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INFLUÊNCIA DA FIBRA DE MANDIOCA NA ABSORÇÃO DOS NUTRIENTES, NA ENERGIA DA DIETA E NA GLICEMIA PÓS-PRANDIAL DE CÃES ADULTOS

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Março de 2023

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Dissertação apresentada como um dos requisitos à obtenção do grau de Mestre em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul

Orientador: Luciano Trevizan

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¶A sua grandeza é todo o amor dentro de você / Enfrentar a fraqueza, faz ela desaparecer / Cantando eu vou - espalhando o bem por aí. ¶

INFLUÊNCIA DA FIBRA DE MANDIOCA NA ABSORÇÃO DOS NUTRIENTES E ENERGIA E NA GLICEMIA PÓS-PRANDIAL EM CÃES ADULTOS[1]

RESUMO

Considerando os benefícios das fibras solúveis na saúde intestinal, há demanda de fontes sustentáveis com a mesma funcionalidade das alternativas disponíveis, como polpa de beterraba e fibra de cana. Este trabalho busca avaliar o impacto da fibra de mandioca (FM) moída em duas granulometrias sobre a digestibilidade dos nutrientes, variações na glicemia pós-prandial e interferência no tempo de consumo do consumo da refeição. Adotou-se um delineamento em quadrado latino balanceado incompleto, aplicando-se 6 dietas a 12 cães em 3 períodos para obtenção de 6 repetições por tratamento. Dois fatores foram testados: inclusão de FM a 7,5% e 30%, amplitude utilizada a fim de magnificar as diferenças entre os tratamentos, e a granulometria de 180 µm e 500 µm. Inclusão e granulometria foram controlados pela comparação às dietas controle, as quais continham mesma inclusão de fibra dietética total (FDT), fibra solúvel e insolúvel que as dietas testes. As seis dietas foram assim divididas: dieta controle sem inclusão de fonte de fibra (2,83% FDT), 7,5% de inclusão de FM moída a 500 µm (4,48% FDT), 30% de inclusão de FM moída a 500 µm (9,62% FDT), 7,5% de FM moída a 180 µm 7,5% (4,48% FDT), ou de inclusão de um mix de polpa de beterraba e fibra de cana a 7,5% (5,19 % FDT) e 30% (12,5 % FDT). Os cães foram alimentados por 15 dias e a digestibilidade da energia e nutrientes, escore fecal, número de defecações, glicemia pós prandial e tempo de consumo da refeição foram avaliados durante esse período. As médias do estudo foram submetidas a ANOVA e as médias dos fatores foram comparadas por contrastes ortogonais assumindo significância ao $P < 0{,}05$ e tendência $P < 0{,}10$. Conforme observado (Souza *et al*., 2021, Bazolli *et al*., 2015), os coeficientes de digestibilidade foram prejudicados pela inclusão de fibra nas dietas ($P < 0{,}05$), exceto a proteína bruta. A digestibilidade é menos afetada pela menor granulometria, em relação à maior. A glicemia média foi maior nos animais alimentados com FM a 180 µm ($P < 0{,}05$), comparando-se à FM a 500 µm. Os cães consumindo as dietas com 30% de inclusão de fibra levaram mais tempo para ingerir todo o alimento. A inclusão de 30% de FM (9,6 FDT) reduz a área abaixo da curva da glicemia no pós prandial, conforme observado em estudos utilizando fibra solúvel (Massimino *et al.* 1998; Deng *et al*. 2013). Os achados neste estudo demonstram que a FM é uma boa opção de fonte de fibra solúvel e insolúvel em dietas para cães.

Palavras-chave: digestibilidade; escore fecal; saciedade; área abaixo da curva; curva glicêmica

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[1] Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, (61p) RS. março de 2023.

INFLUENCE OF CASSAVA FIBER ON NUTRIENTS AND ENERGY ABSORF ON AND POSTPRANDIAL GLYCEMIA OF ADULT DOGS[2]

ABSTRACT

Considering the benefits of fibers on intestinal health, there is a need to alternative sustainable fiber sources with similar functionality as the available sources, as beet pulp and sugar cane fiber. This work seeks to evaluate the impact of cassava fibre (CAS) in two granulometries on the digestibility of nutrients, variations in postprandial glycemia and food consumption in dogs. An incomplete balanced Latin square design was adopted, applying 6 diets to 12 dogs in 3 periods to obtain 6 replicates per treatment. Two factors were tested: inclusion of CAS at 7.5% and 30%, to amplify the difference between treatments, and granulometry of 180 µm and 500 µm. Both factors were controlled by comparison to control diets, which contained the same inclusion of total dietary fibre (TDF), soluble and insoluble fibres as the test diets. The six diets were divided as follows: control diet (CO), without inclusion of fibre source (2.83 % TDF), 7.5% CAS diet ground at 500 µm (4.48 % TDF), 30% CAS ground at 500 µm (9.62% TDF), 7.5% CAS ground at 180 µm (4.48 % TDF) and a mix of beet pulp and sugar cane in 7.5% (5.19 % TDF) and 30% inclusion (12.5 % TDF). Dogs were fed for 15 days and the digestibility of energy and nutrients, faecal score, number of defecations, postprandial glycemia and time of meal consumption were recorded in the period. The data were submitted to ANOVA and the means of the factors were compared by orthogonal contrasts assuming significance at $P < 0.05$ and trend $P < 0.10$. Like seen before (Souza *et al*., 2021, Bazolli *et al*., 2015), digestibility coefficients were impaired by the inclusion of fibre in the diets ($P < 0.05$), except crude protein. Fine grounding of CAS affects digestibility in a mildly than coarse grounding as seen before (Average glycemia was higher in dogs fed CAS 180 µm diet ($P < 0.05$), compared to CAS 500 µm diets. Dogs fed 30% fiber inclusion spent more time to eat a full meal. The inclusion of 30% CAS inclusion diet (9.6% TDF) decreases area under the glycemic curve in the postprandial period, like observed is studies using soluble fiber (Massimino *et al.* 1998; Deng *et al*. 2013). The findings in this study demonstrate that cassava fiber is a good soluble and insoluble fiber source for dog’s diets.

Keywords: digestibility; fecal score; satiety; area under the curve; glycemic curve

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[2] Master dissertation in Animal Science – Animal production, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS. (61p) March 2023.

SUMÁRIO

CAPÍTULO I

INTRODUÇÃO

As fibras podem ser divididas em fibras solúveis e fibras insolúveis. As fibras solúveis são evidenciadas como aditivos alimentares sacietógenos, promotoras da saúde intestinal, servindo como substrato para a seleção de microrganismos desejáveis no intestino, os quais produzem substratos nutritivos aos colonócitos. As fibras insolúveis, por sua vez parecem estar relacionadas ao controle da glicemia, diminuição do tempo de trânsito intestinal. Nas dietas voltadas para a perda de peso, as fibras parecem ser uma boa alternativa para a diluição dos nutrientes, permitindo que o animal consuma maior quantidade de alimento de menor densidade calórica. A obesidade nos animais de companhia é uma condição de caráter epidêmico, que afeta grande parte da população mundial. As fibras têm sido propostas como ingredientes alimentares que substituem ingredientes com alta disponibilidade energética para a formulação de alimentos com baixa densidade calórica. Dessa forma, o processo de emagrecimento fica mais confortável para o animal e seu tutor, pois é possível comer maiores volumes e sentir-se mais saciado, diminuindo o comportamento incessante de busca por alimento – uma reclamação recorrente dos tutores de animais de companhia.

A digestibilidade da energia e dos nutrientes tende a ser prejudicada com a inclusão crescente de fibra na dieta. No caso de dietas voltadas para animais obesos, a diminuição da energia poderia ser uma característica interessante, contudo, a redução da absorção de aminoácidos teria um impacto negativo. Da mesma forma as fibras podem impactar na digestibilidade de outros nutrientes essenciais. É necessário, portanto, avaliar a relação adequada para a adição de fibra com o consumo e aproveitamento dos nutrientes.

Pelo custo e disponibilidade, o mercado encoraja a busca de novas fontes de fibras alimentares. A fibra de mandioca é um coproduto da indústria alimentícia e vem sendo sugerida com uma opção às fontes convencionais utilizadas no mercado, por sua relação favorável entre a porção de fibra insolúvel/solúvel (12,5:1). Por ser uma fonte nova, restam dúvidas sobre o impacto do tamanho da partícula na absorção dos nutrientes. Além do processo de moagem ser financeiramente mais oneroso, sugere-se que o tamanho da partícula também afete o tempo de passagem do alimento no trato gastrointestinal, uma vez que a inclusão de fibra modifica as características do alimento extrusado que pode interferir na forma como o animal se alimenta. Poucos estudos relacionados a tamanho de partícula da fibra de mandioca em cães foram encontrados. Considerando os benefícios das fibras na alimentação humana e animal, e o surgimento de novas de fontes sustentáveis na indústria alimentícia, este trabalhando busca avaliar o impacto da fibra de mandioca, moída em duas diferentes granulometrias, sobre a digestibilidade aparente dos nutrientes, glicemia pós prandial e interferência no consumo alimentar de cães adultos saudáveis.

REVISÃO BIBLIOGRÁFICA

Fibras

As fibras são definidas como porções de polissacarídeos da parede celular de plantas comestíveis, lignina, e substâncias associadas, resistentes à digestão e absorção no trato gastrointestinal presentes em grãos integrais, nozes e sementes, vegetais e frutas (Makharia *et al*., 2018). As fibras alimentares são compostas de celulose, hemicelulose, gomas, pectinas, lignina e oligossacarídeos, provindas de diversas fontes.

Solubilidade

As fibras podem ser classificadas pela solubilidade em água em fibras solúveis ou insolúveis (Tabela 1). O aumento da solubilidade é relacionado positivamente com suscetibilidade a degradação microbiana através da fermentação.

Tabela 1 – Fibras dietéticas [1]

Fibras insolúveis	Fibras solúveis	
Celulose	Viscosas	Não viscosas
Hemicelulose	Pectina	Dextrina
Lignina	B-D-Gluganas	Amido resistente
Amido resistente	Galactomanose	Polidextrose
Arabinoxilanos	Glucomanose	Inulina
Polissacarídeos não-amiláceos	Psyllium	FOS

[1]Adaptado de Mudgil (2017).

As fibras predominantemente insolúveis, como a lignina, a celulose e hemicelulose não são amplamente fermentadas pelo microbioma intestinal de cães e gatos. As fibras insolúveis diminuem o tempo de passagem do alimento pelo trato gastrointestinal, contribuem diretamente no aumento da massa fecal e na diluição energética da dieta (Marx *et al.,* 2022). As fibras insolúveis não possuem característica de viscosidade no trato gastrointestinal. A viscosidade está relacionada a capacidade de ligação à água. Sugere-se que a capacidade de ligação à água também está diretamente relacionada à susceptibilidade à degradação microbiana, característica das fibras solúveis. A viscosidade formada no trato gastrointestinal pode dificultar a absorção de nutrientes por tornar difícil o acesso do alimento às enzimas digestivas.

