acids (e.g., lactate or succinate or BCFA) are found in much smaller amounts in the bowel intestine and are the result of protein

fermentation by gut microbiota. For example, the fermentation of valine and leucine, that are released during bacterial death, result in the BCFA isobutyric and isovaleric (McDonald et al., 2002).

In the present study, the consumption of diets with guava fibre (mostly non fermentable) and similar in protein content resulted in a linear decrease in acetic and propionate acids concentration ($P<0.05$) without changes in BCFA production in comparison with the control diet. This could be due to a variation in starch fermentability, in accordance with Bradford et al, (2007) because the GF12 diets have less maize and as consequence less fermentable starch.

Calabro et al, 2013, evaluating the effect of purified cellulose, carboxymethylcellulose and sugarcane fibre providing ≤850 g/kg of TDF DM also found that fibre inclusion resulted in lower SCFA concentration, like in our study, but they also found an increase in BCFA concentration, which we did not find. Since we did not observe changes in fecal pH or BCFA (and only mild changes in SCFA) It is likely that the guava fiber inclusion level was not high enough to result in modification of VFA production.

Due to the expense and difficulties associated with *in vivo* systems for estimating fibre fermentation, *in vitro* systems using dog and cat faecal inoculum have been used (Fahey et al, 1990[a]; Swanson, 2002; Fischer et al, 2012; Calabro et al, 2013; Musco, 2013;). Several *in vitro* and *in vivo* studies using dogs and few studies using cats have examined the fermentative characteristics of several fibre sources, including fruits, such as pea fibre, sugarcane fibre, apple pomace, grape pomace, tomato pomace, fruit blend and carrot pomace. In this study, guava presented a ratio of 50.76:1 (I:S), suggesting that this fibre might result in little microbial fermentation, which is supported by our faecal VFA and pH analyses.

Table 4. Lactic acid, faecal pH, faecal ammonia and volatile fatty acids (VFA) concentrations of dogs fed diets with different inclusion levels of guava fibre

Item	Experimental diets*				S.E.M†	P value	Contrasts‡	
	CO	GF3	GF6	GF12			Linear	Quadratic
Lactic acid (mmol/kg of faecal DM)	2.76	2.22	2.38	2.77	0.11	0.677	ns	ns
Faecal pH	6.16	6.25	6.20	6.16	0.04	0.959	ns	ns
Ammonia (mmol/kg of faecal DM)	144.6	151.6	133.8	133.6	4.74	0.444	ns	ns
Short-chain fatty acids, mmol/g of faecal DM								
Acetic	368.4	325.3	298.2	272.9	13.74	0.015	0.007	ns
Propionic	220.1	201.5	185.8	167.8	7.25	0.039	0.006	ns
Butyric	71.7	64.8	64.9	61.3	2.63	0.370	ns	ns
Total	660.2	581.6	550.5	487.5	21.81	0.070	ns	ns
Branched-chain fatty acids, mmol/g of faecal DM								
Isobutyric	9.6	9.7	6.2	7.6	0.60	0.211	ns	ns
Isovaleric	14.0	6.2	10.4	11.1	0.66	0.051	ns	ns
Valeric	1.2	7.6	1.4	1.8	0.16	0.302	ns	ns
Total	24.7	26.8	18.2	20.1	1.30	0.116	ns	ns
Total VFA	684.9	608.4	567.0	522.6	21.47	0.069	ns	ns
Short-chain fatty acids, molar proportion (%)								
Acetic	55.8	55.9	54.2	56.0	0,02	0.170	ns	ns
Propionic	33.3	34.7	33.8	34.4	0.01	0.716	ns	ns
Butyric	10.9	11.1	11.8	12.6	0.01	0.423	ns	ns
Branched-chain fatty acids, molar proportion (%)								
Isobutyric	38.8	36.2	34.2	37.9	0,02	0.581	ns	ns
Isovaleric	56.5	23.2	57.4	55.1	0,02	0.836	ns	ns
Valeric	4.7	28.5	7.7	8.9	0.01	0.204	ns	ns

* CO: control, without fibre; GF3: supplemented with 3% of guava fibre; GF6, supplemented with 6% of guava fibre; GF12, supplemented with of 12% of guava fibre.

† S.E.M. = standard error of the mean (n = 6 dogs per treatment).

‡ Linear and quadratic effect of guava fibre addition.

§ ns= not significant (P>0.05).

‡ Linear and quadratic effect of guava fibre addition

Faecal consistency and digesta mean retention time (DMRT)

Guava fibre addition increased faecal DM excretion linearly (P=0.004). The faecal moisture of dogs fed the control diet averaged 61.2%, which was significantly higher than the average moisture content of dogs fed the diets GF3 (58.2%), GF6 (59.2%) and GF12 (57.4%) (Table 5). This study showed guava to have a significant effect on faecal moisture, even though there were no changes in faecal water output.

Mean faecal bulk was higher in dogs fed GF6 and GF12 diets compared to the control (P<0.001). Usually, insoluble dietary fibre sources are able to increase up to 20 times the faecal volume and weight, thanks to their ability to retain water, which can also help in the management of digestive disorders like constipation (Musco, 2013). Burkhalter et al. (2001), Diez et al. (1998), and Cole et al. (1999) also found that a diet containing no added fibre resulted in lower faecal excretion compared with fibre-containing diets in dogs.

From a commercial point of view, faecal characteristics are important factors to consider. In the present study, guava fibre did not affect stool quality measured by a visual scale and not did it result in an increased number of defecations or statistical differences in faeces weight. Other studies have reported the positive effect in faecal formation and quality of insoluble fibre as increased the frequency of well-formed faeces compared to the control group (Wichert et al., 2002; Prola et al., 2010), but we did not observe this in the current study. Non-fermentable fibres may decrease gastric transit time, dilute diet caloric density, increase faecal bulk and moisture and aid in laxation (Wenk, 2001; Davidson et al, 1998). In the present study, fibre inclusion affected the DMRT. The CO diet resulted in an average retention time of

34 ± 3.8 hours, compared to 31.5 ± 4.5 hours in dogs fed G12 (P=0.046), thus, 12% guava fibre inclusion decreased gastrointestinal retention time in the dogs in approximately 2.5 hours when compared to CO diet. However, no statistical differences were found among the treatments, concluding that our results, similar to what has been found in humans (Burrows, 1982) indicate that there are wide individual variations to be considered.

Palatability test procedure

In the palatability test, for first choice, 84% of the animals preferred the CO diet to 16% that preferred the GF6 diet; and for food intake, 81% consumed CO diet while only 16% consumed the GF6 (Figure 3). Then, the palatability comparison CO *versus* GF6 had significant differences (P<0.001). However, there were no statistical differences (P>0.05) when comparing CO *versus* GF12 diet: in the first choice test, 58% of animals to 42% preferred the CO diet and for food intake, 61% preferred the CO diet.

Diet palatability usually is related with the perceptions derived at the time food is consumed and accounts for the flavour and the animals' perception of the appearance, temperature, size, texture and consistency and perhaps prior experiences (Greg et al., 2015). It is also possible to correlate palatability with acceptability. Fibre in pet foods can affect how crisp the product will be, due to its effect on texture, specific volume and expansion (Mendonça, 2000).

Table 5. Faecal production, faecal traits and digesta mean retention time of dogs fed diets with different additions of guava fibre.

