UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

EFEITOS DA FIBRA DE LARANJA NA DIGESTIBILIDADE DOS NUTRIENTES, PRODUTOS DE FERMENTAÇÃO NAS FEZES E TEMPO DE TRÂNSITO GASTROINTESTINAL DE CÃES

Lara Mantovani Volpe

Médica Veterinária

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Orientador: Prof. Dr. Aulus Cavalieri Carciofi

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: AVALIAÇÃO DOS EFEITOS DA FIBRA DE LARANJA NA DIGESTIBILIDADE DOS NUTRIENTES, PRODUTOS DE FERMENTAÇÃO DE FEZES E TEMPO DE TRÂNSITO GASTROINTESTINAL DE CÃES

AUTORA: LARA MANTOVANI VOLPE
ORIENTADOR: AULUS CAVALIERI CARCIOFI

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Jaboticabal, 30 de setembro de 2020

DADOS CURRICULARES DO AUTOR

LARA MANTOVANI VOLPE - Nascida em 03 de abril de 1989, em Franca – SP, graduada em Medicina Veterinária pela Universidade Federal de Uberlândia (UFU) em março de 2014. Foi bolsista de iniciação científica nesta mesma instituição na área de nutrição de cães e gatos sob a supervisão da Profa. Janine França. Participou do programa de aprimoramento profissional (residência) em Nutrição e Nutrição Clínica de Cães e Gatos no Hospital Vetrinário Governador Laudo Natel, sob orientação do professor Dr. Aulus Cavalieri Carciofi, pela Faculdade de Ciências Agrárias e Veterinárias da UNESP - câmpus Jaboticabal (2018). Realizou estágio no Serviço de Nutrição Clínica de Cães e Gatos do hospital veterinário Red Bank Veterinary Hospital, Tinton Falls, Nova Jersey, Estados Unidos da América, supervisionada pela dra. Martha Cline e no Serviço de Nutrição Clínica de Cães e Gatos da Ontario Veterinary College, Universidade de Guelph, Canadá, supervisionado pela professora Dra. Adronie Verbrugghe. Atualmente faz mestrado em Medicina Vetrinária com ênfase em Nutrição de Cães e Gatos no Laboratório de Pesquisa em Nutrição e Doenças Nutricionais da Faculdade de Ciências Agrárias e Veterinárias – UNESP, câmpus de Jaboticabal, sob orientação do professor Dr. Aulus Cavalieri Carciofi (linha de pesquisa: fibras para cães); também atua como analista de treinamento técnico na empresa Adimax Pet, trabalhando na área de treinamento técnico e de produtos.

EPÍGRAFE

"Tão importante como a chegada é a caminhada, e não há caminho sem metamorfoses"

Adaptado de Kamila Behling

DEDICATÓRIA

Aos meus pais, Pedro e Débora e ao meu irmão, Breno, que nunca mediram esforços, amor e compreensão para a realização de mais este sonho.

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Por fim, agradeço à maravilhosa vida que eu tive durante os meus anos em Jaboticabal e presente na FCAV. Obrigada!

SUMÁRIO

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Avaliação dos efeitos da Fibra de Laranja no processo de extrusão, digestibilidade de nutrientes, produtos da fermentação das fezes e tempo de trânsito gastrointestinal de cães"**, protocolo nº **07365/19**, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 13 de junho de 2019.

Vigência do Projeto	14/06/2019 a 28/02/2020
Espécie / Linhagem	Caninos/ beagles
Nº de animais	50
Peso / Idade	09 - 16 kgs / 2 - 6 anos
Sexo	Machos e fêmeas
Origem	Laboratório de Nutrição de Cães e Gatos

Jaboticabal, 13 de junho de 2019.

Fabiana Pilarski

Prof.ª Dr.ª Fabiana Pilarski
Coordenadora – CEUA

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EFEITOS DA FIBRA DE LARANJA NA DIGESTIBILIDADE DOS NUTRIENTES, PRODUTOS DE FERMENTAÇÃO NAS FEZES E TEMPO DE TRÂNSITO GASTROINTESTINAL EM CÃES

RESUMO – Fibras são utilizadas em alimentos comerciais de cães para favorecer a saúde intestinal pelo fornecimento de substrato para melhor atividade metabólica da microbiota presente neste órgão. Fibras solúveis e fermentáveis podem apresentar melhor este efeito, sendo em geral mais caras e menos disponíveis comercialmente. O Brasil é o maior produtor mundial de laranja, de cuja fruta é possível se obter fibra com importante fração solúvel, com potencial de emprego em alimentos para cães. Esta dissertação apresenta dois capitulos, no primeiro é apresentado levantamento bibliográfico da fibra e seus efeitos na nutrição de cães, incluindo informações da fibra de laranja, ingrediente que foi objeto de estudo do segundo capítulo. O segundo capítulo reporta estudo que teve por objetivo comparar os efeitos de duas inclusões de fibra de laranja (1 e 3%), em comparação com alimento controle negativo (sem adição de fonte fibras) e dois controles positivos, representados pela inclusão de 3% de polpa de beterraba ou 1% de inulina purificada, totalizando cinco rações extrusadas para cães. Os alimentos foram testados em 40 cães adultos e a digestibilidade foi determinada pelo método de coleta total de fezes. Fezes recém eliminadas foram recolhidas para análises de pH e produtos de fermentação. O tempo de trânsito gastrointestinal foi determinado utilizando-se marcadores plásticos. O estudo seguiu delineamento em blocos casualizados, com 4 blocos de 10 cães, 2 cães por ração em cada bloco totalizando 8 cães por ração. Os resultados foram submetidos a análise de variância, quando diferenças foram obtidas no Teste F, médias foram comparadas pelo teste de Tukey ($P<0.05$). Foi possível identificar que as dietas com 1 e 3% fibra de laranja apresentaram aumento significativo na produção de ácidos graxos voláteis, ácidos graxos de cadeia curta totais, ácido acético e ácido butírico em comparação com controle ($P<0,05$). Fezes com maior teor de água foram dos animais que receberam o tratamento 3% Fibra de Laranja, e mesmo alimento foi o que apresentou maior consumo de FDT. Os tratamentos 3% Fibra de Laranja e 3% Polpa de Beterraba apresentaram menor digestibilidade de matéria seca, matéria orgânica e proteína bruta. Não houve diferença significativa no escore fecal e no tempo de trânsigo gastrointestinal entre os tratamentos. Os resultados quanto à digestibilidade de nutrientes, teor de água nas fezes e produção de ácidos graxos de cadeia curta (AGCC) nas fezes foram semelhantes aos obtidos por outros trabalhos que avaliaram os efeitos de fontes de fibras na alimentação de cães. A presente disseração concluiu que o uso de fibra de laranja aumenta a produção de AGCC, o teor de água nas fezes, se tratando, portanto, de uma boa fonte de fibras na a alimentação de cães.