As fibras predominantemente solúveis, como as pectinas, gomas, algumas hemiceluloses e inulina, aumentam a viscosidade do bolo alimentar, aumentando o tempo de trânsito intestinal, tornando a absorção da glicose mais lenta (Mudgil, 2017). As fibras solúveis são fermentáveis no intestino grosso e modulam a microbiota intestinal (Wambacq, 2016; Alves *et al*., 2021). As fontes de fibras solúveis possuem variadas características de fermentabilidade, servindo de substrato para fermentação das bactérias intestinais produtoras de ácidos graxos voláteis no intestino grosso de cães (Wambacq, 2016). Os ácidos graxos voláteis, por sua vez, nutrem os colonócitos, contribuindo com um ambiente intestinal saudável (Fritsch *et al*., 2019). A alta fermentação, contudo, pode deixar as fezes mais amolecidas, devido à retenção de água no lúmen. As fibras insolúveis, em contraponto, contribuem para fezes mais secas, pois tem a propriedade de retenção hídrica (Donadelli & Aldrich, 2019).

As características fecais dos animais de companhia é motivo de preocupação dos tutores, pois podem refletir a saúde intestinal do animal, além de facilitar o processo de higienização do local onde vivem. A relação entre fibra insolúvel e solúvel adequada é crucial nas características fecais desejáveis (pouca quantidade e formato bem definido) dos cães (Marx *et al*., 2022).

Digestibilidade

Por dificultar a ação das enzimas digestivas sobre o bolo alimentar, as fibras aparentam interferir na digestibilidade de outros nutrientes. Ramos e colaboradores (2019) verificaram a redução da digestibilidade de matéria seca e energia metabolizável da dieta com a inclusão de 15% de casca de soja na alimentação de cães adultos. Outro estudo comparou diferentes fontes de fibra na dieta de gatos e revelou diminuição na digestibilidade da digestibilidade de todas as classes nutricionais com a adição de 12% de fibra de cana (características insolúveis semelhantes à celulose). A utilização da fibra também diminuiu concentração média de glicose e área abaixo da curva da glicose pós prandial em relação às demais dietas que incluíram farelo de trigo e polpa de beterraba (Fischer, 2011). Contudo, está descrito no NRC (2006) que a digestibilidade da proteína bruta pode ser subestimada pela excreção de nitrogênio microbiano no colón pela fermentação de fibras solúveis. Em um estudo realizado com fibra de mandioca em cães, verificou-se a diminuição da digestibilidade da maioria dos nutrientes e energia metabolizável das dietas com inclusão de 120 g/kg (8,3% de fibra dietética total) (Souza *et al*., 2021).

O grau de lignificação e a estrutura dos tecidos da planta influencia a digestibililidade da parede celular. Massas celulares mais densas são mais resistentes à ação das enzimas (McDonald *et al*., 2011). O processamento também interfere na taxa de passagem do alimento no trato gastrointestinal (Willmann *et al*., 2010; Bazolli *et al*., 2015; De Cuyper *et al*., 2018). Sugere-se que menor tamanho de partícula de fibra adicionada às dietas de cães favorece a formação de *kibbles* menos densos e duros e que a fonte de fibra influencia a expansão no processo de extrusão (Monti, *et al*., 2016). É necessário que o processamento seja realizado na granulometria mais adequada para o objetivo final (perda ou ganho de peso, manutenção da saúde intestinal), buscando equilíbrio na taxa de passagem e degradação microbiana do alimento, otimizando o aproveitamento (Gomes *et al*., 2012).

Fibras e saciedade

A inclusão de fibra na dieta dos cães pode impactar a ingestão de alimento. A adição de alimentos ricos em fibra permite o aumento do volume da ingesta, mas com baixo incremento calórico, apresentando-se como uma boa estratégia para promover maior saciedade e bem-estar dos animais obesos em restrição calórica (Linder & Mueller, 2014). Um estudo comparou 3 dietas: dieta com alta fibra + alta proteína, baseada em celulose, polpa de beterraba, frutooligossarideos (FOS) e psyllium; dieta com alta fibra + alta proteína contendo celulose e polpa de beterraba; e a dieta com alta fibra + moderada proteína contendo essencialmente celulose. Foi observado o consumo voluntário dos cães, obtendo como resultado maior saciedade na dieta com alta fibra + alta proteína. Os resultados demonstram a influência das fibras solúveis sobre a saciedade (Weber *et al*., 2007), já que diminuem o tempo de esvaziamento gástrico e mantêm a liberação de insulina e a glicemia estáveis. O resultado foi novamente encontrado no estudo de Bosch *et al.* (2009), que verificou menor consumo voluntário de cães consumindo uma dieta contendo altos níveis de fibra fermentável (8,5% polpa de beterraba e 2% inulina), quando comparados à dieta contendo alto nível de fibra de baixa fermentação (8,5% celulose), embora não tenha sido detectada diferença no perfil sanguíneo dos hormônios sacietógenos.

Outros fatores que afetam consumo alimentar

Acredita-se que o controle da saciedade em mamíferos ocorre em diversas áreas do sistema nervoso central, destacando-se os centros localizados no hipotálamo. Olfato, paladar, textura e visualização têm papel importante no estímulo da quantidade de alimento ingerido em humanos e animais, variando de acordo com a espécie (McDonald *et al*., 2011).

Algumas teorias sobre o controle do consumo em animais não ruminantes são discutidas. Entre as teorias de consumo de regulação a curto prazo, estão a teoria termostática e a quimiostática. A teoria termostática propõe que os animais se alimentam para se manter aquecidos e reduzem o consumo para evitar a produção de calor. De acordo com a teoria quimiostática, a presença de nutrientes do alimento no trato digestivo promove a liberação de substâncias sinalizadoras que fazem com que o animal pare de comer e uma queda nestas substâncias estimula o consumo. Pequenas doses de insulina, que diminui a glicose sanguínea, fazem com que o animal sinta fome e inicie a refeição. Alguns receptores de glicose localizados no intestino e no fígado parecem provocar uma resposta mais rápida à ingestão de alimento. Outra possível sinalização entre o cérebro e o intestino é a colecistocinina (CCK), hormônio peptídico liberado quando aminoácidos ou ácidos graxos atingem o duodeno e que age no hipotálamo, inibindo o consumo. Outros hormônios como a grelina e somatostatina também estão envolvidos no controle do consumo através de seus efeitos no hipotálamo (McDonald *et al.*, 2011). A grelina é sintetizada e liberada principalmente no estômago e está relacionada ao controle do consumo alimentar.

Os animais, em especial as aves, demonstram capacidade de ajuste do consumo quando expostos a dietas de diferente incremento calórico. Nos mamíferos, o efeito pode ser bem observado durante a lactação, que normalmente está associada no evidente aumento do consumo voluntário. Sugere-se que esse mecanismo de controle do consumo esteja menos evidente em algumas espécies pelo efeito da seleção artificial voltada para alto consumo, ganho de peso e conversão alimentar nos animais de produção. Contudo, quando a diluição calórica da dieta é muito grande, fatores físicos adicionais atuam na diminuição do consumo. A distensão mecânica do esôfago, estômago, duodeno e intestino delgado, através de receptores locais, estimulam o nervo vago e o centro da saciedade no hipotálamo, fazendo com que o animal pare de comer. Esse conhecimento pode ser utilizado como estratégia no auxílio do controle do consumo em animais obesos. A obesidade nos animais de companhia é uma condição de caráter epidêmico, que afeta grande parte da população mundial. Linder & Mueller (2014) sugerem que 34 a 59% dos cães e 25 a 63% dos gatos apresentam sobrepeso ou obesidade. Em um trabalho realizado no município de São Paulo, estimou-se que a obesidade atinge em torno de 15% da população canina (Porsani, 2019) e menciona que problemas decorrentes da obesidade refletem dificuldades no sistema respiratório, músculo-esquelético, circulatório, reduzindo a qualidade e a expectativa de vida. Trata-se de uma patologia multifatorial, que envolve fatores genéticos, raça, idade, sedentarismo, fatores relacionados aos tutores, consumo calórico e composição dos alimentos ingeridos. A vida urbana e o sedentarismo contribuem com o ganho de peso, resistência insulínica e dislipidemia (Aptekmann *et al*., 2014).

A ideia de promoção da saúde e qualidade de vida através da nutrição tem se expandido, surgindo a necessidade de formulações voltadas ao bem-estar e à longevidade (Saad & França, 2013). Desta forma, seguem as discussões sobre as diferentes estratégias nutricionais para a perda de peso.

A alimentação dos cães e gatos selvagens é primordialmente composta de altos níveis proteicos e lipídicos. Hoje, a maioria das rações comerciais para carnívoros domesticados têm o amido como fonte energética principal (França *et al*., 2011). Ë reconhecido que cães domésticos apresentam diferenças metabólicas quando comparados aos ancestrais. A presença de carboidratos solúveis na alimentação de cães parece não impactar negativamente o metabolismo de animais saudáveis (Laflamme, 2022). Porém, para animais obesos ou com transtornos lipídicos há recomendação de cautela na inclusão de carboidratos solúveis (Laflamme, 2022). As dietas para controle de diabetes tendem a ter em sua composição uma menor quantidade de amido e maior teor de carboidratos de digestão mais lenta, assim como fibras, promovendo maior sensação de saciedade e favorecendo o controle da glicêmico. Em humanos, as fibras em geral estão associadas à diminuição do colesterol sanguíneo, a incidência de doenças cardiovasculares e diabetes (Makharia *et al*., 2018).

Nas dietas voltadas para a perda de peso, as fibras são uma boa alternativa para a diluição dos nutrientes, permitindo que o animal consuma maior quantidade de alimento de menor densidade calórica (Marx *et al*., 2022).

Fontes de fibra na formulação de rações

As fontes de fibras utilizadas na alimentação animal provêm de resíduos da indústria alimentícia humana (Embrapa, 2023). A polpa de beterraba é citada como principal fonte de fibra utilizada em dietas para em cães (Sunvold *et al*., 1995) e possui características principalmente solúveis. Como fonte de fibra insolúvel, destaca-se a fibra de cana (Tabela 1). O Brasil é o maior produtor mundial de cana de açúcar, que serve de matéria prima para o álcool e o açúcar e seus subprodutos são utilizados na cogeração de energia elétrica, na alimentação animal e como fertilizantes (Embrapa, 2023).

Considerando os benefícios das fibras na saúde animal, estudos buscando novas fontes de fibra como aditivos nas dietas têm sido estudados (Kröger, Vahjen & Zentek, 2017; Donadelli & Aldrich, 2019).

A mandioca é produzida por diversos países, sendo o Brasil o segundo produtor mundial, e é alimento básico para milhões de pessoas (Embrapa, 2023). É uma planta que se desenvolve em solos menos exigentes e possui destaque de produção no norte do Brasil, onde é fonte de subsistência para a população, principalmente na produção de farinhas. As farinhas de mandioca são ricas em fibras e energia (Oliveira *et al*., 2021). A mandioca é utilizada como importante fonte energética na alimentação animal (Embrapa, 2003). A fibra de mandioca é obtida da porção cortical da raiz, após processo de secagem e moagem (Souza *et al*., 2022) e apresenta uma característica de solubilidade intermediária entre a polpa de beterraba e a celulose (Tabela 2), as quais são as principais fontes de fibras utilizadas como aditivos nas rações e gatos (Donadelli & Aldrich, 2019).