Item	Experimental diets*				S.E.M†	P value	Contrasts‡	
	CO	GF3	GF6	GF12			Linear	Quadratic
Faecal traits								
Dry matter (%)	38.8	41.8	40.8	42.6	0.45	0.004	0.008	ns
g faeces/kg$^{0.75}$ day^{-1} (As-fed basis)	14.1	11.9	17.3	19.0	0.75	0.001	<0.001	ns
g faeces/kg$^{0.75}$ day^{-1} (DM basis)	5.5	5.0	6.8	8.1	0.31	<0.001	<0.001	0.035
Faecal score¶	3.9	4.0	4.0	3.9	0.01	0.645	ns	ns
Mean weight of each defecation (g)	80.9	70.9	70.9	78.7	2.85	0.583	ns	ns
Number of defecations per day	1.29	1.31	1.46	1.63	0.06	0.079	ns	ns
Digest mean retention time (h)	34.0	34.1	33.9	31.5	1.19	0.046	ns	ns

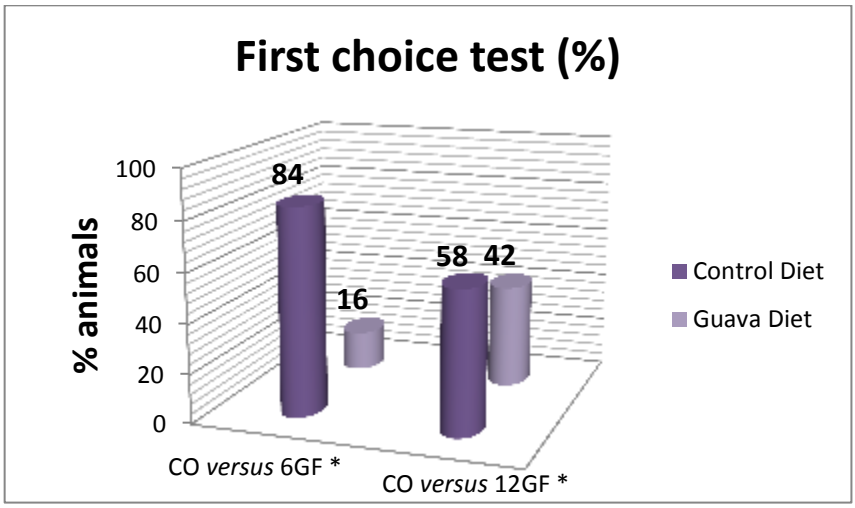
* CO: control, without fibre; GF3: supplemented with 3% of guava fibre; GF6, supplemented with 6% of guava fibre; GF12, supplemented with of 12% of guava fibre.
† S.E.M. = standard error of the mean (n = 6 dogs per treatment).
‡ Linear and quadratic effect of guava fibre addition.
§ ns = not significant (P>0.05).
¶ 0 = watery liquid, which can be poured; 1 = soft, unformed; 2 = soft, malformed stool, which assumes shape of container; 3 = soft, formed and moist, which retains shape; 4 = well-formed and consistent stool, which does not adhere to the floor; and 5 = hard, dry pellets, which are small and hard mass.

Koppel et al, (2015), using high trained human panellists, evaluated the same experimental extruded canine diets used in the present study for sensory characteristics. They found that fibre inclusion appeared to influence aroma and flavour properties; the treatment with the highest amount of guava fibre was the most bitter and had most aftertastes, including off-notes such as stale and oxidized oil. The coating process was shown to be very important in reducing or eliminating off-notes both in flavour and aroma, and also altering the appearance. It is noteworthy that the sensory evaluation is not in agreement with the palatability test results in our study, since dogs did not show a preference towards the CO diet when compared to GF12.

According to Araujo et al, (2004), there are new methods to assess diet palatability in dogs such as the cognitive assessment protocol. This method may provide good results with less variation and fewer test animals than the method used in this study, however, it does not simulate real-life conditions and it is limited to foods with an obvious difference and to dogs exclusively with a higher functioning cognitive ability. Thus, the two pan test still has value (Greg et al., 2015).

One limitation of the current study is that we did not compare the diets with fibre (GF3, GF6, and GF12) amongst themselves and the CO *versus* GF3. Those results could contribute to a better understanding the effect of guava fibre on palatability in dogs, especially since we cannot find an explanation as to why the control diet was preferred over the GF6 but not over the GF12.

A)



B)

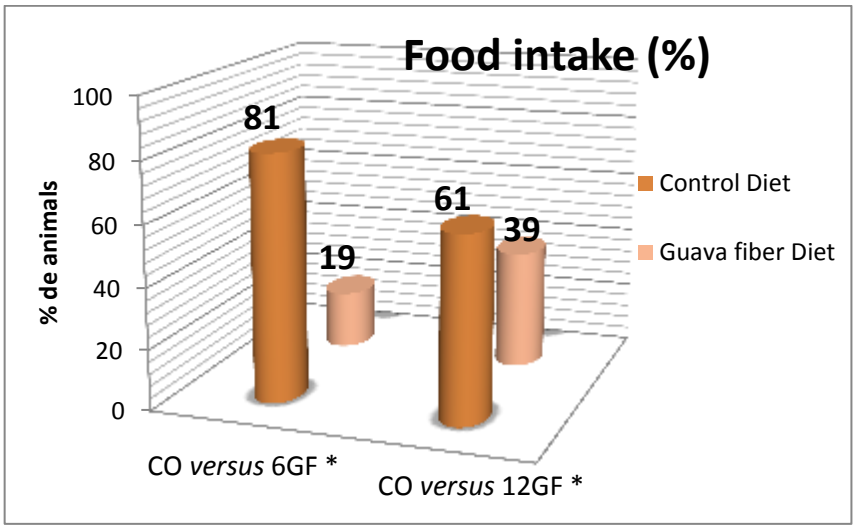


Figure 3. Palatability test of guava fibre diets used in this study: (A) First Choice and (B) Food Intake. *CO: control, without added fibre; GF6, supplemented with 6% of guava fibre; GF12, supplemented with of 12% of guava fibre.

Conclusions

The guava fibre source used in this study had good water-holding capacity during extrusion, decreased the some nutrients digestibility, accelerate the intestinal transit time at the highest inclusion level, were only partially degraded by microbiota, and increased faecal bulk.

Even now, we can also suggest that the guava fibre is a degradable material when submitted a high temperature and contain a structural and electrochemical characteristic with lower thermal stability during the extrusion processing.

The results of this study suggest that guava is a novel insoluble fibre ingredient that could be safety used in commercial pet foods without excessively affecting nutrient digestibility and without negative effects on other gastrointestinal function measures.

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CAPÍTULO 4

FONTES DE FIBRA E TAMANHO DE PARTÍCULA SOBRE A DIGESTIBILIDADE DOS NUTRIENTES, PRODUTOS DA FERMENTAÇÃO, PALATABILIDADE DA DIETA E TEMPO DE RETENÇÃO INTESTINAL DE CÃES ALIMENTADOS COM DIETA EXTRUSADA.

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

Running head: Different fiber particle size for dogs

Fiber sources and particles size on nutrient digestibility, fermentation products, diet palatability, and gastrointestinal retention time of dogs fed kibble diet

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Abbreviations: AAFCO, American association of feed control officials; BCFA, branched chain fatty acid(s); CTTDA, coefficients of total-tract apparent digestibility; CP, crude protein; SCFA, short chain fatty acid(s); TDF, total dietary fiber; TGA, thermogravimetric analysis.

Fiber sources and particles size on nutrient digestibility, fermentation products, diet palatability, and gastrointestinal retention time of dogs fed kibble diet

ABSTRACT- Fibrous ingredients have been a trade of work that shows the benefits of fiber in dog and cats diets. Associated with this, many researchers have studied the importance of smaller particles size on animal feeding and performance due to the increased surface area available for enzymatic attack. In accordance with this, the objectives of this study were to determine the influence of two types of fibers particles sizes on: nutrients digestibility of dry dog foods; fecal traits, stool quality and nutrient fermentation; gastrointestinal retention time and palatability. Five experimental diets were formulated and manufactured: a control diet (with no fibrous ingredient added), sugarcane (SC) fiber diet (9% inclusion; large and small particle size), and wheat bran (WB) fiber diet (32% inclusion; large and small particle size). The study followed a two-way factorial design with additional treatment of type control, including 6 animals per treatment. Diets were offered to 30 Beagle dogs for 15 days of adaptation; six days of total feces collection to assess digestibility; and three days of fresh feces collection to determine the fermentation products. All animals received an oral pill containing radiopaque markers to determine gastrointestinal transit time. Diet palatability was measured using a qualified trained panel of dogs by two-pan test. Data were evaluated by orthogonal contrast considering $p<0.05$. The difference of fiber particle size used in this study did not modify the nutrients intake and CTTAD. However, the addition of fiber increased nutrient intake ($P<0.001$) without effect for starch. The coefficients of total tract apparent digestibility of DM, OM and food ME were higher for CO diet ($P<0.001$) but no differences were found for CP, fat, TDF and starch digestibility's for the fiber supplemented diets. Dogs fed small particles had increased the isovaleric acid concentration ($P=0.008$). WB diets resulted in greater lactic acid concentration ($P<0.001$), lower fecal pH, ammonia ($P<0.001$), isovaleric and total branched chain fatty acids concentration ($P<0.001$). SC diets decreased fecal ammonia content ($P<0.001$). The inclusion of both fibers decreased the gastrointestinal retention time compared to CO diet ($P<0.001$). Palatability testing results indicated that the CO treatment was preferred over the fiber diets and the treatment with large SC fiber particles was preferred over the treatment with small particles. The fibers sources in both particles size were stable during the thermogravimetric analysis, being considered safety to dog foods

manufactured. Small particles did not result in improved digestibility of a high fiber diet high and gastrointestinal retention time; however, may have interfered on BCFA and palatability.