Palavras-chave: nutrição, microbiota, prebiótico, ácidos graxos voláteis, atividade intestinal

ORANGE FIBER EFFECTS ON NUTRIENT DIGESTIBILITY, FERMENTATION PRODUCTS ON FECES AND DIGESTA MEAN RETENTION TIME IN DOGS

ABSTRACT – Fibers are used in commercial dog food to promote intestinal health by providing substrate for better metabolic activity of the microbiota present in this organ. Soluble and fermentable fibers can better present this effect and are generally more expensive and less commercially available. Brazil is the world's largest producer of oranges, from whose fruit it is possible to obtain fiber with an important soluble fraction, with potential for use in dog food. This dissertation presents two chapters, the first one presents a bibliographic survey of the fiber and its effects on nutrition of dogs, including information on orange fiber, an ingredient that was the object of study in the second chapter. The second chapter reports a study that aimed to compare the effects of two orange fiber inclusions (1 and 3%), in comparison with negative control food (without addition of fiber source) and two positive controls, represented by the inclusion of 3% of beet pulp or 1% purified inulin, totaling five extruded dog food. Food was tested on 40 adult dogs and digestibility was determined by the total feces collection method. Freshly eliminated feces were collected for pH analysis and fermentation products. Gastrointestinal transit time was determined using plastic markers. The study followed a randomized block design, with 4 blocks of 10 dogs, 2 dogs per feed in each block totaling 8 dogs per feed. The results were submitted to analysis of variance, when differences were obtained in Test F, means were compared by Tukey's test ($P < 0.05$). It was possible to identify that diets with 1 and 3% orange fiber showed a significant increase in the production of volatile fatty acids, total short-chain fatty acids, acetic acid and butyric acid compared to control ($P < 0.05$). Faeces with higher water content were from animals that received the 3% Orange Fiber treatment, and the same food was the one that presented the highest consumption of FDT. The 3% Orange Fiber and 3% Beet Pulp treatments showed less digestibility of dry matter, organic matter and crude protein. There was no significant difference in fecal score and gastrointestinal transit time between treatments. The results, regarding the digestibility of nutrients, water content in feces and production of short-chain fatty acids (AGCC) in feces were similar to those obtained by other studies that evaluated the effects of fiber sources on dog food. The present dissertation concluded that the use of orange fiber increases the production of AGCC and water content in feces, therefore, is a good source of fibers in the feeding of dogs.

Keywords: nutrition, microbiota, prebiotic, volatile fatty acids, intestinal activity.

LISTA DE ABREVIATURAS

AHF: Acid Hydrolysis Fat
BCFA: Branched chain fatty acids
BP: Beet pulp (treatment)
CF: Crude fiber
CO: Control (treatment)
CP: Crude protein
DM: Dry matter
DMRT: Digesta mean retention time
FOS: fructooligosaccharide
GE: Gross energy
IN: Inulin (treatment)
MRR: Marker recovery rate
NNE: non-nitrogen extract
OF: Orange fiber
OF1: 1% Orange fiber (treatment)
OF3: 3% Orange fiber (treatment)
OM: Organic matter
SCFA: Short chain fatty acids
TDF: Total dietary fiber
TIF: Total insoluble fiber
TSF: Total soluble fiber

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CAPÍTULO 1

CONSIDERAÇÕES GERAIS

1. **INTRODUCTION**

Fibers are commonly used in commercial dry dog and cat foods due to their several import physiological functions such as improvement of gastrointestinal health ad function (MARIA et al., 2017), stool consistency (DE GODOY et al., 2013; LOUREIRO et al., 2017), alterations on digesta mean retention time (LOUREIRO et al., 2017), reduction on nutrient digestibility and the energy value of the food (KAWAUCHI et al., 2011), promotion of satiety and hairball control in cats (LOUREIRO e al. 2014).

Due to these functions, fibers are widely used as adjuvants in the treatment of gastrointestinal disorders. To support gastrointestinal health, soluble and fermentable fibers may be more adequate. Soluble fiber may me more fermentable, supplying more energy to saccharolytic microbiota inducing higher production of several fermentation products that are relevant to gut health. Among them higher butyrate and acetate may result in better nutrient and energy supply to colonocytes and enterocytes, allowing better development of the intestinal mucosa, reducing inflammation and increasing local and systemic immunity (ASWHWAR et al., 2016; BIRD et al., 2007; BIRT et al., 2013; BROUNS et al., 2002; de SOUZA THEODORO, 2019; MARIA, 2017; PEIXOTO et al., 2018; SCHEPPACH et al., 1994; ZAMAN & SARBINI et al., 2016).

Brazil is the biggest orange producer of the world, yielding in 2017 approximately 17,459,809 tons of this fruit (IBGE, 2018). Great part of this production is intended to orange juice manufacture for exportation. In 2018, approximately 1,083,032 tons of orange juice were exported, raising a total of around 2 billion dollars to the country (CITRUS-BR, 2018). In the context of the modern agriculture, sustainable disposal of co-products are very relevant, so along the years several uses of the citrus pulp, generates after juice is squeezed, was developed, standing out the production of the citrus pulp pellet, largely used as feedstuff for cattle where it can contribute to a reduction on production costs (BAMPIDIS & ROBINSON, 2006), Recently, a special co-product rich in soluble fiber was developed from the orange bagasse: the orange fiber. This product was preliminary tested in one PhD Thesis of our laboratory, with promising results. The mechanism for obtaining orange fiber and its composition will be discussed over the next chapters.

Soluble and fermentable fiber sources usually are expensive. Companies may use the strategy to include in formulations co-products with some proportion of soluble fibers. Nowadays the most common ingredient used for this purpose is beet pulp, with approximately 24% of soluble fibers (CALABRÒ et al., 2013). Purified sources are also used, including several gums like psyllium gum, as well as many prebiotic fibers as fructooligosaccharide (FOS) and mannooligosaccharide. FOS is a soluble fiber extracted from vegetables (eg, *Cichorium intybus L*), several studies in dogs characterized this prebiotic, showing it effects on altering gut bacteria and fermentation products (FLAM et al., 2001; FULLER et al., 2016; HESTA et al., 2000; MENSINK et al., 2015; MÜLLER et al., 2018).

Taking this in consideration, the present Dissertation studied the effects of orange fiber, a semi-purified soluble source of fiber and compared it with beet pulp and FOS on nutrient digestibility, fermentation products on feces, and gastrointestinal transit time of dogs.

2. LITERATURE REVIEW

Brazil is the second largest country in population of dogs, cats and ornamental birds, and the fourth largest in the world in total population of pets, with approximately 52.2 million of dogs and 22.1 million of cats (ABINPET, 2018). This population drives the market, making Brazil the 3rd largest country in the world in sales of pet products and services, with a total gain of 20.3 billion of Reais in 2017. The market categories are divided into Pet Services, Pet Care, Pet Vet and Pet Food – this last one represents 68.6% of the total revenue mentioned above. The growth between years 2016 and 2017 in the Pet Food segment was 9.9%, highest in comparison to other sectors such as Pet Vet (7.0%) and Pet Care (5.5%). In 2016, the Brazilian pet food industry produced 2.58 tons of feed and 2.66 tons in 2017, an increase of 3%. When considering sales in comparison to the world market, Brazil presents 5.1% of the total and represent the 3rd position, behind only United States (41%) and United Kingdom (5.3%) (ABINPET, 2018).

Along with the Pet Food market, there is also a growing demand for high quality foods, with the aim of increasing the longevity and life expectancy of animals and decreasing the incidence of diseases (BONTEMPO, 2005). The development of dog and cat nutrition has been following the same pattern observed for human nutrition. The concepts of nutrition are expanding beyond the limit of promoting satiety and guaranteeing the supply of all essential nutrients and animal's survival, aiming to maximize the production and use of foods that promotes well-being and improve health, in addition to reducing the risk of diseases (FAHEY, 2003). More recently, it has been defined that providing adequate health care and a nutritionally balanced diet for pets is part of the human responsibility to maintain their well-being (GRZÉSKOWIAK et al., 2015).

2.1 Fiber for dogs

Fiber inclusion in diets for dogs and cats brings several benefits such as normal functioning and health of gastrointestinal tract, stool formation and consistency, energy dilution and appetite regulation (KIENZLE, 2001; MONTI, 2015). Each of these benefits will be discussed along this chapter. Also, fibers can be classified as insoluble or soluble, and non-fermentable or fermentable.