Tabela 2 – Composição química de fibra de mandioca, celulose, polpa de beterraba e fibra de cana[1]

Item, g/kg MS	Fibra de mandioca[2]	Celulose	Polpa de beterraba[3]	Fibra de cana[4]
Fibra dietética total	350	929	620	908
Fibra insolúvel (FI)	324	928	371	908
Fibra solúvel (FS)	25,9	1	245	0
Proporção IF:SF	12,5:1	929:3:1	3,5:1	908

[1]Adaptado de Souza *et al*. (2021) e Calabrò (2013)
[2]Outros componentes analisados da fibra de mandioca (g/kg de matéria seca): matéria seca = 960,7, proteína bruta = 45,4, extrato etéreo = 21,1, cinzas = 58,7, amido = 501,2.
[3]Outros componentes analisados da fibra de beterraba (g/kg de matéria seca): matéria seca = 905, proteína bruta = 75, extrato etéreo = 5,5, cinzas = 64.
[4]Outros componentes analisados da fibra de cana (g/kg de matéria seca): matéria seca = 982, proteína bruta = 17, extrato etéreo = 13, cinzas = 56.

Em estudo anterior em cães, a fibra de mandioca demonstrou propriedades prebióticas e de suporte à saúde intestinal, aumentando a diversificação da microbiota e melhorando a consistência fecal de cães adultos, sugerindo como uma boa opção como fonte fibras na alimentação animal (Souza *et al*., 2021).

O conhecimento de que uma maior granulometria aumenta o tempo de trânsito gastrointestinal e diminui consumo alimentar, aliado a uma nova fonte de fibra, sugere estudos mais aprofundados sobre o efeito do tamanho de partícula mais adequado para a fibra de mandioca.

HIPÓTESES E OBJETIVOS

Portanto, o objetivo deste estudo foi verificar se a fibra de mandioca poderia ser uma fonte alternativa ao mix de polpa de beterraba e fibra de cana de açúcar; se a fibra de mandioca influencia a digestibilidade dos nutrientes e energia das dietas; nas características fecais; se a inclusão da fibra de mandioca influencia a glicemia pós prandial, o tempo que o animal leva para fazer uma refeição e a ingestão hídrica 6 horas após o alimento. Além das fontes e das diferentes inclusões de fibra, também foi verificado se a granulometria da fibra de mandioca (180 µm e 500 µm) poderia interferir nos resultados.

Nossas hipóteses são que a fibra de mandioca tem efeito semelhante a mistura de polpa de beterraba e fibra de cana de açúcar, influenciando negativamente a digestibilidade dos nutrientes e energia, reduzindo a área abaixo da curva glicêmica no pós-prandial e aumentando o tempo para o consumo alimentar dos cães. Maiores inclusões de fibra, independente da fonte, tendem a influenciar as medidas de forma mais acentuada. Acredita-se, também, que o menor tamanho de partícula diminuirá a digestibilidade e aumentará a área abaixo da curva de forma mais sutil que a fibra de maior granulometria, diminuirá tempo de consumo e aumentará consumo hídrico durante as 6 horas que sucedem à refeição dos cães, devido a menor distensão gástrica.

CAPÍTULO II

Influences of cassava fiber on nutrients and energy absorption of diet and post prandial glycemia of adult dogs.

Este capítulo é apresentado de acordo com as normas de publicação da **Journal of Animal Science**

Running title: Cassava fiber in diets for dogs

Influences of cassava fiber on nutrients and energy absorption of diet and post prandial glycemia of adult dogs.

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Lay Summary

Considering the benefits of diet fiber on animal health and the urge of new sustainable, low price fiber sources, this study evaluated the impact of cassava fiber (CAS) in two sized on nutrients absorption, feces characteristics, time of meal consumption and postprandial glycemia in adult dogs. Absorption of the nutrients were impaired by inclusion of fiber on diets, except for protein. Inclusion of 30% CAS fiber increased consumption time and reduced glycemia response. Fecal scores remained in a desirable range for companion dogs. The findings demonstrate that CAS is a good fiber source for dog's diets.

Teaser text

Considering the benefits of diet fiber on animal health and the urge of new sustainable, low price fiber sources, this study evaluated the impact of cassava fiber (CAS) in two sized on nutrients absorption, feces characteristics, time of meal consumption and postprandial glycemia in adult dogs. The findings demonstrate that CAS is a good fiber source for dog's diets.

ABSTRACT

Considering benefits of fiber on animal health, this study evaluated the impact of cassava fiber (CAS) in two granulometries on digestibility of nutrients and postprandial glycemia in adult healthy dogs. An incomplete balanced Latin square design was adopted, applying 6 diets to 12 dogs in 3 periods to obtain 6 replicates. Two factors were tested: inclusion of CAS at 7.5% and 30% and granulometry of 180 µm and 500 µm, compared to a mix of beep pulp and sugarcane fibers (BP), as control diets. The six diets were divided as follows: control diet, without fiber source (CO), 7.5% inclusion of CAS ground at 500 µm, 30% inclusion of CAS ground at 500 µm, 7.5% inclusion of CAS ground at 180 µm, 7.5% or 30% inclusion of BP. Dogs were fed diets for 15 days and digestibility of energy and nutrients, fecal score, postprandial glycemia and time of meal consumption were recorded. Data was submitted to ANOVA and complemented by orthogonal contrasts assuming significance at $P < 0.05$ and at $P < 0.10$. Analyses demonstrated similar content of nutrients in all diets, except for higher insoluble fiber and gross energy in BP diets. Digestibility coefficients were impaired by inclusion of fiber on diets ($P < 0.05$), except for crude protein. Fine grounding affected digestibility mildly, compared to coarse grounding, showing higher average glycemia ($P < 0.05$). Inclusion of 30% CAS fiber increased consumption time and reduced the area under the curve, like previous studies with fiber (Kimmel *et al.*, 2000 and Nelson *et al.*, 2000). Fecal scores remained in a desirable range for companion dogs (2-2.5 in WALTHAM scale). The findings demonstrate that CAS is a good fiber source for dog's diets.

Key words: keywords: digestibility, fecal score, satiety, area under the curve, glycemic curve

Introduction

Obesity in companion animals is an epidemic condition, that affects great portion of population (Souza *et al.,* 2019). Soluble fibers, as beet pulp, are satiating and intestinal health promotors (Fritsch *et al*., 2019). Insoluble fibers can be used for energy dilution of diets and higher fecal bulk (Marx, *et al*., 2022). Fibers play an important role on diets for weight loss, allowing the animal to eat a large meal with lower calorie ingestion. Energy and nutrients digestibility appears do decrease according to the greater fiber inclusion (Prola *et al.,* 2010; Ramos *et al*., 2019). It is required balance between fiber inclusion with consumption and nutrient absorption. Brazil is the world's second main product of cassava and it has been used for animal feeding (EMBRAPA, 2023). Cassava fiber (CAS) have been suggested as a good fiber source with similar effects as actual sources (as cellulose and beet pulp) since CAS has a good proportion of insoluble and soluble fiber (12.5:1). Cassava is a widely available product for human nutrition and the coproduct could be useful to animal nutrition, looking from a sustainable perspective. Since it is a new product, we wonder about the impact of particle size on digestion of nutrients and energy. In addition to the high cost of grounding process, it is suggested that the granulometry affects gastrointestinal transit time and nutrient absorption (Bazolli *et al*., 2015; Willmann *et al*., 2010), but there are few studies regarding particle size and CAS on non-ruminant (Souza *et al*., 2022). Considering the benefits of fiber on human and animal nutrition and the need of new sustainable sources of fiber to supply the industry demand, the objective of the study was to observe the impact of two inclusions (7.5% and 30%) of CA in two granulometries (180 µm and 500 µm) on digestibility of nutrients, and postprandial glycemia response in dogs; stool score and frequency, time of food intake in dogs and postprandial water intake were complementary assessments. We also observed the voluntary water intake up to 6 hours after meal. We expect that digestibility of energy and nutrients, as well glycemic index of diet would be impaired by higher fiber inclusions (for both CA and BP) and coarse particle size. The greater inclusion of fiber would result in a longer meal, since diets are energetic diluted.

Material and Methods

The procedures used in this study were approved by the Universidade Federal of Rio Grande do Sul Ethics Committee on Animal Use, protocol number 41825, and were performed in accordance with ethical standards and animal welfare.

Experimental design

The study was conducted in a balanced incomplete Latin square design as proposed in the study of Ai *et al.* (2013). It was used with six dietary treatments (6 diets) and 12 dogs, in 3 periods of 14 days (8 days for diet adaptation, 5 days for feces and urine collection and the last day for glycaemic index, time of meal consumption and water consumption up to 6 hours) to obtain 6 replications for each treatment, according to the recommendations of the American Association of Feed Control Officials protocols (2019). Thirty days before starting study and 15 days between each experimental period dogs were washed out in a control diet.

Animals

Twelve healthy adult Beagle (5 males and 7 females), aged between 1 to 7 years, weighing between 8.6 and 11.9 kg, with a BCS of 5 ± 2 (Laflamme, 1997) were used to test the inclusion of cassava fiber in the diet. During the experimental period, dogs were separated in groups and remained together in an outdoor area for socialization. During the night, meals, and digestibility assay they were housed individually in stainless steel metabolic cages (0.80 by 0.70 by 0.90 m) equipped with feeders, drinkers, feces and urine collectors in a controlled room at 24˚C, with a light: dark cycle of 14:10 h. Dogs were fed individually according to their metabolic weight once a day, at 8:00 AM. Before the assay, dogs were adapted to the cages and blood collection using food as a treat for positive reinforcement. Water was provided ad libitum throughout the experimental period. All dogs were previously vaccinated and evaluated by complete blood counts, serum biochemistry profiles, coproparasitological tests, and clinical examination to ensure they were healthy.

Dietary Treatments

Experimental diets were prepared using basically the same ingredients, except for the inclusion of dietary fiber sources (Table 1). Two factors were tested: fiber source inclusion at 7.5% or 30% and particle size of CA fiber ground 500 µm or 180 µm. The combination of BP and sugar cane was used as control diets, resulting in six diets as follow: Control diet, with no fiber added (CO), 7,5% cassava fiber ground 500 µm or 180 µm (CAS500/7.5; CAS180/7.5), 30% cassava fiber inclusion ground 500 µm (CAS500/30), 7.5% or 30% beet pulp + sugar cane fiber (BP/7.5; BP/30). The proportion of BP and sugarcane fiber added on diets was 1:1.3. Diets were formulated to meet the Association of American Feed Control Officials (AAFCO, 2019), according to dog's maintenance energy requirement estimated of 140 kcal of metabolized energy per $kg^{0.75}$ per day.

Table 1 - Ingredients and chemical composition of experimental diets.