Keywords: canine, digestibility, fibers geometric mean, food preference, intestinal activity.

Introduction

Fibers are structural carbohydrates, mainly originating from plant cell walls. The energy available from fibers is limited, and because of this, fibers were considered be good ingredients in reduced energy diets (Mcnamara, 2014). In addition it has been found that fibers reduce the energy digestibility. According to NRC, (2006), each percentage point of added fiber causes 1.43% of reduction of energy. Furthermore, fiber is added due to the influence on gastrointestinal tract healthy maintenance (Reinhart and Sunvold, 1996), food energy dilution (Bissot et al., 2010) and improvement of carbohydrates metabolism (Carciofi, 2005). Fibers in combination with proteins help regulate satiety levels in dogs (Weber et al., 2007), the digestive process, and the glycemic response in dogs and cats (De Godoi et al., 2013; Campbell et al., 2009). Depending on the fiber type and consumed amount, different effects on nutrient digestibility and fecal formation can be affected by the fiber type and consumed amount (Burkhalter et al., 2001).

The fiber processing changes the physiochemical properties, enabling to increase the fermentability, solubility and viscosity, altering effects in the animals. (Zhang et al., 2009; Redgwell et al., 2011; Robin et al., 2012). Depending of amount and source, the fibers may decrease food preference (Carciofi, 2005) and most often decrease the intestinal transit time (Fahey Jr et al., 1990a, 1992; Hernot et al., 2005).

Currently, there are a few information about the particle size of fibers included in dry dog food. With the exception of purified celluloses (Wichert et al., 2002), only one study involving different particles sizes of cereal starches is available: Bazolli et al. (2015), reported that different particle sizes of broken rice, maize and sorghum grain affected nutrient digestibility and fecal characteristics of dogs. However, studies involving fibrous ingredient particle size are unavailable for dogs.

According to the hypothesis of this study, small particles of fibers could alter its functional properties by increasing the superficial area of the material for microbial activity, and results in better diet acceptability and stool quality. Thus, the effect of two different

sources and particle sizes of sugarcane and wheat bran fiber on nutrients digestibility, fecal fermentation products, palatability, and gastrointestinal retention time of extruded diets for adult healthy dogs were investigated.

Material and Methods

Animals and experimental design

The study was conducted in the College of Agrarian and Veterinary Sciences, UNESP- Univ Estadual Paulista, Campus of Jaboticabal, Department of Clinical and Surgery, Laboratory of Research on Nutrition and Nutritional Diseases of Dogs and Cats, and all procedures received ethical approval from the Ethics Committee for Animal Well-Being (Protocol number 07895/14).

Adult clinically healthy Beagle dogs (n=30) were used, with an average body weight of 12.75 ± 0.59 kg, a mean age of 7 ± 2.0 yr, and a mean body condition score of 5.0 ± 0.2 on a nine-point scale (Laflamme, 1997).

The dogs were housed in pairs in 1.5 x 4.0m kennels with a solarium and were randomly allotted to the experimental treatments. The dogs were exercised daily in a 200m^2 grassy area. During the collection period, the animals were kept in individual stainless steel metabolic cages (0.9m cm x 0.9m x 1m) equipped with a system to separate feces and urine for collection.

The energy intake was determined individually for each dog by a standard equation for energy requirements (ME, kJ = 523 × $kg^{0.75\ -1}$ day^{-1}) for dogs maintenance (NRC, 2006). Daily caloric intake was divided into 2 equal meals and fed at 10 a.m. and 4 p.m.

The dogs were randomized into five treatment groups, each involving a different dietary fiber addition. The study followed a completely block randomized design with five diets and six dogs per treatment, totalizing 2 blocks of 15 animals.

Each trial had duration of 30d and started with a 15d adaptation period followed by a 6d of fecal collection period to assess nutrient digestibility. Dogs stayed 3d in the kennel with no access to grass, prioritizing animal welfare and then, animals were relocated in the cages for more 6d to determine fermentation end products and gastrointestinal retention time of food.

Diets

Five diets were formulated to attend the requirements for adult dog in maintenance (FEDIAF, 2013 – European Pet Food Industry Federation): 1) Control, with no fibrous ingredient added; 2) 9% sugarcane fiber large particle size (SC_L); 3) 9% sugarcane fiber small particle size (SC_S) 4) wheat bran large particles (WB_L) and 5) wheat bran small particles (WB_S) - inclusion level 32% for both treatments. Fiber sources were provided already ground to desired particle sizes by the supplier (Dilumix, Leme, SP, Brazil) and sizes were determined using laser diffraction particle size analysis (BOAC, 2009). The analyzed chemical composition and particle size of the fiber sources used in the experiment are represented in Table 1.

Ingredients were previously analyzed and diets were balanced to reach 16% of TDF, which is a level typically used in commercial high-fiber dog diets. The formulation and chemical composition of the experimental diets are shown in Table 2.

The ingredients, except fiber source, were weighed, mixed, and ground using a hammer mill (Model 4, D'Andrea, Limeira, Brazil) fitted with a 0.8 mm screen (Sistema Tigre de Mistura e Moagem, Tigre, Sao Paulo, Brazil). The other ingredients and fiber sources were mixed, compounding the final diet.

Diets were extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with a processing capacity of 250 kg/h. A pre-conditioner was used to treat the diets with steam and water prior to extrusion. The pre-conditioner residence time was approximately 3.5 min, and downspout temperature was 83.95°C. The extruder screw speed was set at 465 rpm, and the die open area was 15.9 mm^2/ton/h. Extruder die temperature ranged between 118.7–130.3°C and die pressure between 52.45–70.6 bars. The processing conditions were not changed for any treatment in order to isolate the influence of fiber. Each dog food was processed separately in two different days. Four samples were collected per diet each day. Pooled samples from both days were combined into one batch per diet to measure the palatability.

After extrusion, kibbles were dried in a forced air dryer at 105°C for 30 min and coated with fats and palatability enhancers at 2% of the ingredients. Fish oil was mixed with dry ingredients before extrusion.

Table 1. Chemical composition and particle size of the fiber sources.

Item	Fiber Sources	
	Sugarcane[1]	Wheat Bran[1]
Chemical composition (%, as-fed basis)[2]		
Moisture	5.70 ± 0.21	7.11 ± 0.22
Protein	2.94 ± 0.14	16.38 ± 0.27
Starch	0.65 ± 0.16	24.93 ± 2.19
Fat	1.12 ± 0.01	4.71 ± 0.66
Total dietary fiber	86.99 ± 0.30	39.52 ± 1.55
Insoluble fiber	86.99 ± 0.31	37.80 ± 1.29
Soluble fiber	0.00	1.71 ± 0.26
Cellulose	45.81 ± 1.61	7.18 ± 5.53
Hemicellulose	28.19 ± 1.77	29.03 ± 1.00
Lignin	9.30 ± 0.33	4.65 ± 0.02
Fiber particle size (µm)		
Large size particles [3]	394 ± 317.84	345 ± 241.90
Small size particles [4]	196 ± 188.75	143 ± 101.56

[1] Vit2be Fiber, wheat bran fiber, Dilumix, Leme, SP, Brazil.
[2] Analyzed in duplicate.
[3] Ground in roller mill fitted with a sieve of 500µm
[4] Ground in roller mill fitted with a sieve of 250µm

Digestibility protocol

The coefficients of total tract apparent digestibility (CTTAD) of nutrients were calculated according to the quantitative collection of feces protocol and calculation procedures described by the Association of American Feed Control Officials (AAFCO, 2008). The ME content was calculated considering the values of digestible energy and digestible protein of the diets (NRC, 2006).