2.1.1 Insoluble fibers

Insoluble fibers have more limited water absorption capacity and do not form viscous solutions; they are generally non-fermentable (ex: cellulose), but there are also fermentable (ex: cabbage fiber) and moderately-fermentable (ex: beet pulp, rice bran) sources (REIHNART & SUNVOLD, 1997). Their physical properties increase fecal mass and decrease gastrointestinal transit time. Insoluble fibers are widely used in diets for weight loss programs, because of their ability to reduce energy digestibility resulting in low energy dense foods. In dogs, each percent of crude fiber in the food decrease in 1.43% the digestibility of the energy (KIENZLE, 2001; NRC, 2006). Additionally, it is claimed that these fibers may promote satiety, also favoring obtain the energy deficit required to weigh loss, although this is still controversial (LOUREIRO et al., 2017. In their work, German et al. (2010), concluded that animals that received diets with high fiber and high protein had greater and faster percentage of weight loss than those that received medium fiber and high protein. In another study, Weber et al. (2007), found that diets with high protein and high fiber content promote increased satiety in obese dogs.

Digestion mean retention time (DMRT) in gastrointestinal tract is another parameter influenced by fiber intake. Digesta mean retention time comprises the period between food ingestion and excretion of feces. This period does not correspond to the permanence of the digesta in each specific organ of the animal (stomach, small intestine and large intestine), but the general time from the entry of the food to the elimination of undigested components (feces) (BURROWS et al., 1982; LOUREIRO et al., 2017). Sources of insoluble and non-fermentable fibers tend to pass more quickly through the gastrointestinal tract, leading to decreased DMRT (BURROWS ET AL., 1982). In a master's dissertation from our research group with insoluble fibers, the radiopaque marker method was successfully used, demonstrating a reduction in DMRT of dogs when fed diets supplemented with sugarcane fiber, an insoluble fiber source (SILVA, 2013). In another study using the same methodology for DMRT, Monti (2015) found that concentrations of 12% of guava fiber (a source of insoluble fiber) also results in shorter transit time. The interference of fiber on DMRT, however, depend on the amount added, in their study using beet pulp, Fahey et al. (1990) showed that DMRT were not decreased until dietary fiber level exceeded 10%. Along to the decrease in DMRT, the increase in insoluble fibers in a diet also leads to an increase in fecal production, as demonstrated by Sa et al. (2012).;

2.1.2 Soluble fibers

Soluble fibers are those that form a viscous solution when in contact with water; its physical properties affect gastric emptying and gastrointestinal transit time (ANDERSON et al., 1990; REIHNART & SUNVOLD, 1997). Soluble fibers can be either fermentable (ex.: pectin, guar gum), non-fermentable (ex: carboxymethylcellulose, methylcellulose, xanthan gum; psyllium grum), or moderately-fermentable (ex: gum arabic) (REIHNART & SUNVOLD, 1997).

Fermentable fibers are generally more soluble and are thus classified according to their capacity for bacterial fermentation in the animal's colon; the substrate, which is not digested by enzymes of dogs and cats, travels through the gastrointestinal tract to colon, where it is used as an energetic substrate by bacteria that produce short chain fatty acids (SCFA), which are in turn important sources of energy for colonocytes (ASWHWAR et al., 2016; BIRD et al., 2007; BIRT et al., 2013; MARIA et al., 2017; PEIXOTO et al., 2018; ZAMAN & SARBINI et al., 2016).

Fermentable fibers are also highly capable of interfering in the health of the animal's gastrointestinal tract as they stimulate growth and/or metabolic activity of beneficial bacteria present in the intestinal lumen (GOMES et al., 2009; PAWAR et al., 2017; SWANSON et al., 2002). The large intestine of animals contains several species of bacteria, most of which are anaerobic. In dogs and cats, the most common are *Streptococcus, Lactobacillus* and *Bacteroides* (COMAN et al., 2019; HOLSCHER, 2017). These bacteria are capable of producing antibacterial substances and enzymes that prevent proliferation of harmful microorganisms, such as *Eschechia coli, Clostridium sp and Salmonella*. So, the growth of beneficial bacteria acts indirectly on immune system (DE SOUZA THEODORO et al., 2019).

The beneficial microbiota present in the intestine is also capable of producing substances that interact with the animal's immune system in order to modulate production of cytokines, increase proliferation of mononuclear cells, increase macrophage phagocytosis and promote synthesis of larger quantities immunoglobulins (DE OLIVEIRA et al., 2019; DE SOUZA THEODORO et al., 2019; MACFARLANE & CUMMINGS, 1999).

Fermentation products are substrates made by colon bacteria, important for the intestinal and general health of animals. The qualitative and quantitative aspects of fermentation products are also important markers of the health of the gastrointestinal tract (SUNVOLD et al., 1995b; SUNVOLD et al., 1995d). Examples of fermentation products are volatile fatty acids – composed of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA). Short-chain fatty acids – ex: acetate, propionate, and butyrate - are considered sources of energy for intestinal cells, promote reduction on the limenu of the colon pH and, consequently, inhibit the growth of pathogenic microorganisms (HOLSCHER et al., 2017). Acetic and propionic acids are readily absorbed and enter the bloodstream, providing extra energy for the animal (GOMES et al., 2009). Butyrate is the main energetic substrate for colonocytes, and it can represent up to 70% of the energy used by the colon mucosa (GIBSON et al., 2004; TENG, et al., 2018). It is in adition an important regulator of the growth and differentiation of intestinal cells (GIBSON et al., 2004; NRC, 2006; TENG, et al., 2018). This short-chain fatty acid, as well as the others SCFA, is produced by beneficial bacteria present in the colon lumen when in presence of fermentable fibers, and the supply of butyrate helps maintain mucosal integrity, playing an important role in maintaining a normal cell phenotype and reducing risk of colonic carcinomas (SCHEPPACH, 1995; GOMES et al., 2020). The SCFA also favor blood flow and muscle activity in the colon, stimulate mucin production and enterocytes proliferation (GIBSON, 2004). *In vitro* and *in vivo* studies demonstrate that the intermediate and final products of the fermentation carried out by the intestinal microbiota depend on the chemical composition of the carbohydrate. For example: starch fermentation produces a high amount of butyrate, while pectin fermentation produces a greater amount of acetate (TESHIMA, 2003).

2.2 Orange Fiber

Despite the reduction in production during 2019 due to weather-related problems, Brazil is the largest producer and exporter of orange juice in the world (USDA, 2020). In 2018, the production of oranges was 16,713. 534 tons, adding a total of R $ 9,450,570.00 to the country (IBGE, 2019).

Orange (*Citrus sinensis*) is one of the most cultivated species in Brazil. The orange juice industry receives fruits daily, washes and prepares them. Subsequently, the fruits go through a process in which they are squeezed. The orange juice, together with the orange fiber is drained through tubes. The juice is filtered, pasteurized and prepared for commercialization. Orange fiber is a co-product of juice processing. The remaining co-products (peel, bagasse, seeds) are pelleted and used, mainly, in the feeding of ruminants (AREAS, 1994).

Orange fiber represents the solid residue present in the orange juice. This is squeezed out of the vesicle of the fruit, along with the juice. Therefore, this fiber-rich by-product is widely obtained in the orange juice industry. Figure 1 represents the equatorial section of the orange where the juice vesicles can be seen, and Figure 2 represents the mechanism for obtaining orange fiber. There are no studies regarding the chemical composition of this fiber, however, Areas (1994) studied orange pulp (in his study, orange pulp is comprised of orange fiber and the membrane that separates pulp into segments) and found a chemical composition of 10.7% crude protein, 1.2% fat, 9.9% of total sugars and 71.1% of total dietary fiber (TDF). Within TDF, the same author found 55% of pectic substances and 45% of the combination of cellulose + hemicellulose + lignin.