Fiber inclusion	0%	7.5%			30%	
Source	Control	CAS500	CAS180	BP	CAS500	BP
Ingredient, % as is						
Broken rice	54.6	49.1	49.1	48.6	30.1	29.2
Poultry byproducts meal, low ash	20.9	20.2	20.2	20.2	18.6	18.6
Corn gluten	10.0	9.15	9.15	9.14	8.42	8.42
Bovine meat and bone meal	0.36	1.41	1.41	1.41	1.30	1.30
Palatability enhancer	2.00	1.83	1.83	1.83	1.7	1.69
Poultry fat	8.89	7.55	7.55	7.54	6.94	6.94
Soybean oil	1.54	1.72	1.72	1.71	1.58	1.58
DL-Methionine	0.13	0.10	0.10	0.10	0.09	0.09
Potassium chloride	0.38	0.32	0.32	0.32	0.29	0.29

Premix mineral/vitamin[1]	0.50	0.45	0.46	0.45	0.42	0.42
Sodium chloride	0.50	0.45	0.46	0.45	0.42	0.42
Antifungal	0.15	0.14	0.14	0.14	0.13	0.13
Antioxidant	0.05	0.05	0.05	0.05	0.04	0.04
Beet pulp[2]	0	0	0	1.50	0	5.98
Sugar cane fiber	0	0	0	1.97	0	8.02
Cassava fiber 180[3]	0	0	7.50	0	0	0
Cassava fiber 500[3]	0	7.50	0	0	30.0	0
Starch	0	0	0	4.65	0	16.9
Total	100	100	100	100	100	100
Analyzed chemical composition of experimental diets, % DM basis						
Dry matter	94.2	90.2	93.3	90.8	94.3	95.3
Organic matter	93.9	93.8	93.6	93.2	93.6	93.3
Ash	6.10	6.18	6.39	6.76	6.45	6.70
Crude protein	23.2	27.9	24.9	26.9	26.8	26.4
Acid hydrolyzed fat	13.2	13.5	12.5	14.0	13.3	13.2
NDF	10.32	12.98	11.14	11.7	15.7	16.5
ADF	1.34	2.46	1.86	2.41	5.38	4.62
IF	1.74	3.37	3.28	4.03	8.41	11.2
SF	1.08	1.1	1.2	1.19	1.2	1.29
TDF	2.83	4.48	4.48	5.19	9.62	12.5
I/S	1.61	3.06	3.06	3.39	7.01	8.71
Gross energy (kcal/kg)	4361	4470	4583	4743	4535	4693

[1]Premix mineral/vitamin (content per kg) – vitamin A (10,800 UI), vitamin D3 (980 UI), vitamin E (60 mg), vitamin K3 (4.8 mg), vitamin B1 (8.1 mg), vitamin B2 (6.0 mg), vitamin B6 (6.00 mg), vitamin B12 (0.29 mcg), pantothenic acid (12 mg), niacina (60 mg), folic acid (0.8 mg), biotin (0.084mg), manganese (7.5 mg), zinc (100 mg), iron (35 mg), copper (7.0 mg), cobalt (10 mg), iodine (1.5 mg), selenium (0.36 mg), choline (2.400 mg), taurine (100 mg), and antioxidant (150 mg).
[2]Beet Pulp – 51.8% insoluble fiber; 9.55% soluble fiber; 61.4% total dietary fiber;
[3]Cassava fiber – 23.9% insoluble fiber; 1.65% soluble fiber; 25.5% total dietary fiber.

Experiment 1 – Digestibility assay

After 8 days of adaptation with experimental diet, the total content of feces and urine were collected for 5 days. In the beginning of the assay and at the end, capsules containing iron oxide were administered to the dogs to establish the 5-day digestibility period. Defecation frequency was observed, and feces were scored, using the WALTHAM Feces Scoring System (Moxham, 2001). Total urine collection was performed daily in the morning in a plastic bucket containing 0.5 g of thymol. An aliquot of 3 ml of urine was used to measure the pH using a bench pH meter (AKSOVR pH Plus, São Leopoldo, Brazil) and urinary density by a portable refractometer (BEL ENGINEERINGVR model RPI, China) and samples were frozen at -20°C until analysis.

Chemical analysis

Fecal samples were dried in the stove for 55°C for 72 hours, then grounded though a 1 µm screen in a Wiley hammer mill (DeLeo Equipamentos Laboratoriais, Porto Alegre, Brazil) and sent to the lab for chemical analysis. Diets and feces were analysed for dry matter (AOAC 934.01), nitrogen was analysed (method 954.01) and then crude protein was calculated as N x 6.25 (AOAC, 1995), acid-hydrolysed ether extract (AOAC 954.02) ash (AOAC, 1995), acid detergent fiber (ADF) and neutral detergent fiber (NDF), according to Silva & Queiroz (2002); Cassava fiber and beet pulp were analysed for soluble and insoluble fiber based on Prosky *et al*., 1988 (AOAC 991.43). Gross energy in the diets and feces were determined in an adiabatic bomb calorimeter (Parr Instrument Co, model 1261, Moline, IL, USA).

Experiment 2 – Post prandial glycemic index

At the last day of the period (14°), one drop of blood of each dog was used to measure the glycemia using glycosometer (Accu-Chek Guide Me, Roche, German). Glucose was measured in the morning before the meal, time 0, and 15, 30, 45, 75, 165, 255, and 345 minutes after the meal to establish a glycaemic curve. Maximum glycemia, time for maximum glycemia, minimum glycemia, time for minimum glycemia and average glycemia was also recorded.

Experiment 3 - Time for meal consumption and water intake

At the last day of experiment (14º), dogs were distributed in the treatments according to the experimental design described. The entire meal (140 kcal of metabolized energy per $kg^{0.75}$ per day) was offered to the dogs at approximately 7:30 h and the time taken by the dog to eat the entire meal was recorded individually. Water intake was recorded up to 6 hours after the meal. Water bowls were filled out with 500 ml after the morning meal and subtracted to the remaining volume in the bowl after 6 hours. Extra water filling was added during the day, if needed. An extra 500 ml water bowl was left in the room to measure evaporation and the result was subtracted from the initial volume. The procedure was replicated in the other two subsequent periods to achieve 6 replications per diet to evaluate time for meal consumption, and volume of water consumed.

Statistical Analyses

Coefficients of digestibility were obtained based on grams of nutrients and kcal ingested and grams and kcal excreted in the feces. Data was distributed in excel tables to organize data of feces and diets. Data was displayed in excel tables to observe maximum glycemia, time for maximum glycemia, minimum glycemia, time for minimum glycemia, average glycemia and to calculate area under the curve (AUC). To provide better analyses, AUC was divided in the time lapses starting at point 1 (before meal) and ending at 345 minutes after meal (point 8), leading us to 7 intervals of analyses in the glycaemic curve. The basal glycaemic area (point 0-15 min) was excluded to measure the post prandial increment on glycemia.

To data analyses, it was adopted a model for balanced incomplete latin square experimental design. The individual observations data of all assays were submitted to ANOVA procedure, considering the periods and diets as fixed effects and animals as a variable effect. For glycemic curve data, the mixed procedure and repeated measures were used, since the samples was collected in different times for the same animals. To complement the results, it was applied the orthogonal contrasts technique, as follows: CO vs. other diets; CAS diets vs. BP diets; CAS500/7.5 vs. CAS500/30 diets, CAS180/7.5 vs. CAS500/7.5 and CAS500/30 and between BP (7.5 and 30% inclusion).

Data was analyzed using SAS 9.4 (SAS Inst. Inc., Cary, NC) procedures, assuming significance as $P < 0.05$ and trend as $P < 0.10$.

Results and discussion

All dogs remained healthy and were able to eat all energetic demand in one meal per day during the entire study. The chemical analyses demonstrated similar content of nutrients in all diets, except for higher insoluble fiber and gross energy in BP diets. We could, maybe, attribute the higher insoluble fiber content to precision errors of total dietary fiber method, or to the variation naturally found on plants and manufacturing process of the source. It is also known that CAS has a high portion of starch in its composition (501.2 g/kg in dry matter basis). To formulate equal diets, starch was added to BP diets, and it could be the cause to higher gross energy found on both BP diets. Digestible and metabolizable energy of BP and CAS diets were not affected (Table 2).

During the extrusion process, we observed an alteration on kibbles on BP30. We suppose it could be an expansion issue, due to higher fiber inclusion (12.5% of total dietary fiber) or to the big particle size of beet pulp, like reported before (Alam et al., 2014). The kibble expansion seems to had occurred normally on CAS500/30 diets (9.6% of total dietary fiber), like observed in a previous study with in a diet containing 12% of CAS ground at 125 µm, resulting in 8.32% TDF (Souza, *et al*., 2022).

The intent of diet calorie dilution trough fiber inclusion was achieved, since dogs ate more natural matter according to fiber inclusion (Table 2). We expected no difference in dog nutrient intake between diets, but we couldn´t achieve that is most of the nutrients. Despite energy differences between diets, dogs remained in constant body weights.

Experiment 1 – Digestibility and fecal characteristics

Dogs fed the highest fiber inclusion (30%) showed higher DM intake (Table 2). Energy and nutrients absorption was impaired by fiber inclusion and higher particle size ($P < 0.05$) and demonstrated no relation with fiber source (BP or CAS). We did expect the digestibility of energy and nutrients of diet to be impaired by higher fiber inclusions (for both CAS and BP) and higher particle size (Souza *et al*., 2021, Bazolli *et al*., 2015). Apparent protein digestibility was not affected by 7.5% fiber inclusion or higher granulometry, but it was affected by 30% fiber inclusion (BP and CAS).

Mineral apparent digestibility was greater on CAS500/30, comparing to BP30. Prola *et al*. (2010) correlated fecal excretion of mineral to fecal bulk, fecal water, DM, and type of fiber in diet. On a previous study (Cargo-Froom *et al*., 2019) suggests further studies are needed to better understand the relationship between total diet fiber and mineral absorption and questioned if minerals may have been over supplemented on animals extruded diets.

Defecation was more frequent and grams of feces (as is) per day was higher on diets containing 30% fiber inclusions (Table 3), as expected and previous observed with insoluble fibers (Loureiro *et al.* 2017, Marx *et al*., 2022). We could not observe difference on DM of feces between diets, demonstrating fecal bulk was basically humidity caught by fiber.

The fecal score of dogs fed CO was better formed than diets ($P < 0.05$), which presented drier feces (Table 3). Fecal scores of dogs eating BP were found to be the same of dogs eating CA diets. Also, the granulometry of the fiber source did not interfere on fecal score.

Table 2 – Nutrient intake and digestibility of nutrients and energy of dogs fed the experimental diets.

Fiber inclusion	0	7,50%			30%			Constrasts				
Source	**CO**	**CAS500**	**CAS180**	**BP**	**CAS500**	**BP**	**SEM[1]**	**CO vs diets**	**CAS500 vs BP**	**CAS500 vs CAS180**	**CAS500 7.5% vs CAS500 30%**	**BP 7.5% vs BP 30%**
Daily nutrient intake, g/day										P-value		
DM	185	185	187	193	218	218	4.83	<.0001	<.0001	<.0001	<.0001	<.0001
NM	195	214	207	214	230	234	7.55	0.0003	0.4373	0.0155	0.0110	0.057
OM	174	174	175	181	203	204	4.51	<.0001	<.0001	<.0001	<.0001	<.0001
AHF	24.6	25.0	26.1	25.8	28.6	27.2	0.64	<.0001	<.0001	0.99	<.0001	<.0001
PB	43.0	51.7	50.4	51.8	57.6	53.3	1.27	<.0001	<.0001	0.0015	0.0028	<.0001
FDA	2.51	5.08	3.76	4.95	10.6	12.7	0.985	<.0001	0.0004	<.0001	<.0001	<.0001
FDN	21.0	25.8	21.9	23.7	39.6	37.9	0.72	<.0001	<.0001	<.0001	<.0001	<.0001
NFE	107	97.7	89.0	103	117	123	2.61	0.49	0.0002	<.0001	<.0001	<.0001
Ash	11.3	11.4	12.6	12.4	14.6	13.9	0.32	<.0001	<.0001	0.82	<.0001	<.0001
Apparent total digestibility, %												
DM	89.4	84.9	86.1	86.0	80.0	79.3	0.80	<.0001	0.4519	0,0002	<.0001	<.0001
OM	93.2	89.4	90.6	90.0	83.5	83.3	0.63	<.0001	0.05	<.0001	<.0001	<.0001
AHF	95.1	92.8	94.0	94.0	90.7	89.9	0.44	<.0001	0.78	<.0001	<.0001	<.0001
PB	85.1	84.6	84.6	85.2	83.1	80.5	0.97	0.13	0.29	0.08	0.006	0.13
FDA	27.7	4.28	25.6	4.65	-10.5	-2.27	11.4	0.009	0.62	0.06	0.68	0.36
FDN	84.0	70.5	67.1	68.6	54.5	51.7	2.70	<.0001	0.49	0.067	<.0001	0.0012
NFE	96.0	91.1	92.8	91.4	81.9	83.1	0.75	<.0001	0.0009	<.0001	<.0001	<.0001
Ash	30.8	17.7	22.6	28.1	30.7	20.9	4.87	0.17	0.04	0.56	0.63	0.7
DE	92.0	89.0	90.3	89.8	83.8	83.3	0.58	<.0001	0.1217	<.0001	<.0001	<.0001
DE kcal/kg	4261	4407	4559	4516	4124	4047	28.7	0.0187	0.44	<.0001	<.0001	<.0001
ME, kcal/kg	4014	4112	4274	4230	3950	3797	26.5	0.14	0.32	<.0001	<.0001	<.0001

[1]SEM - standard error of the mean. DM - dry matter. OM - Organic matter. AHF - acid hydrolyzed fat; CP - crude protein; NFE. nitrogen-free extractive; DE. digestible energy; ME. metabolizable energy; CO – Control diet; CAS500-7.5 – Cassava fiber ground 500 µm included at 7.5%; CAS500-30 – Cassava fiber ground 500 µm included at 30%; CAS180-7.5 – Cassava fiber ground 180 µm included at 7.5%; BP7.5 – Beet pulp included at 7.5%; BP30 – Beet pulp included at 30%.