All feces were removed from the cages and discarded before 8a.m. and total fecal output for each dog was collected from this point onwards for the 6d. Feces were collected twice per day, weighed and frozen at −15°C. Afterwards, the feces were dried in a forced-air oven (Fanem, São Paulo, Brazil) at 55ºC for 72 h. Properly dried, fecal samples and diets were ground in a cutting mill (Mod MA-350, Marconi, Piracicaba, Brazil) fitted with a 1 mm screen and analyzed for DM by oven-drying the sample (method 934.01) and for ash content by muffle furnace incineration (method 942.05).

Table 2. Ingredients, chemical composition and starch gelatinization degree of experimental diets for dogs with different sources and particle sizes of fiber.

Item	Experimental Diets[1]				
	CO	SC_L	SC_S	WB_L	WB_S
Ingredients (%)					
Corn grain	57.82	47.46	47.46	30.30	30.30
Chicken by product meal	31.80	32.56	32.56	26.12	26.12
Sugarcane fiber [2]	-	9.00	9.00	-	-
Wheat bran	-	-	-	32.00	32.00
Chicken fat	6.44	7.20	7.32	7.64	7.64
Liquid palatant [3]	2.00	2.00	2.00	2.00	2.00
Common Salt	0.50	0.50	0.50	0.50	0.50
Choline Chloride	0.20	0.20	0.20	0.20	0.20
Potassium chloride	0.65	0.65	0.65	0.65	0.65
Vitamin and mineral premix [4]	0.30	0.30	0.30	0.30	0.30
Fish oil	0.15	0.15	0.15	0.15	0.15
Mold inhibitor [5]	0.10	0.10	0.10	0.10	0.10
Antioxidant [6]	0.04	0.04	0.04	0.04	0.04
Total	100	100	100	100	100
Chemical composition (%, DM basis)					
Moisture	5.90	5.40	6.19	5.30	6.42
Ash	6.00	5.48	5.87	6.14	6.06
Crude protein	25.21	25.07	25.88	25.01	25.40
Crude fat	15.30	15.03	14.71	14.48	15.56
Total dietary fiber	10.75	16.47	16.75	18.01	17.99
Insoluble fiber	10.75	16.47	16.75	17.22	17.11
Soluble fiber	0.0	0.0	0.0	0.80	0.88
Starch	41.20	37.82	38.00	34.26	34.54
Starch gelatinization degree (%)	92.82	93.77	94.56	91.93	94.01

[1] CO: control diet, without added fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles.
[2] Vit2be Fibers, Dilumix, Leme, SP, Brasil.
[3] Liquid palatant, SPF do Brazil, Descalvado, Brazil.
[4] Supplied per kilogram of diet: vitamin A, 18,000 IU; vitamin D, 1,200 IU; vitamin E, 200 IU; thiamin, 6 mg; riboflavin, 10 mg; pantothenic acid, 40 mg; niacin, 60 mg; pyridoxine, 6 mg; folic acid, 0.30 mg; vitamin B12, 0.1 mg; iron, 100 mg; copper, 10 mg; magnesium, 10 mg; zinc, 150 mg; iodine, 2 mg; selenium, 0.3 mg;
[5] MoldZap: ammonium dipropionate, acetic acid, sorbic acid and benzoic acid. Alltech do Brasil Agroindustrial Ltda, Curitiba, Brazil;
[6] Banox: butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and calcium carbonate. Alltech do Brasil Agroindustrial Ltda, Curitiba, Brazil.

CP was analyzed by the Kjeldahl method (method 954.01), and acid hydrolyzed fat was assessed using a soxhlet apparatus (method 954.02) according to the Association of Official Analytical Chemists (AOAC, 1995). OM was calculated as DM minus ash. Dietary fiber (total, soluble, and insoluble) was measured by using a combination of enzymatic and gravimetric procedures (AOAC, method 991.43, 1995). The total amount of starch was determined according to the method described by Hendrix (1993). The degree of gelatinization of the starch was determined by the amyloglucosidase method by Sá et al, (2013).

Neutral detergent fiber (NDF) was determined using α-amylase and without the addition of sodium sulphite, following Van Soest et al. (1991). Acid detergent fiber (ADF) was determined using the method described by Goering & Van Soest. (1970) and acid detergent lignin was determined by solubilization of cellulose with sulphuric acid, according to Van Soest & Robertson (1985). The cellulose content were calculated subtracting ADF less lignin and the hemicellulose content were calculated subtracting NDF less ADF The analyses, with the exception for diets TDF, were carried out in duplicate and repeated when the coefficient of variation among the duplicates was higher than 5%.

Fermentation end products

Fresh fecal samples were collected immediately after elimination to measure lactic acid, ammonia and volatile fatty acids (VFAs) as short chain fatty acids and branched chain fatty acids. Approximately 10 g of fresh feces were mixed with 30 mL of a 16% (v/v) formic acid solution, precipitated at 4°C for 72 h, and the supernatant was centrifuged (5804 R, Eppendorf, Hamburgo, Brazil) three times at 4500 G at 15°C for 15 minutes. The VFAs were analyzed in the supernatant by gas chromatography (model 9001, Finnigan, San Jose, USA) according to Erwin et al. (1961), using a glass column 2 m in length and 3.17 mm in width, covered with 80/120 Carbopack B-DA/4% Carbowax 20M. Nitrogen was the carrier gas with a flow rate of 25 mL/min. Working temperatures were 220°C at injection, 210°C in the column, and 250°C in the flame ionization detector.

Lactic acid was measured following the Pryce modified method, (1969), using phosphoric acid 85% (Molar mass 98) as precipitating reagent, dissolving 23.29 mL in distilled water to complete 1000 mL. The optical density was measured with l-cm cuvettes, at

a wavelength of 565 nm using a colorimetric method (Spectrophotometer Quick – Lab, Drake, São José do Rio Preto, Brazil).

The ammonia content was determined in the same extracts used for VFAs. The extracts were thawed at room temperature, diluted into distilled water (2:13 v/v) and ammoniac nitrogen was distilled using potassium hydroxide 0.2 N and boric acid 0.9 N in a nitrogen system (TE Tecnal – 036/1, Piracicaba, Brazil). For titling, was used chloridric acid 0,005 N according to Vieira, (1980).

Fecal pH was determined by mixing 9mL of distilled water with 4 g of fresh feces (v/w) and measuring the pH in the final solution using a calibrated pH meter (DM20, Digicrom Analítica Ltda, São Paulo, Brazil).

Fecal characteristics

Fresh feces were analyzed for DM by oven-drying the sample (AOAC method 934.01). Fecal samples were scored according to the following system by Carciofi et al. (2008): 1 = watery, liquid that can be poured; 2 = soft, unformed, stool assumes shape of container; 3 = soft, formed, moist, softer stool that retains shape; 4 = hard, formed, dry stool, remains firm and soft; 5 = hard, dry pellets, small, hard mass.

Digesta mean retention time

Digesta retention time in the gastrointestinal tract was evaluated according to an adaptation of the method described by Burrows et al. (1982). For the assay, dogs were restricted to their cages and the time of each defecation was recorded. The animals were fed at 10 a.m. and gelatin capsules containing 10 radiopaque markers (Sitzmarks, Konsyl Pharmaceuticals Inc., Fort Worth, Texas USA) were mixed with the food. The markers were 4.5 mm in diameter and had densities of 1.25 g/mL. On each day a different marker format was utilized, allowing three consecutive observations of the retention time.

The time of the marker administration was registered, and the animals and their deposition were observed at 2 h intervals until the last marker was recovered in the feces. All feces were collected, weighed and the time of sampling was recorded. When the exact fecal elimination time was not observed within those time windows, the average time between

samplings was considered for the purposes of this experiment. All feces were radiographed and the markers counted.

The digesta mean retention time was computed as the time interval (in hours) between the food intake plus capsule administration and the time of excretion of the feces containing the last marker recovered. The mean food retention time was the average for the three days of observation. In addition, the marker recovery rate was computed as:

$$\text{Markers recovery rate } (\%) = \frac{\text{number of markers recovered on feces}}{\text{total of markers orally dosed}} \text{ x } 100$$

To validate the observation a minimum recovery rate of 0% of the markers was established. The number of defecations per day and the weight of each defecation were also recorded.