Since this natural ingredient contains pectin, its intestinal fermentability (and consequent production of short chain fatty acids) is probably elevated, especially regarding the production of the main fatty acid used by intestinal cells, the butyrate. In the study conducted by Hallman et al. (1996), the use of pectin resulted in a greater production of butyrate than acetate and propionate, and in the in-vitro study carried out by Sunvold et al. (1995), there was an increase in the production of short chain fatty acids, propionate, acetate and butyrate during the incubation of citrus pectin.

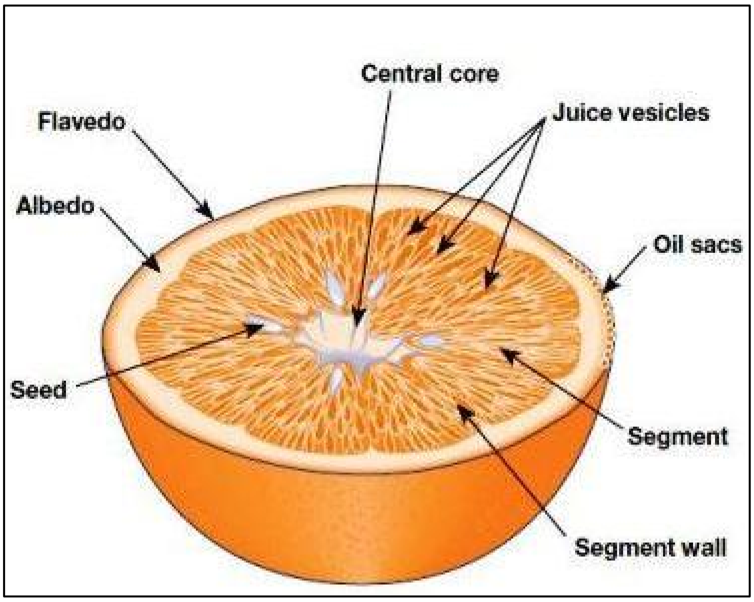

Figure 1: Anatomy of the orange in equatorial section. When squeezing the orange, the juice vesicles are excreted together with the liquid part, making up the solid residue of the juice production (Orange Fiber). Source: Citrus-BR, 2018.

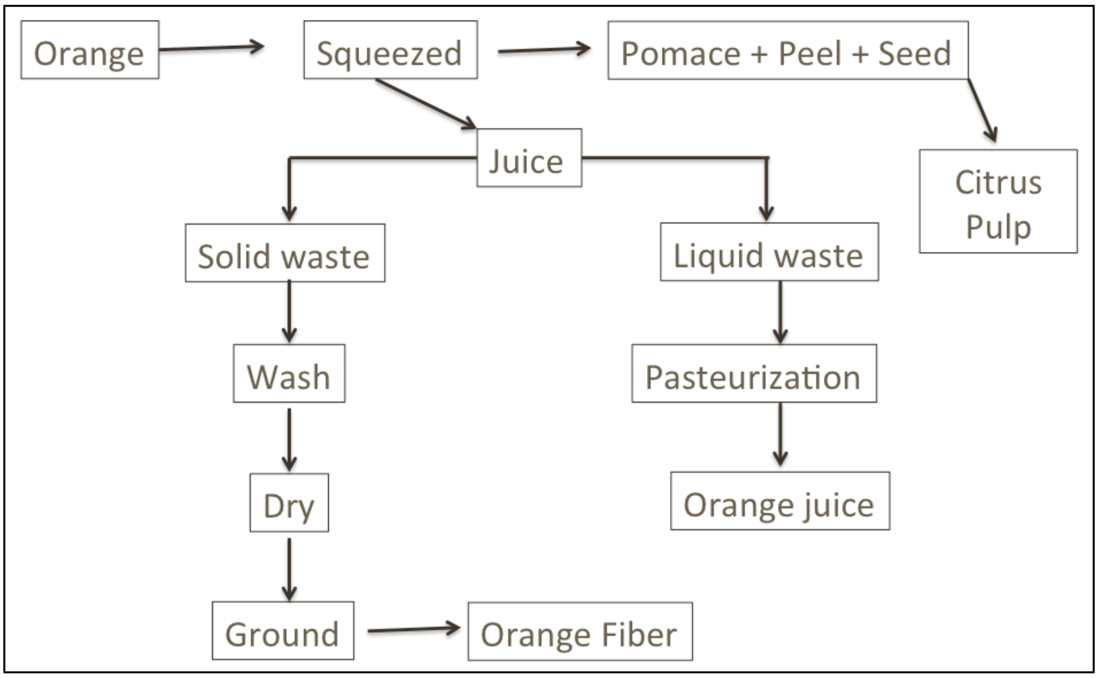

Figure 2: Mechanism for obtaining orange fiber. After extracting the juice, its solid residue is washed, dried and ground. Source: CITROSUCO, R. Alberto Berniche, Matão/SP, Brazil .

2.3 Beet Pulp

Beet pulp is a source of fiber commonly used in pet food industry that contains relatively high concentrations of cellulose, pectin and hemicellulose (WEN et al., 1988). Beet pulp contains about 80% of TDF and, of these, about 80% of insoluble fiber and 20% of soluble fiber, but its chemical composition can vary depending on the raw material (FAHEY et al., 1990b; SUNVOLD et al., 1995a, b, c). Widely used in dog food due to its soluble fraction and moderate fermentability, beet pulp promotes desirable effects on feces consistency depending on dosage (SUNVOLD et al., 1995a). In a study by Fahey et al. (1990a), it was observed that levels of 12.5% of beet pulp caused a greater volume of feces, greater frequency of defecation and decrease in digesta mean retention time (DMRT); other studies showed the same effects on using beet pulp as a fiber source in dog food (FAHEY et al., 1990b; LOUREIRO et al., 2017). However, the study done by Fahey et al. (1992)

authors found that concentrations of 2.5 and 7.5% of beet pulp did not show any influence on the digesta mean retention time of adult dogs.

Another benefit of using beet pulp is its fermentability. Studies show that the use of this ingredient in the feeding of dogs and cats provided an increase in the production of lactate and volatile fatty acids, including the short-chain fatty acids acetate and propionate (FISCHER et al., 2012; HALLMAN et al., 1996; KRIGER et al., 2017; MIDDELBOS et al., 2007). However, the production of butyric acid - the most preferably used by colonocytes - did not show a significant increase when compared to other sources of fermentable fibers (FISCHER et al., 2012; KROGER et al., 2017). Due to this increases on organic matter fermentation on colon, increases in fecal moisture of dogs was observed in diets supplemented with beet pulp (DIEZ et al., 1998; FAHEY et al., 1990a; FAHEY et al., 1990b)

Regarding the interference on the digestibility of other nutrients, studies indicate that the use of beet pulp interferes with apparent digestibility of dry matter, organic matter and energy (DIEZ et al., 1998; FAHEY et al. 1990a; FAHEY et al., 1990b; FAHEY et al., 1992; FISCHER et al., 2012).

2.4 Inulin

Inulin is a fructooligosaccharide (FOS) extracted from vegetables (eg, *Cichorium intybus L* - popularly known as chicory), with a degree of polymerization of 2 to 60 sugar units (DIEZ et al., 1998; HIDAKA & HIRAYAMA, 1991; NOGUEIRA, 2002). The structural conformation of their osidic bridge (ß1-2) makes it resistant to hydrolysis by alimentary enzymes (HESTA et al., 2000). According to Roberfroid (1993), inulin has the same characteristics as dietary fibers and the author proposes it be classified as such.