Despite of the differences described, all fecal scores remained in a desirable range, suggesting a good proportion between insoluble and soluble in the experimental diets.

Table 3– Fecal score, number of defecations per day, fecal dry matter content and content of feces as is of dogs fed the experimental diets.

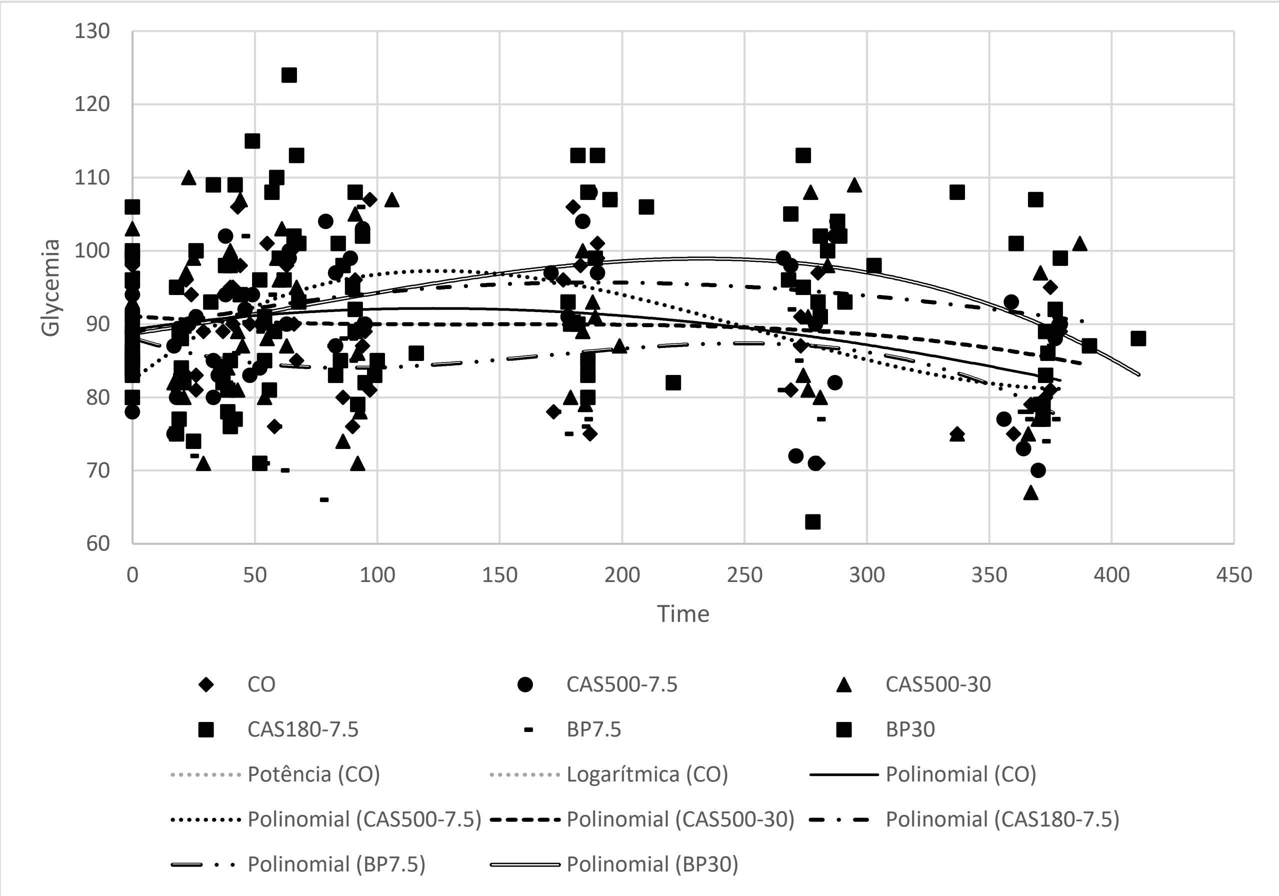
Fiber inclusion	0	7.50%			30%			Constrasts				
Source	CO	CAS500	CAS180	BP	CAS500	BP	SE	CO vs diets	CAS500 vs BP	CAS500 vs CAS180	CAS500 7.5% vs CAS500 30%	BP 7.5% vs BP 30%
Fecal score[1]	2.74	2.55	2.50	2.57	2.21	2.06	0.09	0.0012	0.993	0.0329	0.0009	0.013
Number of defecations per day	1.25	1.31	1.42	1.22	1.85	1.69	0.09	0.02	0.45	0.47	0.0081	0.0002
Feces, dry matter, grams per day	19.3	28.2	25.4	27.5	43.7	45.7	2.1	0.45	0.44	0.78	0.40	0.73
Feces, as is, grams per day	60.2	84.9	80.0	84.0	132	146	5.1	<.0001	<.0001	<.0001	<.0001	<.0001

CO – Control diet; CAS500-7.5 – Cassava fiber ground 500 µm included at 7.5%; CAS500-30 – Cassava fiber ground 500 µm included at 30%; CAS180-7.5 – Cassava fiber ground 180 µm included at 7.5%; BP7.5 – Beet pulp included at 7.5%; BP30 - Beet pulp included at 30%.
DM – Dry matter
[1] Scored as: 1 = very hard and dry stool. 2 = hard. dry. firm stool. 3 = soft. moist stool. well formed. 4 = soft and shapeless stool. 5 = liquid stool. diarrhea.

Experiment 2 – Glycemia index

The higher minimum glycemia was observed on CO diet by the time of 165 to 255 minutes after meal (Graphic 1). Lower particle size did not affect AUC and average glycemia, when compared to CO and presented the largest largest AUC in every time lapses (Table 4), with significant higher values of AUC 75 to 165 minutes after meal (Graphic 2).

Graphic 1 - Glycemic curve over 345 minutes after meal in beagle dogs.



CO – Control diet. CAS500-7.5 – Cassava fiber ground 500 µm included at 7.5%. CAS500-30 – Cassava fiber ground 500 µm included at 30%. CAS180-7.5 – Cassava fiber ground 180 µm included at 7.5%; BP7.5 – Beet pulp included at 7.5%; BP30 – Beet pulp included at 30%.

CAS500/30 presented the smallest AUC and average glycemia between all diets, like previous studies using fermentable fiber (Massimino *et al.* 1998; Deng *et al*. 2013). We observed lower AUC in several time lapses, comparing CAS500/30 to CAS500/7.5, mostly (Graphics 2). We could attribute to the higher fiber inclusion, but we did not see similar patter when comparing

BP/7.5 to BP/30 (Table 5). Kimmel *et al.* (2000) and Nelson *et al.*; (2000) observed lower mean, maximum blood glucose concentrations and AUC in dogs fed the high insoluble fiber. Monti *et al.* (2016) concluded that the glycemic curve is influenced mainly by the starch content of the diet, instead of fiber inclusion on dogs.

Table 4 - Values of glycemic curve and time of meal consumption on dogs.

Fiber inclusion	0	7.5%			30%			Constrasts				
Source	CO	CAS500	CAS180	BP	CAS500	BP	SE	CO vs diets	CAS500 vs BP	CAS500 vs CAS180	CAS500 7.5% vs CAS500 30%	BP 7.5% vs BP 30%
								P-value				
Average glicemya, mg/dL	92	90.5	92.5	86.2	89.7	87.3	1.87	0.07	0.085	0.04	0.10	0.12
Maximum glycemia, mg/dL	104	102	105	97.2	103	98.6	2.62	0.22	0.39	0.09	0.29	0.10
Point of maximum glycemia	4.76	5.50	5.20	3.10	5.51	3.84	0.74	0.65	0.10	0.56	0.10	0.23
Minimum glycemia, mg/dL	79.2	76.5	75.6	75.8	77.6	75.6	2.07	0.09	0.58	0.82	0.69	0.48
Point of minimum glycemia	6.47	5.76	3.91	4.48	4.05	4.38	0.94	0.05	0.62	0.30	0.30	0.75
Meal intake, min	4.22	2.65	2.46	2.38	5.50	5.96	0.76	0.56	0.68	0.04	0.003	0.01
Voluntary water intake, ml	486	534	395	428	371	382	39.6	0.11	0.40	0.21	0.006	0.4