Palatability Testing Procedure

The palatability tests were performed in Panelis, Diana Group (Descalvado, São Paulo, Brazil) and were measured for the all fiber treatments (CO, SC_L, SC_S, WB_L, and WB_S) by two-pan method (Griffin, 2003). First, the diets were coated with 6 D'TECH in the same company with a commercial palatant enhancer and then, the food was providing to dogs. Each test was carried out with 38 dogs housed in individual kennels.

In the morning, after a 12 h fast, the dogs received two pans, each containing one of the experimental foods, and were allowed to eat for 30 min. The position of the food pans was alternated at the evening meal.

The amount of food offered in each pan surpassed the consumption capacity of the animal to ensure there would be leftovers to measure. After 30 min the pans were removed, the remains weighed and consumption rate was calculated (Equation (1)). Due to the large differences in body weights the results were calculated as relative consumption of each diet, and the mean intake of the two meals for each dog was compared.

The combinations of tests were:

- CO diet vs. SC_L diet,
- CO diet vs. WB_L diet,

- SC_L diet vs. SC_S diet,
- WB_L diet vs. WB_S diet.

$$\text{Relative intake (\%)} = \frac{\text{Food A intake}}{\text{Food A intake+Food B intake}} \times 100 \qquad (1)$$

Material Characterization

- Thermogravimetric analysis (TGA)

This measurement was carried out using 5g of sample heated until 800 °C in an aluminum crucible with N_2 atmosphere. The carbonization behaviors of the samples were monitored by TGA (TA Instrument, Delaware, USA, SDT/Q600) and the test was performed at a constant heating rate of 10°C min^{-1} under an air flow (70 cm^3 min^{-1}). This analysis was carried out in the Laboratory of Physical Chemistry of Materials, Chemistry Institute of Araraquara, UNESP- Univ Estadual Paulista.

- Photomicrography

The samples were imaged by the Laboratory of Scanning Electron Microscopy of UNESP, Campus de Jaboticabal, to characterize a high-resolution imaging of surfaces (Figure 2).

Statistical analysis

The experiment followed a randomized complete block design. The analysis of variance was performed using SAS software (version 9.1; SAS Institute, Cary, NC, USA). The analysis of variances considered the effects of treatments (diets). When diet effects for digestibility, fecal traits, fermentations products and digesta mean retention time were detected, orthogonal contrasts were used to evaluate the difference between sugarcane and wheat bran, large and small particle size, and fiber addition and CO diet. The palatability tests

were compared using the Chi^2 and T- student test. Values of $P< 0.05$ were considered significant.

Results and discussion

Chemical composition and digestibility

The experimental diets contained similar amounts of moisture, ash, protein and fat. The fiber sources varied from 10.75% (CO) to 16.5% (SC) and 18% (WB) of dietary fiber content (Table 2). Only WB presented a few amount of soluble fiber (0.88%) and contained a little more fiber.

All dogs remained healthy throughout the study and consuming properly all diets. No significant differences were found among the treatments (CO, SC_L, SC_S, WB_L, WB_S) for BW at the start and end of the experiment (P= 0·900, 0.864, 0.705, 0.609 and 0.645, respectively; data not shown). During the 30d, the food and ME intake was lower for control diet ($P<0.001$) (Table 3).

All nutrients intake were higher for SC and WB diets ($P<0.001$) in comparison with CO diet (Table 4) and no difference were found among different particle sizes treatments. WB diets presented higher TDF intake ($P<0.001$), which was expected according to diet formulation (inclusions of 32% WB versus 9% SC) and lower OM, CP and GE digestibility compared to SC diets.

The CTTAD of diets supplemented with SC and WB were lower for DM, OM and food ME ($P<0.05$), tending to decrease the GE digestibility ($P=0.085$). These reduction resulted in a decrease in metabolizable energy of fiber diets ($P<0.001$), being lower for WB diet ($P<0.001$). No differences were found among CO and fiber diets for CP, fat, TDF and starch for CTTAD. Carciofi et al. (2008) already published similar results using six different starch sources in extruded diets for dogs (cassava flour, brewer's rice, corn, sorghum, peas or lentils), showing a reduction in the DM, OM, and GE digestibility in the diets with higher TDF (14.13 % DM).

On the other hand, Sá et al. (2013) found that dogs fed WB diets (25% inclusion) presented reduction in the CTTAD of DM, OM, CP, fat and energy. Burrows et al. (1982),

Fahey et al. (1990), Lewis et al. (1994) and Kienzle et al. (2001) also reported that divergent fibers sources progressively decrease canine's nutrient apparent digestibility. In the current study, no differences were found in TDF digestibility of SC and WB and starch digestibility was not influenced by fiber addition.

Other studies with cats already demonstrated the effect of fiber in lowering the DM, OM, and GE digestibility (Fischer et al., 2012; Sunvold et al., 1995[a]; Fekete et al., 2004; Prola et al., 2009). Fischer et al. (2012) included 24.9% of WB and 12.8% of SC and reported that while SC reduced the CTTDA for CP and fat, the WB did not influenced the CP and fat digestibility, being similar to the control diet. Sunvold et al. (1995[b]) and Fekete et al. (2004) observing feline responses fed with 10% (DM basis) of peanut hull, alfalfa meal and dried sugar beet pulp reported differences in protein and fat digestibility according to the fiber source utilized, which they attributed to the apparent digestibility of fiber.

Regarding particle size, this parameter did not affect the dog's nutrients digestibility in this study. In the literature, however, have only one data with the same specie involving particle sizes of ingredient reporting the opposite: Bazolli et al. (2015) using a formulation for dogs with maize, rice and sorghum grounded into 3 different particles (approximately 300, 450, and 600 μm), reported a reduction for nutrients digestibility for diets containing maize and sorghum with greater cereal mean geometric diameter. For rice diets, only GE digestibility was reduced at the largest size. For another species as pigs, a study using pellet diets determined the effects of particle size of corn and sorghum (Healy et al. 2014). The data shown that increases in digestibility were found with particle size reduction from 900 to 500 μm. The pigs had growth performance improved as the particle size of the cereal grains was reduced well, below 800 mm and the increased cost of milling in this case was easily justified by improved gain feed.

Table 3 Body weight, dry matter and metabolizable energy intake of dogs fed diets with different sources and particle sizes of fiber

Item	Experimental diets[1]					SME[2]	P value	Contrasts		
	CO	SC_L	SC_S	WB_L	WB_S			SCxWB[3]	LxS[4]	COxFiber[5]
Body weight (kg)										
Initial	13.3	12.35	11.65	13.04	12.98	0.26	0.261	ns	ns	ns
Final	13.2	12.39	11.91	13.22	13.46	0.29	0.305	ns	ns	ns
P value [6]	0.999	0.864	0.705	0.609	0.645					
Food intake (Mean of 27 days of study)										
Dry matter ($g \bullet kg^{0.75} \bullet day^{-1}$)	28.6	36.4	38.2	38.4	38.8	0.94	<0.001	ns	ns	<0.001
Metabolizable energy ($kcal \bullet kg^{0.75} \bullet day^{-1}$) [7]	111.10	134.6	139.8	131.03	134.27	2.79	0.005	ns	ns	<0.001

[1] CO: control diet, without added fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles.
[2] SEM = standard error of the mean (n = 6 dogs per treatment).
[3] Sugarcane diets versus wheat bran diets;
[4] Large particle size versus small particle size fiber sources;
[5] Control diet versus fiber supplemented diets.
[6] P value by F test.
[7] Calculated with the food metabolizable energy values determined in vivo.