Inulin, as well as other fructooligosaccharide such as oligofrutose and short-chain fructooligosaccharide are examples of dietary constituents that beneficially alter microbial populations in the gut and help prevent the invasion of pathogenic bacteria, been classified as a prebiotic (PROPST et al., 2003). Studies have shown that the use of inulin in the feeding of dogs and cats has led to an increase in the total production of short chain fatty acids and propionate in the feces of animals (CALABRÓ et al., 2013; HESTA et al., 2000; PROPST et al., 2003), but only one study showed an increase in the production of acetate (CALABRÓ et al., 2013) and butyrate (MIDDEBLBOS et al., 2007).

Regarding the digestibility of nutrients, the use of inulin reduced apparent digestibility of proteins (DIEZ et al., 1998; HESTA et al., 2000; MIDDELBOS et al., 2007; PROPST et al., 2003). Some authors even found a decrease in the digestibility of dry matter (PROPST et al., 2003), organic matter (DIEZ et al., 1998; PROPST et al., 2003) and fat (DIEZ et al., 1998; HESTA et al., 2000). Depending on the quality of the raw material, inulin can contain from 92% to 99.5% of oligofructose and 0.5 to 8% of sugars (FRANCK, 2002).

3. HYPOTESIS

It is hypothesis of the present study that orange fiber, a semi-purified ingredient constituted by the walls of juice vesicles and fruit segments is fermented by the intestinal microbiota of dogs, reducing the digestibility of nutrients, increasing moisture and feces production, and support the formation of fermentation products in the colon. The fermentation profile of the orange fiber will be different from that of inulin or beet pulp, allowing the formation of greater amounts of butyrate with possible advantages for intestinal health.

4. OBJECTIVE

Based on the previous considerations, the present research aimed to evaluate the effects of two inclusions of orange fiber (1 and 3%), comparing the ingredient with a negative control (diet without addition of fiber source) and two positive controls, the beet pulp as a moderately soluble fiber source and purified inulin, a recognized prebiotic for dogs, studying these ingredient effects on apparent nutrient and energy digestibility, fermentation products on feces, and gastrointestinal transit time.

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ORANGE FIBER EFFECTS ON NUTRIENT DIGESTIBILITY, FERMENTATION PRODUCTS IN FECES AND DIGESTA MEAN RETENTION TIME IN DOGS

ABSTRACT – Fermentable fibers are used in commercial dog food to promote intestinal health by providing substrate for better metabolic activity of the gut microbiota. Brazil is the world's largest producer of oranges, from whose fruit it is possible to obtain fiber with a relevant soluble fraction. The present study compared the effects of two inclusions of orange fiber (1 and 3%, on as fed basis) with a negative control (without addition of fiber source) and two positive controls, beet pulp (3%) and purified inulin (1%), totaling five extruded diets for dogs. The experiment followed a randomized block design with 4 blocks of 10 dogs, 2 dogs per food in each block, totaling 8 dogs per diet. Apparent total tract nutrient digestibility was determined by total feces collection. Feces pH and fermentation products content were also measured. The digesta mean retention time (DMRT) were evaluated by plastic markers. Results were submitted to analysis of variance and means compared by Tukey's test ($P<0.05$). The inclusion of 3% fiber source on diets with 3% orange fiber and beet pulp reduced DM, OM, and energy digestibility ($P<0.05$). Diets with 3% orange fiber, beet pulp, and 1% inulin presented lower crude protein digestibility than control ($P<0.05$). Dietary fiber digestibility was higher for orange fiber supplemented diets than beet pulp and inulin ($P<0.05$). Beet pulp and 3% orange fiber inclusions resulted in increased moisture content on the feces of dogs ($P<0.05$) but did not alter DMRT ($P>0.05$). Total short-chain fatty acids were higher than control in the feces of dogs fed both orange fiber levels and the beet pulp supplemented diet ($P<0.05$), presenting the inulin supplemented diet fed dogs intermediate values. Butyrate was higher on feces of dogs fed the diets supplemented with 1% and 3% of orange fiber ($P<0.05$), and similar values than control was observed for beet pulp and inulin fed animals (P>0.05). It was concluded that orange fiber presented higher apparent total tract dietary fiber digestibility than beet pulp and have a fermentation profile on the colon that promotes the generation of butyrate, effects not observed for inulin and beet pulp.

Keywords: nutrition, microbiota, prebiotic, volatile fatty acids, intestinal activity.

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INTRODUCTION

Fibers are commonly used in commercial dry dog and cat foods due to their several import physiological functions such as improvement of gastrointestinal health an function (MARIA et al., 2017), modulation of gut microbiota (DESAI et al., 2016; PANASEVICH et al., 2015), stool consistency (DE GODOY et al., 2013; LOUREIRO et al., 2017), alterations on digesta mean retention time (LOUREIRO et al., 2017), reduction on nutrient digestibility and the energy value of the food (KAWAUCHI, et al., 2011), promotion of satiety and hairball control in cats (LOUREIRO e al. 2014).

To support gastrointestinal health, soluble and fermentable fibers are more adequate, supplying energy to saccharolytic microbiota inducing higher production of several fermentation products that are relevant to gut health (CUI et al., 2019; MAKKI et al., 2018; SWANSON et al., 2001). Among them higher butyrate and acetate may result in better nutrient and energy supply to colonocytes, allowing better development of the intestinal mucosa structure and barrier (DONOHOE et al., 2011; TOPPING AND CLIFTON, 2001). The supply of butyrate and the induced changes on gut microbiota may reduce inflammation and increase local and systemic immunity on animals, my increasing gut and general health (BROUNS et al., 2002; de SOUZA THEODORO, 2019; MARIA, 2017; PEIXOTO et al., 2018; SCHEPPACH et al., 1994). For this reason, soluble and fermentable fibers are widely used in foods for the treatment of diseases such as obesity and therapies for glycemic control (CANFORA et al., 2015; DE VADDER et al., 2014; FISCHER et al., 2012), as well as intestinal diseases such as colitis (LECOINDRE & GASCHEN, 2011; LEIB et al., 2000; PANASEVICH et al., 2015b).

Brazil is the biggest orange producer, yielding in 2017 more than 17 million tons of this fruit (IBGE, 2018). Most part of this production is intended to orange juice manufacture, and approximately 1,080,000 tons of orange juice were exported in 2018 (CITRUS-BR, 2018). In the context of responsible agriculture practices, sustainable use of co-products are relevant, and the citrus pulp pellet, obtained after orange squeezed is largely used as feedstuff for cattle (BAMPIDIS & ROBINSON, 2006). Recently, a special co-product rich in soluble fiber was developed and called orange fiber. Different from citrus pulp pellet, which comprises the orange seeds, flavedo, albedo, central core, and the solids retained after juice filtration, the orange fiber is derived only by the material obtained by the juice filtration process, mainly integrated by the walls of juice vesicles and fruit segments, with elevate content of pectic substances and soluble fiber. Studies on dogs showed pectin are largely fermented by gut microbiota, with relevant production of short-chain fatty acids (SCFA) (HALLMAN et al., 1996; SUNVOLD et al., 1995a). Pectin fermentation is known to induce butyrate production, with possible implications to gut health (CHEN et al., 2018; SUNVOLD et al., 1995a). This open an opportunity to explore this raw material as a soluble and fermentable fiber source for dogs, but orange fiber was not studied previously for animals.