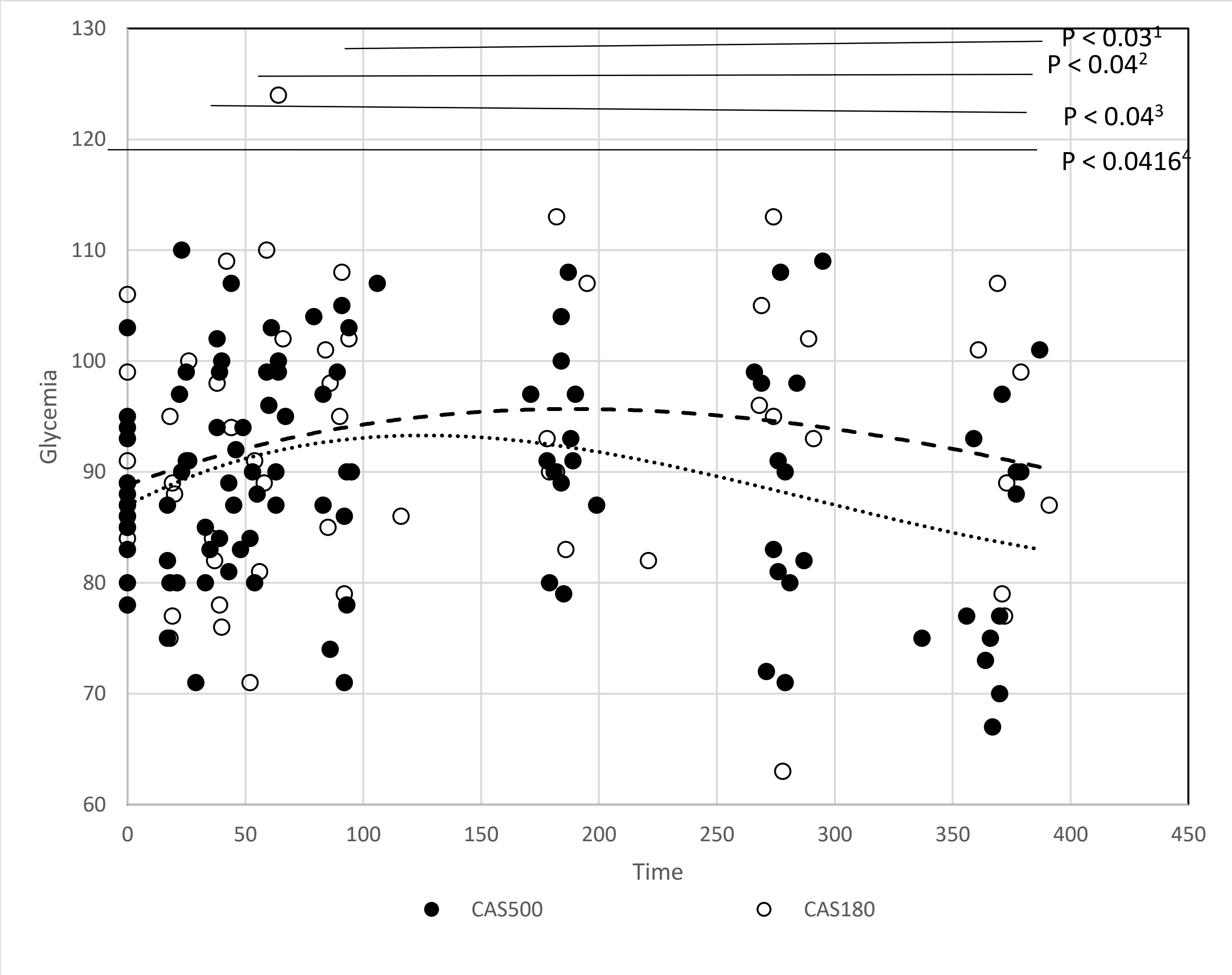
CO – Control diet; CAS500 – Cassava fiber ground 500 µm inclusion; CAS180 – Cassava fiber ground 180 µm inclusion; BP – Beet pulp + cane fiber inclusion

Table 5– Area under the curve without basal glycaemic area of dogs fed the experimental diets.

Fiber inclusion	0	7.5%			30%			Constrasts				
Source	CO	CAS500	CAS180	BP	BP	CAS	SE	CO vs diets	CAS500 vs BP	CAS500 vs CAS180	CAS5007.5% vs CAS500 30%	BP 7.5% vs BP 30%
Time lapse. min								P-value				
AT 0-345	33859	33673	34937	32035	33759	31862	33354	0.4836	0.412	0.0416	0.126	0.1874
AT 15-345	31878	31653	32800	30498	31229	29873	1041	0.42	0.40	0.0439	0.1139	0.55
AT 30-345	30246	30053	30937	28689	29783	28248	1034	0.40	0.466	0.08	0.12	0.38
AT 45-345	28211	28300	29417	27102	28306	26303	983	0.70	0.66	0.04	0.08	0.34
AT 75-345	25054	25410	26514	24320	25943	22630	1003	0.92	0.73	0.03	0.41	0.26
AT 45-165	20148	20865	20324	19291	20406	19447	725	0.89	0.45	0.80	0.08	0.2
AT 75-165	17175	17899	17469	16588	17903	15658	628	0.90	0.61	0.29	0.0062	0.12
AD 45-75	6969	8186	7942	7520	6771	8066	648	0.28	0.12	0.80	0.89	0.44
AD 15-345	18526	19642	21065	19871	17995	18523	1437	0.57	0.57	0.25	0.62	0.39
AD 45-255	20148	20865	20324	19291	20406	19447	725	0.89	0.45	0.80	0.08	0.20
AD 45-345	19170	20331	21500	20023	19524	18842	1158	0.48	0.68	0.177	0.37	0.77
AD 75-165	17175	17899	17469	16588	17903	15658	628	0.90	0.61	0.29	0.0062	0.12
AD 75-345	18359	19558	20443	19102	19214	17018	1078	0.55	0.88	0.1126	0.1196	0.95

CO – Control diet. CAS500-7.5 – Cassava fiber ground 500 µm included at 7.5%. CAS500-30 – Cassava fiber ground 500 µm included at 30%. CAS180-7.5 – Cassava fiber ground 180 µm included at 7.5%. BP7.5 – Beet pulp included at 7.5%. BP30 – Beet pulp included at 30%. AT – Total area AD – Total area excluding basal area

Graphics 2- Glycemic curve over 345 minutes after meal in beagle dogs fed cassava fiber in two different granulometries.



CAS180 – Cassava fiber ground 180 µm included at 30%; CAS500-7.5 – Cassava fiber ground 500 µm included at 7.5%.

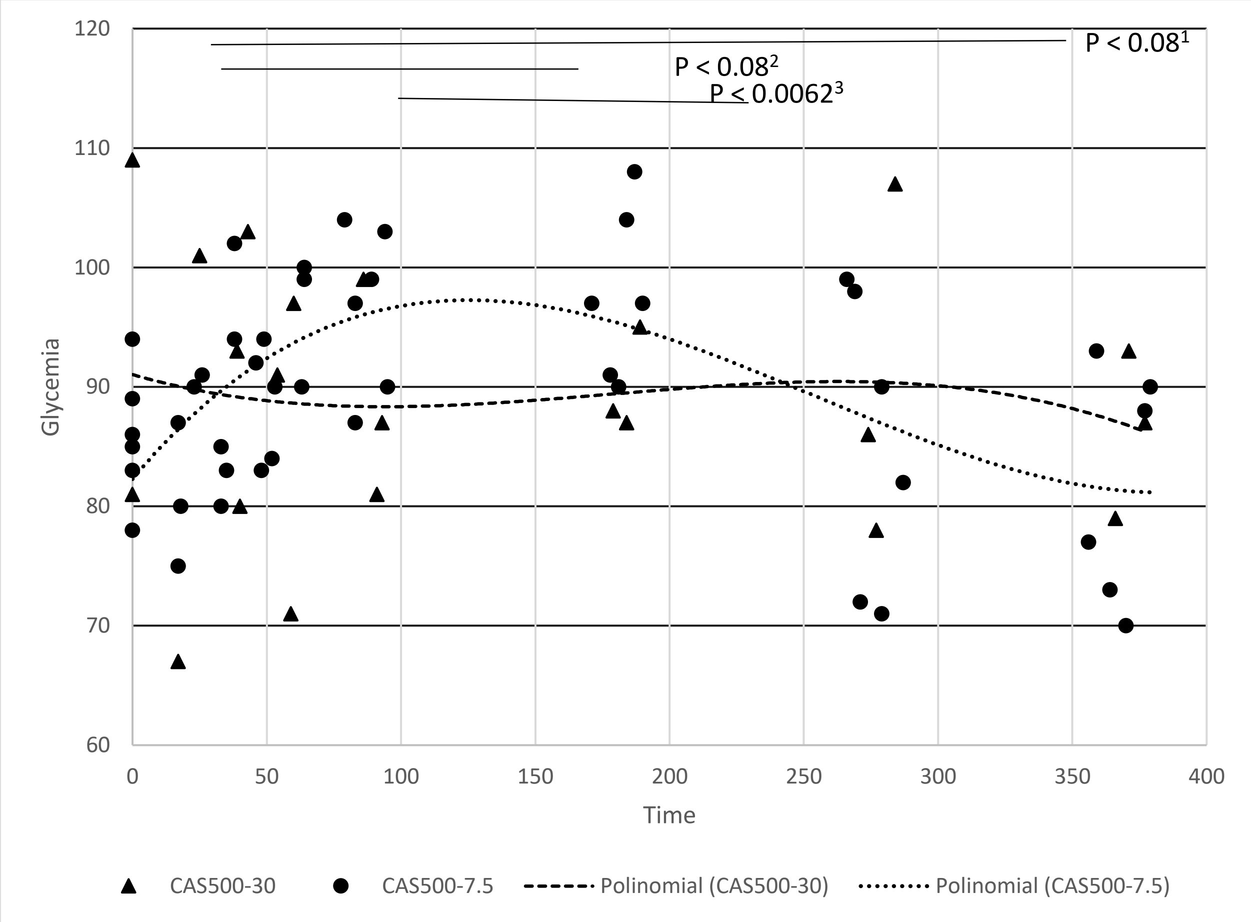
[1] Total area in 75-345 minutes.

[2] Total area in 45-345 minutes.

[3] Total area in 15-345minutes.

[4] Total area in 0-345minutes.

Graphic 3 - Glycemic curve over 345 minutes after meal in beagle dogs fed cassava fiber in two different inclusions.



CAS500-30 – Cassava fiber ground 500 µm included at 30%; CAS500-7.5 – Cassava fiber ground 500 µm included at 7.5%.
Significance P<0.05 Tendency P<0.1
[1] Total area in 45-345 minutes
[2] Total area in 45-165 minutes
[3] Total area in 75-165minutes

Experiment 3 - Time for meal consumption and water intake

The dogs ate all meals as soon as the food was offered. Time for dogs to eat the entire meal was expected to be longer on diets with higher particle size of fiber and 30% inclusion of fiber, because of the high volume of diets. This expectation was indeed observed on dogs who ate both BP30 and CAS500/30. Surprisingly, the shortest time was not recorded on diet with no fiber inclusion. This result could be explained by secondary effects of fibers on palatability. The study by Souza *et al*. (2022), verified positive effect on palatability in 12% CA inclusion diets for dogs. Dogs fed fine particle size of CAS ate faster ($P < 0.05$) when compared to CAS ground 500 µm.

There was no difference in voluntary water intake between CO and other diets. Voluntary water intake recorded up to 6 hours after meal was lower on CA500/30 compared to CAS500/7.5 and BP sources (Table 4).

We know that water balance is a regulated by the central nervous system, kidneys and hormones and behavior situations, as pain and stress could affect water intake in dogs (McGrotty & Randell, 2019). We recorded higher water intake on dogs fed diet with no fiber inclusion ($P < 0.05$) and we assumed it could be due to the low humidity on diet, like observed by Ramsay & Thrasher (1991), but we did not see a proportional correlation between DM and water intake in other observations. Even we could not see statistical significance, we couldn't help noticing that the lowest volume intakes were observed on 30% fiber inclusion diets (Table 4). We wonder if water intake could be related to gastric distention and satiety, like it has been described in humans (Camps *et al.,* 2018; Marciani *et al.*, 2012).

Conclusion

The findings in this study demonstrate that cassava fiber is an alternative to beet pulp and sugar cane fiber mix, containing a good proportion of insoluble and soluble fiber. The CAS 500/7.5 impaired digestibility of nutrients and energy, except on crude protein – desirable characteristics for weight reduction diets. The inclusion of 30% (9.6% of total dietary fiber) decreases area under the glycemic curve in the postprandial period and increases the time spent by the dogs to eat a full meal - conditions that could affect satiety, however unknown. Fine grounding of CAS also impairs digestibility in a mild way, produces lower feces content and presents higher glycemic index when compared to coarse grounding.

The proportion of insoluble/soluble fiber in a range of 1.6:1 to 8.7:1 in the experimental diets of this experiment leads to desirable fecal scores.