Table 4. Nutrient intake, apparent total tract digestibility, and metabolizable energy content of experimental diets for dogs with different sources and particle sizes of fiber

Item	Experimental diets[1]					SME[2]	P value	Contrasts		
	CO	SC_L	SC_S	WB_L	WB_S			SCxWB[3]	LxS[4]	COxFiber[5]
Nutrient intake during the digestibility study ($g \bullet kg^{0.75} \bullet day^{-1}$)										
Dry matter	28.6	36.5	38.5	39.4	39.0	0.85	<0.001	ns[6]	ns	<0.001
Organic matter	26.8	34.4	36.1	36.8	36.6	0.80	<0.001	ns	ns	<0.001
Crude protein	8.3	10.7	11.3	11.0	11.0	0.23	<0.001	ns	ns	<0.001
Acid-hydrolyzed fat	4.3	5.4	5.7	5.7	6.1	0.12	<0.001	ns	ns	<0.001
Total Dietary Fiber	3.1	6.0	6.4	7.1	7.0	0.28	<0.001	<0.001	ns	<0.001
Starch	11.4	13.8	14.6	13.0	13.5	0.33	0.034	ns	ns	0.006
Apparent total tract digestibility (%)										
Dry matter	80.9	77.2	74.7	73.4	75.3	0.62	0.001	0.052	ns	<0.001
Organic matter	84.2	79.7	77.3	75.6	77.7	0.65	<0.001	0.019	ns	<0.001
Crude protein	82.5	83.2	80.8	78.8	80.7	0.47	0.021	0.007	ns	ns
Acid-hydrolyzed fat	89.5	90.5	90.9	89.2	91.4	0.51	0.626	ns	ns	ns
Total Dietary Fiber	22.9	24.1	17.6	24.1	30.4	1.47	0.158	ns	ns	ns
Starch	99.7	99.6	99.7	99.6	99.7	0.02	0.372	ns	ns	ns
Gross energy	84.5	80.3	78.2	75.8	77.9	0.51	0.020	0.009	ns	0.085
Food ME (kcal/g, DM basis)	3.88	3.70	3.62	3.46	3.47	0.03	<0.001	<0.001	ns	<0.001

[1] CO: control diet, without added fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles.
[2] SEM = standard error of the mean (n = 6 dogs per treatment).
[3] Sugarcane diets versus wheat bran diets;
[4] Large particle size versus small particle size fiber sources;
[5] Control diet versus fiber supplemented diets.
[6] not significant (P>0.05).

Fermentation end products

Lactic acid concentrations, fecal pH, ammonia content and VFA are represented in Table 5. A direct correlation was found between available acid lactic and fecal pH (r_2=0.9909; Figure 1).

The large supply of organic matter to the colon associated with carbohydrates fermentation promotes SCFA and this production resulted in reduced fecal pH, which inhibits pathogenic bacteria's. The SCFA are also correlated with other positives effects in the host, as: colon integrity maintenance, proliferation of colonic epithelial cells, prevention of colonic carcinomas and attenuation of negative effects from protein degradation (TELLEZ et al., 2006; NRC, 2006). In the current study, the reduction in fecal pH was not enough to increase the SCFA concentrations, as found by Sá et al. (2013) and also agree with this author about reductions in fecal ammonia content in dogs fed wheat bran. SC diets, in the other hand, present higher ammonia content in the orthogonal contrast (P<0.001). This could be associated with the capacity of sugarcane to negatively influence on feces odor or with high protein fermentation occurred due higher inclusion of chicken by product meal in the SC diets (32.17%) in comparison with WB diets (26.12%). The increased concentrations of ammonia may be indicative of more protein being fermented because the sugarcane is less fermentable. One point is important and need to be considered: high ammonia content could bring damaging to the integrity of the intestinal mucosa and health (FLEMMING, 2005) and changes in ammonia levels are already related to alterations in the type and protein consumption, which tends to be higher by the consumption of animal proteins (Tortola et al., 2013).

The use of fecal concentrations of SCFA to estimate intestinal microbiota activity of the large intestine of dogs has been successful evaluated (Swanson et al., 2002), demonstrating that to collect feces from the rectum microbiota of dogs are effective and have fermentation profiles very similar to the transversal colon, reported by Bosch et al. (2008). In general, the fermentation of carbohydrates and proteins in the colon is not similar. Carbohydrates are fermented primarily in proximal colon producing SCFAs, H_2 and CO_2 (both the presence of carbohydrates in the colon and their fermentation can alter the colonic physiology) (Wong et al. 2006). In contrast to carbohydrates, the fermentation of protein and amino acids by proteolytic bacteria in the colon yield BCFAs, H_2, CO_2, CH_4, phenols, and

amines (Wong et al. 2006). Approximately 30% of protein reaching colon and broken down was converted to SCFA. Of all fatty acids generated from protein, the BCFA comprise about one fifth (Macfarlane et al. 1999). The low fermentation rate of the sugarcane fiber can be attributed according to Calabrò et al. (2009) to its particular composition represented in Table 1, which do not support bacterial growth.

A lower concentration of BCFA was verified in fiber diets (P=0.001). Wheat bran present lower isobutyric, isovaleric and valeric acids content (P<0.001) compared to sugarcane diets, having the ability to reduce the putrefactive compounds of feces occasioned by microbial fermentation of non-digestible amino acids.

The sugarcane diets, in comparison with dogs fed wheat bran presented higher molar proportion for acetic and isobutyric acid (P<0.001), but lower molar proportion for valeric acid (P=0.005). It's difficult to explain these datas, but a possibility is that the changes happened with acetic and valeric acid were not enough to impact in a significant statistic difference, but was sufficient to alter the molar proportion.

Regarding particle size, Bazolli et al. (2015) reported dogs fed coarsely ground maize- and sorghum-based diets had an increase in propionate and butyrate. Our study found that the influence of particle size differed depending on the type of fiber used: the particle sizes did not change the SCFA amounts, but presented a difference in the BCFA relative proportion: small particles size diets had lower values of total branched acids content (P<0.05) and resulted in higher molar proportion (P=0.035) for acetic acid.

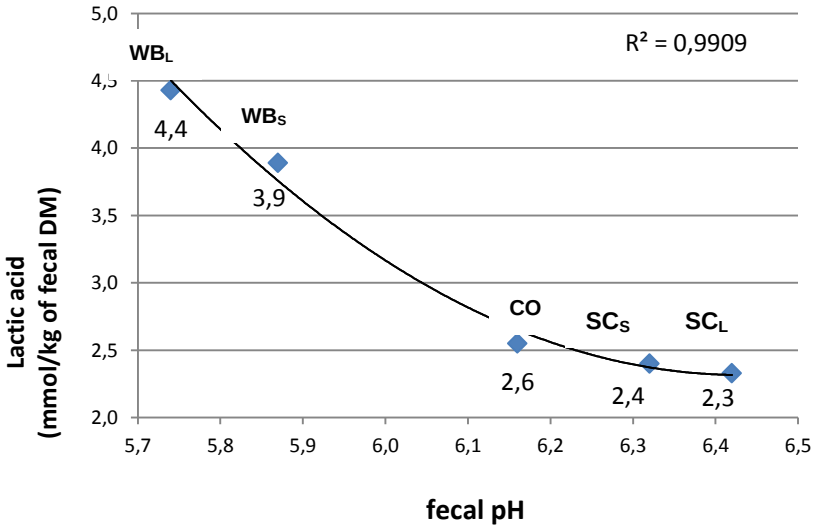

Figure 1. Effect of lactic acid on intestinal pH: relationship between lactic acid concentration and fecal pH of dogs fed sugarcane and wheat bran fibers with two different particle size. CO: control diet, without fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles

Table 5. Lactic acid concentrations, fecal pH, ammonia content and volatile fatty acids (VFAs) of dogs fed diets with different sources and particle sizes of fiber