Common sources of fermentable fiber for extruded dog foods includes beet pulp and inulin. Beet pulp is widely used due to its availability, cost, and scientific background from many publications in dogs (FAHEY et al., 1990b; HALLMAN et al., 1996; KRÖGER et al., 2017; MIDDELBOS et al., 2007). Inulin is a fructooligosaccharide (FOS) extracted from vegetables (eg, *Cichorium intybus*), largely studied as a prebiotic for humans (ASTÓ et al., 2019; VANDEPUTTE et al., 2017), animal models (HOFFMAN et al., 2019; MISTRY et al., 2018; VIDELA et al., 2001), and dogs (DIEZ et al., 1998; SWANSON et al., 2002; ALEXANDER et al., 2018; RÉQUILLÉ et al., 2018). On the published studies, beet pulp and inulin are readily fermented by colon microbiota, increasing SCFA on feces. However, this substrates induce greater increase on acetate and proprionate, but not always increasing butyrate amounts on feces (ALEXANDER et al., 2018; CALABRÓ et al., 2013; HALLMAN et al., 1996; MARIA et al., 2017; SUNVOLD et al., 1995).

Taking this in consideration, the objective of the present study was to evaluate the effects of the arbitrary inclusion of three levels of orange fiber, 0% (negative control), 1%, and 3%, comparing the results of apparent total tract nutrient digestibility, fermentation products in feces, and digesta mean retention time with two positive controls, 3% of beet pulp and 1% of inulin, previously studied fermentable fiber sources to dogs.

MATERIAL AND METHODS

The experiment was conducted at the Laboratory of Research in Nutrition and Nutritional Diseases of Dogs and Cats “Prof. Dr. Flávio Prada”, College of Agrarian and Veterinarian Sciences, Jaboticabal campus, UNESP. All the procedures with dogs followed the ethical principles adopted by the Brazilian College of Animal Experimentation and were previously approved by the Ethics Committee on the Use of Animals (protocol number 07365/19).

Experimental design, ingredients and diets

The experiment included 5 experimental diets and 40 Beagle dogs and was carried out in a randomized block design with 4 blocks of 10 animals each, 2 animals per food in each block, totaling 8 animals (repetitions) per food (treatment). The blocking factor was time, due to impossibility to test the 40 dogs on the same time.

The orange fiber used in the study was donated by Citrosuco S/A Agroindustria (R. Alberto Berniche, Matão/SP). The beet pulp was purchased from the marketing (TECTRON – Nutrição e Saúde Animal – Toledo/PR). The inulin used is a commercial product available on the marketing (ORAFTISIPX, Tienen, Belgium). The chemical composition of the samples used are presented on Table 1.

A control diet (CO) based on polished rice, poultry by product meal and poultry fat, without the inclusion of fiber source was formulated for adult dogs (FEDIAF, 2018). Based on this formulation, the test diets were obtained by the substitution of polished rice by the fiber ingredient in study: addition of 1% of orange fiber (OF1); addition of 3% of orange fiber (OF3); addition of 3% of beet pulp (BP); addition of 1% of inulin (IN). The formulations are shown in Table 2.

Diets were produced at the Feed Facility of the College of Agrarian and Veterinarian Sciences, Jaboticabal campus, UNESP. Ingredients were mixed, grounded in a hammer mill with a 1 mm screen sieve size (Moinhos Tigre, São Paulo, Brazil) and extruded in a single screw extruder (MEX 250, Manzoni, Campinas, Brazil), with a capacity of approximately 250kg/h. The temperature of the preconditioner was maintained for all treatment over 90°C by direct steam injection. Water, steam, extruder screw speed and raw material feed rate were adjusted for the control diet, and kept constant for all other treatments. The mean mass temperature and pressure before extruder die was 134.25±1.36°C respectively. To assure consistent processing and kibble quality, the apparent density of extrudates was obtained weighting the mass of kibbles corresponded to 1 L. Diets presented similar apparent densities, with a mean value of 316.71± 8.90g/L for all treatments. After extrusion, kibbles were dried in a two-pass dryer with forced air heated to 105 ° C for 20 min and then covered with poultry fat and palatant enhancer.

Animals and management

Forty beagle dogs were used, 4.8±1.03 years old, 12.40±2.82 kg of body weight, male and female, and with an appropriate body condition score (4/5 of 9) (LAFLAMME, 1997). The health condition of the animals was previously assessed by physical evaluation, complete blood count, serum biochemistry (alanine aminotransferase, alkaline phosphatase, urea, creatinine), and urine analysis. Each experimental block (period of the experiment) lasted 24 days, distributed as follows:

- days 1 to 10: adaptation to experimental diet.
- day 11 to 15: evaluation of digesta mean retention time.
- days 16 to 18: resting period, for animal welfare.
- days 19 to 24: feces collection for the digestibility test and evaluation of fermentation products.

The dogs were housed during the adaptation and resting periods in kennels with a solarium, with 1.5mx 4.0m. During the days of feces collection to evaluated digesta mean retention time (DMRT), nutrient digestibility and fermentation products dogs remained restricted to individual metabolic cages, measuring 0.9m x 1.0m x 0.9m. The amount of food given to each dog was individually calculated according to the metabolizable energy content of the diet and the individual energy requirement of each animal, stablished by food intake records of the laboratory (NRC, 2006). The dogs were weighed weekly, and the amount of food was adjusted, if necessary, so that the animals kept their body weight constant along the study. The total daily amount of food was provided in a single daily meal at 09:00h. Remaining food was collected after 30 min, weighted and the intake recorded. Throughout the experiment, fresh water was available ad libtum.

Evaluated parameters

Total tract apparent digestibility of nutrients, and fecal characteristics

Total tract apparent digestibility was evaluated by total collection of feces without urine collection, considering the FEDIAF (2018) recommendations. Food consumption was recorded daily, by weighing the offered amount and leftovers. Feces were quantitatively collected, weighed and stored in individual plastic bags in a freezer (-15ºC) for further analysis. At the end of the collection period, feces were thawed and homogenized, making a single sample per animal and period, weighed and dried in a forced ventilation oven (320-SE, FANEM, São Paulo) at 55ºC for 72 hours. The pre-dried feces and diets were then ground in a knife mill (MOD 340, ART LAB, São Paulo) with 1 mm screen sieve, to proceed to laboratory analysis.

Samples were analyzed (AOAC, 1995) by oven drying to obtain dry matter (DM) (method 934.01), by incineration in a muffle furnace for ash content (method 942.05), by the Kjeldahl method for crude protein (method 954.01), and fat was assessed using the acid-hydrolyzed fat (AHF) assay (method 954.02). The organic matter (OM) of the samples was calculated as DM minus ash. Total dietary fiber (TDF), soluble dietary fiber (TSF) and insoluble dietary fiber (TIF) were measured using a combination of enzymatic and gravimetric procedures (method 991.43, AOAC, 2012) The total amount of starch was measured as described by Hendrix (1995). Gross energy (GE) was determined using a calorimetric pump (1281, PARR Instrument Company, Illinois, USA). Additionally, to evaluate processing quality, the degree of starch gelatinization was determined on the food samples by the amyloglucosidase method (Sá et al., 2013). All analysis was conducted in duplicate, and repeated when the variation between replicates was greater than 5%.

Feces characteristics were qualitative assessed during collection by the following score (Carciofi et al., 2008): 0 = liquid feces; 1 = pasty and shapeless stools; 2 = soft, malformed stools that take the shape of the collection container; 3 = soft, formed and moist stools that mark the floor; 4 = well-formed and consistent stools that do not mark the floor; 5 = well formed, hard and dry stools. Values between 3 and 4 are considered adequate.