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CAPÍTULO III

CONSIDERAÇÕES FINAIS

Em estudos com fibras, a caracterização do ingrediente é fundamental para a formulação de dietas com mais precisão. Quando se trata de fibras, a caracterização é difícil, por haver diferentes métodos analíticos e por cada um destes métodos apresentarem erros inerentes à técnica.

Para este estudo as fontes de fibras utilizadas foram amostradas e enviadas a um laboratório para a análise de Weende e fibra dietética total com separação da porção solúvel e insolúvel. Os resultados iniciais demostraram não haver fibra solúvel na polpa de beterraba e nem na fibra de mandioca. Diante da surpresa, as dietas foram reanalisadas, no mesmo laboratório, mas os resultados tiveram uma pequena diferença com a presença de uma pequena porção solúvel, inclusive na polpa de beterraba. As mesmas amostras seguiram para outro laboratório para análise confirmatória. No entanto, as fibras solúveis esperadas apareceram nas análises de FDT, FDN e FDA. Com os ingredientes analisados, as dietas foram formuladas. As dietas com fibra de mandioca foram formuladas com inclusão de 7,5% e 30%. Essa grande amplitude foi estabelecida para que as diferenças entre os tratamentos fossem mais evidentes. A fibra de mandioca é produzida do córtex planta e o conteúdo de amido é variável - cerca de 50% na matéria seca. Para estabelecer teor similar entre as dietas optamos por acrescentar amido de milho às dietas controle. As dietas à base de polpa de beterraba e celulose serviram como dietas controle para as dietas contendo fibra de mandioca. Foram necessários atenção e tempo dispendido para a formulação e fabricação das seis dietas. Foi preciso encontrar os ingredientes específicos, de origem confiável, e em quantidade suficiente para a fabricação. O objetivo da formulação era que as dietas ficassem muito semelhantes em termos de nutrientes, inclusive na proporção de fibra solúvel e insolúvel, o que se tornou o processo dificultoso, devido a composição das diferentes fontes de fibra, incluindo a fibra de mandioca, ainda pouco estudada. A mistura dos ingredientes foi realizada no Laboratório de Ensino Zootécnico e então transportada à fábrica, localizada na cidade de Barra do Ribeiro, para o processo de extrusão.

Os cães permaneceram saudáveis durante o estudo. Nos períodos pré digestibilidade, foram necessários ajustes para a manutenção do peso de alguns animais. Uma das fêmeas apresentava forte tendência para ganho de peso, com ausência de alterações laboratoriais que indicassem patologias endócrinas. Outro fator que influenciou nestes ajustes, foi o fato de o estudo ter sido realizado no inverno, o que facilitava a perda de peso dos cães. Outra cadela apresentou dificuldade em ganhar peso, sem alterações laboratoriais condizentes com alguma patologia. Quando designada a uma dieta com maior inclusão de fibra (30%), aliado a um fator alto utilizado no cálculo de necessidade energética (220) e ao fato de que os cães comiam somente uma vez ao dia, a canina apresentou dificuldade em ingerir a refeição inteira e foi necessário ajuste para o fator 200.

Foram programados os dois seguintes estudos, além dos aqui descritos: um estudo da dosagem sérica de paracetamol para avaliar esvaziamento gástrico pós prandial em cães e a dosagem de hormônios gastrointestinais pré e pós prandiais. O estudo de esvaziamento gástrico foi realizado e encontrava-se em etapa de análise laboratorial no momento da entrega da dissertação. A coleta para dosagem hormonal foi realizada no primeiro período do experimento. Contudo, houve hemólise no contato do soro com o coquetel inibidor de proteases, comprometendo a amostra. Sugere-se que a hemólise tenha ocorrido em função da presença de DSMO no coquetel e foi sugerido pela empresa fornecedora que usássemos um produto liofilizado como substituição (além de outros dois coquetéis inibidores). Devido à grande demora no processo de importação dos coquetéis, o estudo hormonal não foi concluído a tempo e será realizado após a entrega desta dissertação.

A condução das séries experimentais sucedeu-se de forma criteriosa. No dia de coletas seriadas de sangue, grande parte da equipe foi demandada, devido aos diversos fatores que necessitavam maior controle e acompanhamento.

A fibra de mandioca parece ser promissora como fonte alternativa de fibras na alimentação animal, por apresentar características solúveis e insolúveis em proporções interessantes, além da grande disponibilidade da fonte em diversas regiões do mundo.

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APÊNDICE A - Carta de aprovação do Comitê de ética no uso de animais

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA
Comissão De Ética No Uso De Animais

CEUA

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 41825
Título: AVALIACAO DE DIFERENTES ADITIVOS ALIMENTARES SOBRE A SAUDE INTESTINAL E QUALIDADE FECAL DE CAES

Vigência: 01/03/2022 à 31/12/2024

Pesquisadores:

Equipe UFRGS:

LUCIANO TREVIZAN - coordenador desde 01/03/2022
Camila Figueiredo Carneiro Monteiro - desde 01/03/2022
JÉSSICA FERREIRA BARCELLOS - desde 01/03/2022
GIOVANE KREBS - desde 01/03/2022
Caroline Fredrich Dourado Pinto - desde 01/03/2022
JÉSSICA NETO D'AVILA - zzz Outra Função zzz desde 01/03/2022
BIANCA BRUM DE OLIVEIRA - Auxiliar de Biotério desde 01/03/2022
MATHEUS NUNES PERES - zzz Outra Função zzz desde 01/03/2022
ARIANE MIRANDA DA SILVA - zzz Outra Função zzz desde 01/03/2022
ALESSANDRA LATOSINSKI ARAUJO - zzz Outra Função zzz desde 01/03/2022

Comissão De Ética No Uso De Animais aprovou o mesmo em seus aspectos éticos e metodológicos, para a utilização de 14 cães da raça Beagle, provenientes do canil comercial Champions Line Beagle (CNPJ: 304.795.330-91), Porto Alegre/RS, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Sexta-Feira, 24 de Junho de 2022

Maitê de M. Vieira

MAITE DE MORAES VIEIRA
Coordenador da comissão de ética

APÊNDICE B – Normas para redigir o capítulo II – Publicação no periódico Journal of Animal Science

Instructions to Authors

Journal of Animal Science (JAS) publishes original research articles and invited review articles.

The mission of the American Society of Animal Science (ASAS) is to foster communication and collaboration among individuals and organizations associated with animal science research, education, industry, or administration "To discover, disseminate, and apply knowledge for sustainable use of animals for food and other human needs". The *Journal of Animal Science* (*JAS*), which is published by ASAS, accepts manuscripts presenting information for publication with this mission in mind. Its editorial policies are established by the editor-in-chief, managing editor, section editors, and editorial board, subject to review by the publications committee, board of directors, and the membership of ASAS. Views expressed in papers published in *JAS* represent the opinions of the author(s) and do not necessarily reflect the official policy of the institution with which the author is affiliated, ASAS, or the editor-in-chief.

The *JAS* is one of the most frequently cited peer-reviewed, agriculturally oriented research journals in the world, based on statistics published by ISI, Inc. (Philadelphia, PA). Its high impact factor attests to the quality standards maintained by the *JAS* editorial board and by authors who submit manuscripts for publication.

Authors retain the right to make an Author's Original Version (preprint) available through various channels, and this does not prevent submission to the journal, however, preprint servers where papers are formally reviewed and a decision is issued will be considered a previous publication and will not be accepted for review.

Manuscript Preparation (Style and Form)

General

All manuscripts submitted to the Journal must be double-spaced, 12-point Times New Roman font with 1 inch margin all around. Consecutive line and page numbers are required. Greek letters and special symbol are inserted using the symbol palette. Math equations are created with MathType or LaTex.

The layout of the Journal is compatible with the OUP LaTeX template. More information can be found here.

Title Page

Required items on the page are,

1. Running title: short, succinct title no more than 45 keystrokes (characters plus spaces) in length with first and proper nouns capitalized
2. A title with the first word and proper nouns capitalized. Species of subject is encouraged. The title should be unique. The Journal does NOT support multipart series.
3. Full names (given name, middle initial, family name) of all authors
4. Institutions of the authors with location denoted with a symbol (*, †, ‡, §, #,||, and ¶) behind the author last name
5. Department, city, state, country, and postal code (Please note: the country must be listed for each affiliation)
6. Acknowledgements of consortia, grants, experiment station, or journal series number are given as a numerical footnote to the title

Lay Summary

Authors are required to submit a Lay Summary as part of the article, in addition to the main text abstract. The Lay Summary should clearly summarize the focus and findings of the article for non-expert readers, and will be published as part of the article online and in PDF. The Lay Summary follows the Title Page in the main manuscript file and is limited to 200 words. Authors should avoid technical and discipline-specific abbreviations and language whenever possible to engage a larger non-scientific audience. Abbreviations are defined at first use.

Teaser Text

Authors are required to provide 1-2 sentence(s) clearly describing the impact of the research described in the manuscript. Teaser text is used in the Journal table of contents to entice readers. It follows the Lay Summary in the main document file.

Abstract

A single paragraph of no more than 2,500 keystrokes (characters plus spaces) that summarizes the results in an understandable form using statistical evidence (P-values). Abbreviations are defined at first use in the ABSTRACT and again in the body of the manuscript.

Key words

List up to 6 words in alphabetical order and separated by a comma. Capitalize only proper nouns. Do NOT use abbreviations. Place at the end of the ABSTRACT.

List of Abbreviations

A comprehensive list of all abbreviations used in the manuscript and their definition. An example format is MRF, myogenic regulatory factor. The List should not contain standard JAS Abbreviations, diets or treatment descriptions. Abbreviations must be defined at first use in the manuscript text but not in tables and figures unless unique.

Download an MS Excel spreadsheet of *JAS* standard abbreviations.

Plural abbreviations do not contain a final "s" because the context of an abbreviation implies whether it is singular or plural. Use of the standard 3-letter abbreviations for amino acids (e.g., Ala) is acceptable in *JAS*. Use of the internationally recognized chemical symbols for chemical elements (e.g., P and S) is acceptable in *JAS*. Except for N (not italicized), which is the recognized abbreviation for nitrogen and newton (unit of force), chemical symbols for elements are reserved for elements (e.g., C is for carbon and never for control).

Introduction

A clear justification for conducting the research with a stated hypothesis and objective(s) is required. The rationale for the experiments should place the work into the context of existing literature. There is NO word limit on the section but brevity is encouraged.

Materials and Methods

The American Society of Animal Science (ASAS) supports rigor, reproducibility and transparency in science and seeks to ensure that publications of the society reflect these values while also minimizing the burden on authors in preparation of scientific results for publication. There are many available resources describing principles and practices to enhance rigor, reproducibility, and transparency in science. Authors considering the Journal of Animal Science are encouraged to consult these resources when during preparation of their submissions.

The manuscript must include a statement of institutional animal care and use committee (IACUC), or country-specific equivalent, approval of all animal procedures. The *IACUC statement should appear as the first item in MATERIALS AND METHODS* and should specify which publicly available animal care and use standards were followed. A clear description of all biological, analytical and statistical procedures is required with each section denoted by a short descriptive title (i.e., Animals and sampling, Western blot, Immunocytochemistry, Experimental design and analysis, etc). Materials used must include the product name and vendor at first mention. When a commercial product is used as part of an experiment, the manufacturer name and location must be given parenthetically and the generic name should be used subsequently. No ™, ®, or © symbols should be used. Sex, breed, age, species are included in the animal descriptions. Provide evidence of assay validation, or suitable published reference, as well as inter/intra-assay CV, as needed. Appropriate statistical methods should be used with experimental unit defined. Numbers of biological and experimental replicates should be stated. State the threshold for significance ($P < 0.05$) and definition of tendency if used.