Item	Experimental diets [1]					SME[2]	P value	Contrasts		
	CO	SC_L	SC_S	WB_L	WB_S			SCxWB[3]	LxS[4]	COxFiber[5]
Lactic acid (mmol/kg of fecal DM)	2.55	2.33	2.40	4.43	3.89	0.22	0.003	<0.001	ns	ns
Fecal pH	6.16	6.42	6.32	5.74	5.87	0.05	<0.001	<0.001	ns	ns
Ammonia (mMol/kg of fecal DM)	144.7	172.2	199.2	126.3	129.6	6.78	<0.001	<0.001	ns	ns
Short-chain fatty acids (mMol/kg of fecal DM)										
Acetic	368.4	318.1	360.9	346.0	328.4	2.48	0.493	ns	ns	ns
Propionic	220.1	160.4	166.0	222.8	171.1	2.55	0.127	ns	ns	ns
Butyric	71.7	67.2	68.8	75.2	86.4	0.85	0.436	ns	ns	ns
Total	660.2	545.8	595.7	644.0	585.9	8.34	0.310	ns	ns	ns
Branched-chain fatty acids (mMol/kg of fecal DM)										
Isobutyric	9.6	9.1	10.3	4.4	5.4	0.13	<0.001	<0.001	ns	0.006
Isovaleric	14.0	12.1	14.2	6.6	8.7	0.15	<0.001	<0.001	0.008	<0.001
Valeric	1.2	1.1	1.2	1.2	1.8	0.03	0.481	ns	ns	ns
Total	24.7	22.3	25.7	12.1	15.9	0.28	<0.001	<0.001	0.020	0.001
Total VFA	684.9	568.1	621.4	656.1	601.7	8.35	0.330	ns	ns	ns
Short-chain fatty acids, molar proportion (%)										
Acetic	55.8	58.3	60.6	53.7	56.0	0.11	0.002	<0.001	0.035	ns
Propionic	33.3	29.4	27.9	34.6	29.2	0.06	0.140	ns	ns	ns
Butyric	10.8	12.4	11.6	11.7	14.8	0.04	0.315	ns	ns	ns
Branched-chain fatty acids, molar proportion (%)										
Isobutyric	38.8	41.0	40.0	36.0	34.2	0.01	0.006	<0.001	ns	ns
Isovaleric	56.4	54.2	55.2	54.0	55.6	0.01	0.590	ns	ns	ns
Valeric	4.7	5.0	4.8	9.7	11.1	0.03	0.005	<0.001	ns	ns

[1] CO: control diet, without added fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles. [2] SEM = standard error of the mean (n = 6 dogs per treatment). [3] Sugarcane diets versus wheat bran diets; [4] Large particle size versus small particle size fiber sources; [5] Control diet versus fiber supplemented diets. [6] not significant (P>0.05)

Fecal characteristics

The inclusion of sugarcane or wheat bran in the diet did not change the amount of DM excreted as compared with control diet (P>0.05; Table 6). So, increased fresh feces excreted and weight (P<0.001) for wheat bran was really an increase in water content of feces.

The inclusion of both fibers leads to increase the fecal output (P<0.001). The amount of wet feces excreted daily increased with fiber addition. Such effect is called 'bulking effect' of fibers, property usually used for treatment of constipation. The fecal bulking effects appear to be most strongly associated with fiber sources which are insoluble, poorly fermentable and with good water-binding capacity (Diez, 1998).

In this study, sugarcane and wheat bran, both characterized by a higher content of insoluble fiber, no induced increases in the weight of each defecations compared to control diet but, among the fibers, the sugarcane induced decreases (P<0.001) (Table 6). An increased excretion of fresh feces has been reported in dogs either associated with various purified fibers such as cellulose (Burrows et al., 1982), pectins (Lewis et al 1994), maize fiber (Egron et al., 1996) or foodstuffs high in fiber such as beet pulp (Fahey et al., 1990ab, 1992; Sunvold et al., 1995). Although both sources used in this regard produce some increases in stool weight (P<0.001), the fecal score was not affected.

The increase in fecal production verified with fibrous ingredient inclusion, as observed for dry and natural matter, could be explain by the reduction in the dry matter digestibility (P<0.001) and also by the increase in the food intake represented by the DM intake (P<0.001). The insoluble dietary fibers are able to increase up to 20 times its volume and weight, thanks to its ability to retain water that helps in digestive disorders treatment, such as constipation (Musco, 2013).

Particle size also plays an important role in determining fecal weight, transit time, and is determinant of the water-holding capacity of dietary fiber. In this study, no differences were found in sugarcane and wheat bran particle sizes for fecal weight, moisture and water output ($g/kg^{0.75}$/day) in DM and as fed basis.

Gastrointestinal retention time (GIRT)

In general, insoluble fibers may capacity to decrease the GIRT in the hindgut and is generally reported to be accelerated by dietary fiber content (Wenk, 2001; Guerin et al., 2001). The results of the present study confirm this. In pigs, fiber supplementation exerts a direct physical action in the hindgut, which stimulates propulsive colonic motility due to a greater bulk of digest, reducing the nutrients digestibility's (Le Goff et al., 2002; Wilfart et al., 2007). Similar results were obtained in dogs, presenting reduction in GIRT from 37.4 hours to 28.7 hours after fed with wet diet containing 9.0% cellulose (Burrows et al., 1982).

Indeed, analysis of variance in this study showed significant differences between diets for GIRT ($P<0.001$; Table 6): an average of 36.8 h for CO diet; around 25 h for diets with large particles and around 29 hours for diets with small particles. However, differences of fibrous sources (SC or WB) on GIRT is not observed, and has been also reported did not affect dogs (Fahey et al., 1990a; 1990b; 1992; Hill et al., 2000).

Smalls particles tended to increase the GIRT in comparison with large particles, slowing passage of digest. According with this tendency, studies in humans had shown that coarse bran is more effective than fine bran in decreasing GIRT and increasing fecal weight (Kirwan, 1974; Heller, 1980). Finely ground bran, for example, is less effective than coarse bran in holding water and in promoting rapid transit through the human gut (Heller, 1980). Finely ground wood cellulose also has a very low hydration capacity (Van Soest, 1978) and has little effect on fecal volume or transit in humans (Van Soest, 1981). In felines, recent studies have shown that the average fiber length (long or short fiber) can affect stool characteristics of cats, such as stool weight and excretion of water (Prola et al., 2010). Some of these differences may result from ability of the fibers to retain water (Meyer and Tungland, 2001).

Some limitations, however, should be considered when interpreting retention time data. The method considers the time it takes for the stool to be excreted (Lewis et al., 1994), being the animals subject to environmental and behavioral factors, able in some way, to alter the results. Moreover, this method does not discriminate where (stomach, small or large intestine) the food remained more time.

Table 6. Fecal production, fecal traits and digesta mean retention time of dogs fed diets with different sources and particle sizes of fiber

Item	Experimental diets[1]					SME[2]	P value	Contrasts		
	CO	SC_L	SC_S	WB_L	WB_S			SCxWB[3]	LxS[4]	COxFiber[5]
Fecal traits										
Dry matter (%)	38.8	36.1	38.1	33.2	35.5	0.68	0.184	ns[6]	ns	ns
$g \bullet kg^{0.75} \bullet day^{-1}$ (DM basis)	5.5	8.3	9.5	10.3	9.7	0.37	<0.001	0.008	ns	<0.001
$g \bullet kg^{0.75} \bullet day^{-1}$ (as fed)	14.2	23.0	25.0	31.8	27.3	1.23	<0.001	<0.001	0.270	<0.001
Fecal score[7]	3.9	3.9	3.9	4.0	4.0	0.02	0.188	ns	ns	ns
Mean weight of each defecations (g/defecation)	80.9	56.1	66.9	84.7	86.1	3.16	0.004	<0.001	ns	ns
Number of defecations per day	1.3	2.4	2.0	2.3	2.3	0.09	<0.001	ns	ns	<0.001
Digesta mean retention time (h)	36.8	25.3	28.1	25.1	29.5	1.02	0.001	ns	ns	<0.001

[1] CO: control diet, without added fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles.
[2] SEM = standard error of the mean (n = 6 dogs per treatment).
[3] Sugarcane diets versus wheat bran diets;
[4] Large particle size versus small particle size fiber sources;
[5] Control diet versus fiber supplemented diets.
[6] not significant (P>0.05).
[7] 0 = watery liquid, which can be poured; 1 = soft, unformed; 2 = soft, malformed stool, which assumes shape of container; 3 = soft, formed and moist, which retains shape; 4 = well-formed and consistent stool, which does not adhere to the floor; and 5 = hard, dry pellets, which are small and hard mass.

Palatability test procedure

According to the palatability testing results some of the treatments were more palatable than others (Table 7). For example, in the CO treatment the dogs were eaten more than in SC_S or WB_S, thus implying that fiber addition decreased the palatability. This is not a surprising result, given the negative attributes associated with fiber in general, including hardness and bitterness (Koppel et al., 2015). A study conducted by Sá et al. (2013), looked at using wheat bran in dog foods. These authors found that the dogs in the experiment actually consumed the negative control sample less than the wheat bran diet; however, this was not a preference test. Sample WB_L was eaten more than WB_S, and SC_L was eaten more than SC_S. These results indicated that both fiber addition and the size of the fiber particles might have an influence when determining whether the diet is palatable or not. A full set of comparisons among the treatments would be needed in order to determine an order of preference.