Fermentation products on feces

Samples of recently eliminated feces were collected (no more than 15 minutes after elimination) and evaluated for the contents of volatile fatty acids (short-chain fatty acids – SCFA; and branched-chain fatty acids - BCFA), lactate, ammonia and pH. To this, cages were continuously monitored, and feces collected as soon as eliminated. To determine pH, 2.0 grams of feces were diluted (1:3 w / v) in milli-Q water and the pH was measured with a pH meter (model DM20, Digicrom Analítica Ltda , Sao Paulo). To SCFA and BCFA analysis, 10 grams of feces were homogenized and mixed with 30 milliliters of 4.2 N formic acid (1: 3 w / w). The concentrations of SCFA and BCFA were determined by gas chromatography according to ERWIN et al. (1961). To measure lactic acid, approximately three grams of feces were used, immediately homogenized and mixed with nine milliliters of distilled water (w/v). Lactic acid was analyzed according to PRYCE (1969) by spectrophotometric method with a reading at 565nm (500 to 570nm). Samples were quantified by comparing results with a 0.08% standard lactic acid solution. To measure ammonia was adapted the methodology proposed by VIEIRA (1980), the extracts prepared for SCFA were used; two milliliter of the extract were diluted in 13 milliliters of distilled water (2:13 v/v) and the volume submitted to distillation in a nitrogen system (Tecnal TE - 036/1, Tecnal, Piracicaba, Brazil).

Digesta mean retention time

The DMRT in the gastrointestinal tract of the dogs was calculated as described by Loureiro et al. (2017). The animals were fed at 09:00h and immediately received orally gelatin capsules containing 24 markers (Sitzmarks, Konsyl

Pharmaceuticals Inc., Fort Worth, Texas USA). The markers are 4.5 mm in diameter and have a density of 1.25 g / mL. Each day, for three consecutive days, a different marker format was used, allowing three observations of the DMRT. Animals were allowed to eat the total amount of food in 20 min. The time of administration of the marker was recorded, and the animals and their excrement were observed at 15-minute intervals until the last marker was recovered in feces. All feces were collected, weighed and the sampling time recorded. The feces were washed under a sieve using tap water and the markers obtained were counted.

The DMRT was calculated as the time interval (in hours) between the markers and food intake and the excretion time of the feces containing the last marker recovered. The average of the three days of observation was used for statistical comparisons. The marker recovery rate was calculated (recovery rate = number of markers on feces *100 / number of markers orally dosed), and 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.

Statistical analysis

The assumptions of normality of errors and homogeneity of variances were evaluated previously to each analysis. The data obtained were subjected to analysis of variance considering a completely randomized block design. The experimental unit was considered each dog with 8 repetitions per food. When differences were detected at the F test, means of treatments were compared using Tukey test. Values of $P<0.05$ were considered significant. The analyzes were performed using the MIXED procedure of the Statistical Analysis System software (Version 9.2, SAS Institute Inc., Cary, NC, USA).

RESULTS

The chemical composition of the experimental diets is presented on Table 3. Diets presented similar chemical composition, with the exception of a little lower CP content on diet IN. Starch and dietary fiber varied, according to fiber source inclusion on diets. The IN diet also shown lower dietary fiber, may explained by the technical difficult in analysis this ingredient that is purified and partially hydrolyzed. The degree of starch gelatinization of the diets was adequate, indicating consistent extrusion processing.

Dogs showed a proper food intake and maintained a constant body weight throughout the experimental period (data not shown) with no episodes of food rejection or diarrhea. Nutrient intake during the digestibility study and the coefficients of total tract apparent digestibility of nutrients and energy are presented in Table 4. No significant differences were found between the intake of DM, OM, CP, fat and starch (P>0.05). Total dietary fiber intake, however, was higher for dogs fed the OF3 diet, intermediary for dogs fed Control, OF1, and BP diets, and lower for animals fed the IN diet (P<0.05). The lower dietary fiber intake estimated for the IN treatment, however, need to be considered with caution as the fiber analysis of inulin presented technical issues and this non-digestible oligosaccharide corresponded to 1% of the formulation (as-fed basis).

Digestibility of dry matter, organic matter, and gross energy was reduced after the inclusion of 3% of the orange fiber and beet pulp (P<0.05). Experimental diets, however, presented in general elevated digestibility, may explained by the use of polished rice, a carbohydrate source with low dietary fiber content, and the fibers in study was included on treatment foods in low inclusion amounts. Digestibility of crude protein was also high, suggesting the use of a good quality poultry by-product meal. The inclusion of 3% orange fiber and beet pulp also reduced crude protein digestibility in comparison with Control and OF1 diets ($P<0.05$), following the general reduction on diet digestibility. It was unexpected, however, the reduction on CP apparent digestibility after 1% inclusion on inulin, whit a value similar to 3% inclusion of beet pulp and orange fiber ($P<0.05$). This was in line, nonetheless, with the intermediary digestibility of gross energy observed for the IN diet.

Dietary fiber total tract apparent digestibility coefficient was higher for OF1, OF3, and Control diets, intermediary for BP and lower for IN treatment ($P<0.05$). In general, all digestibilities are high, may be explained by the low fiber content of the experimental diets allowing the fermentation on an extensive proportion of the fiber that reached the colon. The lower result observed for the IN diet need to be interpreted. The inulin source used was not quantified by the total dietary fiber assay, due to be a purified and partially hydrolyzed oligofructose material. Due this the estimated dietary fiber content of the diet was low as well as the calculated fiber intake by dogs. This reduced estimation of intake might resulted on an incorrect calculation of the apparent digestibility, that in fact is probably higher. This is reinforced by the increased feces production and moisture content of dogs fed the IN food, in comparison to control ($P<0.05$).

The fecal production and characteristics, and the DMRT of the dogs can be seen in Table 5. Fecal moisture increases on dogs fed OF3 and BP diets, and was intermediary for dogs fed OF1 and IN I comparison with animals fed the Control food ($P<0.05$). Fecal production (as-is basis) was also higher in dogs fed OF3 and BP, due to the lower digestibility and higher moisture content of the feces ($P<0.05$). Although these alterations were observed, fecal score was adequate and similar for all diets (P>0.05). Regarding the number of defecations per day, it was higher for dogs fed OF3 in comparison to OF1, Control and BP (P<0.05), with intermediary values for dogs fed IN. No treatment effect was observed for DMRT (P>0.05).

Although only a tendency was observed for feces pH results on analysis of variance (P=0.068), the Tukey test results indicated lower fecal pH for dogs fed BP than Control, presenting the other treatments intermediary results (P<0.05). The feces of dogs fed both diets containing 3% fiber source (OF3 and BP) presented more lactate, explained by a quantitatively greater organic matter fermentation on colon. Regarding the volatile fatty acids, higher acetate was observed on the feces of dogs fed OF3 and BP, with intermediary amounts for IN and OF1 and lower values for dogs fed the Control food (P<0.05). Propionate was higher for BP, intermediary of OF3 and IN, and lower for OF1 and control (P<0.05). Both diets supplemented with orange fiber (OF1 and OF3) induced an increase on fecal butyrate (P<0.05), with intermediate values on the feces of dogs fed BP, and lower values for IN and Control diets (P>0.05). No effect of diet was observed for branched-chain fatty acids. These alterations resulted in higher total SCFA and total volatile fatty acids in feces of dogs fed OF1, OF3, and BP, with an intermediary concentration on IN and lower for Control (P<0.05). In percentage of the total SCFA, lower proportion of propionate was observed on feces of dogs fed orange fiber supplemented foods, and higher proportion of butyrate was verified on feces of dogs fed orange fiber (OF1 and OF3) and Control diets (P<0.05).