Results

Experimental results are presented in tables and figures. The results should contain sufficient detail to allow the reader to interpret the data. Quantitative measures of significance (*P*-values) should be presented. Authors may use either absolute *P*-values or a defined significance level as long as usage is consistent.

Discussion

The section contains the interpretation of the results. It should be clear and concise, address the biological mechanisms and their significance, and integrate the results into existing literature. The Discussion may offer an interpretation that is consistent with the data. Do NOT include any reference to tables and figures or include *P*-values in the Discussion. Authors have the option to create a single RESULTS AND DISCUSSION section.

Disclosures

All JAS editors, ASAS staff, ASAS Board of Directors, and submitting authors must disclose any actual or potential conflicts of interest that may affect their ability to objectively present or review research or data. A succinct statement detailing any perceived conflict of interest is required. If none, please indicate as such.

Literature Cited

Papers in the section must be published or 'in press'. All references must include the DOI, if available. Authors are encouraged to use the most recent reference style for the Journal of Animal Science in the reference management software of their choice. The format for references are

Journal articles

Perez, V. G., A. M. Waguespark, T. D. Bidner, L. L. Southern, T. M. Fakler, T. L. Ward, M. Steidinger, and J. E. Pettigrew. 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiotia of pigs after weaning. J. Anim. Sci. 89:414–425. doi:10.2527/jas.2010-2839.

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Tables and Figures

Tables and figures should be placed at the end of the manuscript, following the references. Tables and figures should be numbered consecutively, in the order in which they are cited in the manuscript. Tables and figures must be prepared so they can be understood without referring to information in the body of the manuscript.

Tables

1. Tables can be created in in Word using the Table function.
2. All tables should be editable in the manuscript file.
3. Each table should be placed on a separate page. Tables should fit on a single 8.5 X 11-inch page in either landscape or portrait view.
4. Every column should include a heading.
5. Align column values to the decimal point whenever possible. Columns containing a mix of values, symbols and words may be aligned to the center of the heading. Columns using ± should be aligned to the symbol.
6. Units (e.g., kg) are separated from descriptor by a comma.
7. Numerals are used to reference footnotes. Each footnote should begin on a new line immediately below the table.

8. Lowercase, superscript letters are used to indicate significant differences among means within a row or column and to reference footnotes explaining how to interpret the letters.
9. The order of footnotes below the table is numbers first followed by letters and special symbols.
10. If reporting significance, the column heading is P-value.

Figures

1. Figures should be submitted as JPEG, TIFF, or EPS files only.
2. Figures must be high-resolution with a minimum resolution of 300 dpi and a maximum resolution of 600 dpi.
3. All figures must have a title and legend. The legend should be a brief description that allows the reader to interpret the results.
4. Axes descriptors are separated from units (i.e., kg, mm, mL) by a comma. Do NOT place units within parentheses.
5. Use Times New Roman font no smaller than 8 point following figure reduction.

Supplemental Material

Authors may include supplemental tables, figures, or other forms of supplemental material (e.g., detailed data sets, Excel files, videos). Supplemental materials should be included in a separate file.

Supplemental materials must undergo peer review and, thus, should be in a format that is easily accessible (i.e., does not require dedicated software or software that is not generally available) to most reviewers and readers.

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Quantitative Trait Loci and DNA Markers, Microarray and RNA Sequencing Data

Authors of papers that contain original quantitative trait loci (QTL) or DNA marker association results for livestock are strongly encouraged to make their data available in an electronic form to one of the publicly available livestock QTL databases after the manuscript appears on the JAS Advance Articles website (https://academic.oup.com/jas/advance-articles). Similarly, for microarray data and RNA sequencing data, authors are encouraged to submit a complete dataset to an appropriate database.

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The use of names of commercial products should be minimized. When a commercial product is used as part of an experiment, the manufacturer name and location (city and state if in the US; city, administrative region or district [e.g., province], and country if outside the US) or a website address must be given parenthetically at first mention in text, tables, and figures. The generic name should be used subsequently. No ™, ®, or © symbols should be used.

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The mission of the American Society of Animal Science (ASAS) is to “foster the discovery, sharing, and application of scientific knowledge concerning the responsible use of animals to enhance human life and wellbeing” (see ASAS's History and Mission).

The *Journal of Animal Science*, which is published by ASAS, accepts manuscripts presenting information for publication with this mission in mind.

The Editor-in-Chief, Managing Editor, and Section Editors establish the editorial policies of *JAS*, subject to review by the publications committee and ASAS Board of Directors. The views expressed in articles published in *JAS* represent the opinions of the author(s) and do not necessarily reflect the official policy of the institution with which an author is affiliated, the ASAS, or the *JAS* Editor-in-Chief. Authors are responsible for ensuring the accuracy of collection, analysis, and interpretation of data in manuscripts and ultimately for guaranteeing the veracity of the contents of articles published in *JAS*.

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- For American English spelling and usage, consult Merriam-Webster Online.
- For SI units, the National Institute of Standards and Technology provides a comprehensive guide.
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- The hierarchy for brackets and parentheses is [( )]. For example, [(2 + 3) × (12 ÷ 2)] × 2 = 60.
- Meat shear force should be expressed in kilograms (kg), although newtons (N) may also be acceptable.
- Report time using the 24-h system (e.g., 1410 h rather than 2:10 p.m.).
- Use italics to designate genus and species.
- Names of muscles are not italicized.
- Specify the basis (i.e., as-fed or dry matter) for dietary ingredient and chemical composition data listed in text or in tables. Similarly, specify the basis for tissue composition data (e.g., wet or dry basis).
- Calculations of efficiency should be expressed as output divided by input (i.e., gain:feed, not feed:gain).

- A diet is a feedstuff or a mixture of feedstuffs; a ration is the daily allotment of the diet.
- The word "Table" is capitalized and never abbreviated.
- Except to begin a sentence, the word "Figure" should be abbreviated to "Fig."
- Except to begin a sentence, experiment and equation should be abbreviated to Exp. And Eq., respectively, when preceding a numeral (e.g., Exp. 1).
- Avoid jargon unfamiliar to scientists from other disciplines. Do not use the term "head" to refer to an animal or group of animals. Instead, use animal, sow, ewe, steer, heifer, cattle, etc.
- Avoid bi- as a prefix because of its ambiguity; biweekly means twice per week and once every 2 weeks.
- Breed and variety names should be capitalized (e.g., Landrace and Hereford).
- Trademarked or registered names should be capitalized, but no ™ or ® symbols should be used.

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For information on the scientific content of the journal, contact the Editor-in-Chief, Dr. Elisabeth Huff Lonergan, American Society of Animal Science, P.O. Box 7410, Champaign, Illinois 61826-7410; e-mail: elonerga@iastate.edu.

For questions about submitting a manuscript and ScholarOne Manuscripts, contact Bailey Hanna; e-mail: jas.editorialoffice@jjeditorial.com.

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All authors submitting to *JAS* must complete the Care and Use of Animals form certifying that any research that involves animals has followed established standards for the humane care and use of animals and must specify which standards were used. Only investigations that have followed high standards for the humane care and use of animals in research will be reported in *JAS*. Also, the manuscript must include a statement of institutional animal care and use committee (IACUC), or country-specific equivalent, approval of all animal procedures. The IACUC statement should appear as the first item in MATERIALS AND METHODS and should specify which publically available animal care and use standards were followed (e.g., ADSA-ASASPSA Guide for Care and Use of Agricultural Animals in Research and Teaching; Primary Industries Ministerial Council, Model code of practice for the welfare of animals: the sheep). The manuscript should describe anesthetics, analgesics, tranquilizers, and care taken to minimize pain and discomfort during preoperative, operative, and postoperative procedures. If research requires discomfort to the animals or stressful conditions, justification for these conditions must be evident in papers published in *JAS*.

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In the United States, federally funded or regulated research involving human subjects must comply with Code of Federal Regulations (CFR), Title 45 Public Welfare, Part 46 Protection of Human Subjects. However, CFR 45 Part 46.101(b) exempts some research from these regulations. For all exempted research and other details, see this page. Exempted research includes that in which the only involvement of human subjects is for "taste and food quality evaluation and consumer acceptance if 1) wholesome foods without additives are consumed or 2) a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture." If human subjects were used in exempted research and the research was in compliance with CFR 45 Part 46, or equivalent regulations where the research was conducted, authors must state in MATERIALS AND METHODS or acknowledgements that they were in full compliance. If human subjects were used in research that was not exempted in CFR 45 Part 46, or equivalent regulations where the research was conducted, authors must certify that the research received prior approval from an appropriate Institutional Review Board.

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Results of research contained in manuscripts submitted to *JAS* must not have been published in or submitted to another peer reviewed scientific journal prior to receiving a decision from *JAS*. Previous presentation at a scientific meeting or the use of data in field-day reports or similar documents, including press publications or postings to personal or departmental websites, does not preclude the publication of such data in *JAS*.

Articles simultaneously posted to websites and submitted to *JAS* should carry a disclaimer on the website that this version of the paper has not undergone *JAS* peer review and is not to be considered the final published form of the article. If the article has been published in *JAS*, the author should include the complete *JAS* citation.

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Technical Notes. A technical note is used to report a new method, technique, or procedure of interest to *JAS* readers. When possible, a technical note should include a comparison of results from the new method with those from previous methods, using appropriate statistical tests. The advantages and disadvantages of the new procedure should be discussed. When typeset for publication, a technical note shall not exceed 10 pages (approximately 18 Microsoft Word document pages), including tables and figures. "Technical note:" shall be the first portion of the title of such manuscripts. The review process for a technical note will be the same as that for other manuscripts. Information that is more extensive or detailed than necessary for a Technical note may be presented in an e-supplement (see E-Supplements).

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Review Articles

The journal publishes invited review articles only.

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The Editor-in-Chief and Section Editors determine whether manuscripts are suitable for publication in JAS. All communications about a submitted manuscript should maintain confidentiality. Each manuscript will undergo closed scientific review. Manuscripts that are not written clearly, concisely, and coherently, or they are not consistent with guidelines in the current Instructions for Authors, Journal of Animal Science may be rejected without review. Authors whose first language is not English are urged to have an editing service review their manuscripts before they are submitted to *JAS*. For your convenience, JASEdits is available from ASAS.

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Appeals

If a manuscript is rejected, the decision may be appealed to the Editor-in- Chief if the author(s) believe(s) that the judgment was erroneous or biased. A letter presenting the reasons for the appeal should be sent to the Editor-in-Chief within 30 days of the date on the rejection notification. The Editor-in-Chief will decide whether to accept or deny the appeal.

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All revised manuscripts must be returned to Section Editors via *JAS* Scholar- One Manuscripts. Authors will be permitted 15 days to revise and return manuscripts classified as Minor Revision and permitted 35 days to revise and return manuscripts classified as Major Revision. In most cases manuscripts will not be allowed more than a single revision. Unsatisfactory or incomplete revisions will be a cause for rejection of the manuscript.

Manuscripts that exceed the revision-option deadline will be withdrawn. If withdrawn for lack of timely revision, they may be resubmitted for new review. Requests for extensions must be communicated to the Section Editor responsible for the manuscript before the revision-option expires.

Peer Review Policy

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VITA

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