Table 7. Palatability testing results.

Comparison	Treatment[1]	First Choice (%)	Food intake (%)
CO *versus* SC_S	CO	70[2]	80***
	SC_S	30	20
CO *versus* WB_S[3]	CO	75**	88***
	WB_S	25	12
SC_L *versus* SC_S	SC_L	87***	79***
	SC_S	13	21
WB_L *versus* WB_S	WB_L	24	55
	WB_S	76**	45

[1] CO: control diet, without added fiber; SC_L: sugarcane fiber, large size particles; SC_S: sugarcane fiber, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles.
[2] Difference between groups ($p < 0.05$). *** Difference between groups ($p < 0.01$).
[3] This test was not validated due to under consumption of the treatments by the dogs.

Thermogravimetric analysis (TGA) and photomicrography

Thermogravimetric analysis (TGA) is an analytical technique used for characterization of materials and to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated. That way, it's possible to preview the material behavior in the processing before you start the extrusion.

The TGA curves were useful in pinpointing the fibers characteristics when submitted a growing temperatures. The differential weight loss of powder samples is shown in Figure 2. The curves indicated the existence of two degradation stages for all fibers samples. The first event at about 270°C and the second around 350°C. This curve suggest that the sugarcane and wheat bran fibers have higher thermal stability and electrochemical and structural characteristics stables until 250°C, being possible to utilize these safety for dry dog foods, regarding the extrusion process reaches a maximum of 150°C. Both fiber sources presented the same behavior in the test. The particle size did not alter the curve behavior for wheat bran, but for sugarcane, the large particle present a little earlier degradation at 330°C than small particles at 370°C. However, more studies are need to corresponding the attributed of each degradation step.

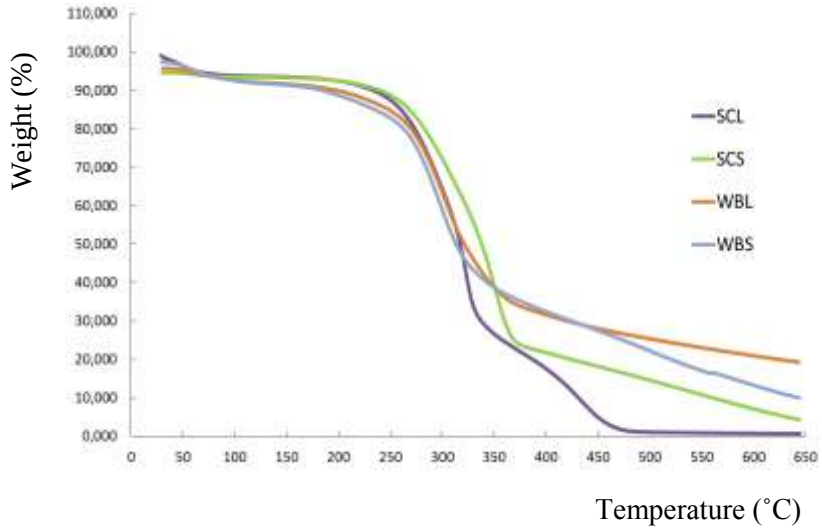

Figure 2. Thermogravimetric (TG) curves profiles of powders obtained by dehydration of sugarcane and wheat bran fiber. For thermogravimetric decomposition, the heating rate was of 10°C /min, between 30°C and 650°C. SC_L: sugarcane, large size particles; SC_S: sugarcane, small size particles; WB_L: wheat bran, large size particles; WB_S: wheat bran, small size particles.

The photomicrographs of the fibers source are represented in the Figure 3. The difference of sugarcane and wheat bran particle size was checked by scanning electron microscopy, where is easily observed.

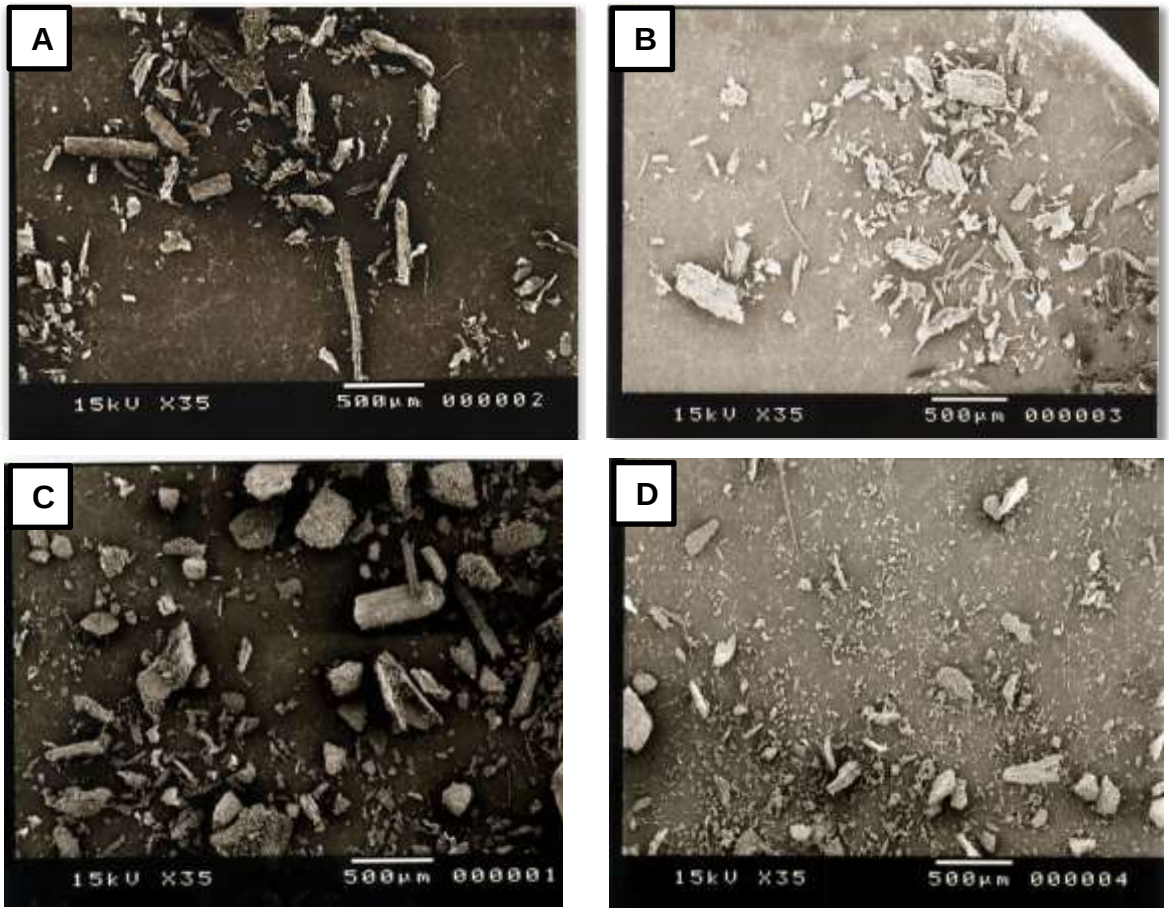

Figure 3. Scanning electron microscopy of sugarcane fiber and wheat bran fiber. A– Sugarcane large particle size B – Sugarcane small particle size; C- Wheat bran large particle size; D- Wheat bran small particle size. Increased 35x (A, B, C, D).

Conclusions

- ✓ Sugarcane and wheat bran added in extruded diets increases the nutrient intake and decrease the digestibility of protein, fiber and starch.
- ✓ Wheat bran reduced the putrefactive compounds of feces caused by microbial fermentation of non-digestible amino acids.
- ✓ Addition of sugarcane and wheat bran in extruded diets significantly influenced dog's gastrointestinal retention time.
- ✓ Fiber addition decreases the diets palatability.
- ✓ At the inclusion amount and particle sizes tested, the particle size of wheat bran and sugarcane fiber did not change significantly nutrient digestibility, fiber fermentability in the intestinal tract, and gastrointestinal transit time.

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