DISCUSSION

The results of the present study confirmed orange fiber as a soluble and fermentable fiber source to dogs. Diets supplemented with orange fiber presented higher dietary fiber apparent digestibility and the feces of dog's greater total short chain fatty acids than the promoted by the intake of diets with beet pulp or inulin.

The study was intended to evaluate fermentable fiber sources included at low levels, similar to the amounts tested to prebiotics such as inulin, that was evaluated in some studies in inclusions amounts close to 1% (PROPST et al., 2003; Swanson, 2002 Propst). Considering inulin is purified and highly soluble and fermentable (CALABRÓ et al., 2013; DIEZ et al., 1998; PROPST et al., 2003), but beet pulp is moderately soluble and moderately fermentable (FAHEY et al.,1990b; FISCHER et al., 2012; HALLMAN et al., 1996; KRIGER et al., 2017; MIDDELBOS et al., 2007; SUNVOLD et al., 1995a, b, c), a dosage three times greater was proposed to this fiber source in order to be comparable with the inulin dosage. Additionally, the intention of the experiment was to evaluate potentially fermentable fiber sources for use in diets with elevate digestibility to dogs, as a tool do modulate fecal short-chain fatty acids and it possible health implications to animals, but not compromising the energy value of the diet. The same two levels were then selected to test the orange fiber, in order to compare results among the three ingredients.

It is well known the effect of fiber reducing total tract apparent digestibility of nutrients (FAHEY et al., 1990; KAWAUCHI et al., 2011; SA et al., 2013). However, not all raw materials have the same effect on this regard (MARIA et al., 2017), and the implications to fiber on nutrient digestibility may vary according to solubility, viscosity development, fermentability by gut microbiota, and other factors (DIEZ et al., 1998; DE GODOY et al., 2013; LOUREIRO et al., 2017). On the current study, fibers included at 3% level (3OF and beet pulp) reduced OM, crude protein, and gross energy digestibility, what is expected. However, crude protein total tract apparent digestibility was also reduced by the addition of 1% inulin. Previous studies with inulin had also shown its potential effects reducing crude protein digestibility (Flickinger, 2003: Propst:).

The apparent total tract digestibility of dietary fiber reflects in fact it fermentability by gut microbiota, resulting on the disappearance of this nutrient along the digestive tract. In general, the values obtained on the current study are elevate, what need to be interpreted considering the low dietary fiber content of the experimental diets, with a maximum value of only 5.8% for the OF3 diet. In this situation of low fiber amount reaching the colon, it is understandable the disappearance of an expressive proportion of these compounds. The higher disappearance of the dietary fiber on the control, OF1 and OF3 treatments reflects a higher fermentability of the natural fiber on rice, and orange fiber in comparison with beet pulp and inulin diets. The results of the IN diet, however, need to be interpreted. The commercial form of inulin used is a purified and partially hydrolyzed oligofructose and was not detected on the dietary fiber assay at the laboratory, as shown on Table 1. Due this, the estimated dietary fiber content of the IN treatment was probably lower than the real, as well as the calculated fiber intake, justifying the reduced estimation of apparent digestibility. Is it possible to see that fermentable material was present on the food and was eaten by dogs due to the reduction on crude protein digestibility and feces DM, and the increase on daily feces excretion per $kg^{0.75}$, and fecal propionate and total short-chain fatty acids when comparing dogs fed Control and the IN treatment.

The higher fermentability of orange fiber may be explained by its chemical composition, with almost three times more fermentable fiber than beet pulp. The rate of insoluble to soluble fiber of orange fiber is 0.9, as the ingredient have more soluble than insoluble fiber, but 4.3 to beet pulp. Additionally, internal data showed that the orange fiber sample used on the current study have 27.6% of pectin on a DM-basis (data not shown), a compound know to be highly fermentable to dogs (SUNVOLD et al., 1995; ZENTEK et al. 1996).

As expected, the addition of fermentable fiber to diets increased fecal moisture, fecal production, and the number of defecations per day (Musco, 2013;, Maria et al., 2017). Fecal score was not altered, may explained by the low inclusion levels tested. Dogs fed Control diet presented dry feces, what may not be adequate especially for animals with difficult defecation. The increase in fecal moisture can be, taking this in consideration interesting in the management of gastrointestinal diseases such as constipation.

The digesta mean retention time reduced in approximately 8h after increase on 3% orange fiber of beet pulp, but the mean value was similar to Control diet. Other studies in dogs also failed to demonstrate an influence of fiber on retention time (Fahey et al., 1992; Hill et al., 2000). It is important to consider, however, the limitations of this measurement, that only shows the time from intake to defecation, not allowing to understand how long food stayed on stomach or each intestinal segment, including the procedure not show the time feces are retained on rectum before elimination, as feces elimination also have behavioral implications.

The main outcome of the present study is the fiber implications on fecal short-chain fatty acid concentration of dogs. The elevated apparent digestibility of dietary fiber in the diets supplemented with orange fiber was followed by an increased concentration of lactate, acetate, propionate, butyrate, and total short-chain fatty acids on feces when compared to Control. This confirmed the hypothesis of the current study, according to previous data that showed high fermentability of pectin from orange to dogs (Sunvold et al., 1995; Hallman et al., 1996; Zentek et al., 1996) and other species (CHENG et al., 2017).

It is especially interesting the elevation on fecal butyrate, as this short-chain fatty acid is considered important, with many physiological functions. Butyrate is the main energy substrate for colonocytes, and it can represent up to 70% of the energy used by the colon mucosa (GIBSON et al., 2004; TENG, et al., 2018). It is in addition an important regulator of the growth and differentiation of intestinal cells (GIBSON et al., 2004; TENG, et al., 2018) may improving the barrier function and intestinal structure (Tan et al., 2014; Ríos-Covián et al., 2016; Peixoto et al., 2018). Another potential role of butyrate are the maintenance of a normal cell phenotype with reduced risk of colonic carcinomas (SCHEPPACH, 1995; GOMES et al., 2020), and reduction of mucosae inflammation (Knudsen et al., 2018; Theodoro et al., 2019).

Beet pulp and inulin supplementation also increased acetate and propionate on feces (specially beet pulp), but not butyrate, with similar values than Control. This differences in fermentation profile can also be observed when short-chain fatty acids as expressed in percentage, as the feces of dogs fed BP and IN diets have proportionally a lower % of butyrate. Some studies had already shown that not all fermentable fiber sources increase butyrate (Fischer et al., 2012; Wambacq et al., 2016; Maria et al., 2017). Beet pulp fermentation on colon usually results in higher acetate and propionate (HALLMAN et al., 1996; KROGER et al., 2017; MIDDELBOS et al., 2007), but not necessarily butyrate. Studies about inulin to dogs also reported increased acetate and propionate, but not butyrate (ALEXANDER et al., 2018; HESTA et al., 2000; PROPST et al., 2003).

CONCLUSIONS

Orange fiber included on diets for dogs was fermentable, increasing the apparent digestibility of dietary fiber and the lactate and short-chain fatty acid on feces. It shown be more fermentable than beet pulp and inulin, increasing the fecal concentration of butyrate.

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Declaration of interest statement

No potential competing interest was reported by the authors

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Table 1: Analyzed chemical composition of fiber sources used on the experiment (g/100g as fed basis)

Item	FIBER SOURCES[1]		
	Orange Fiber	Beet Pulp	Inulin
Dry matter	91.30	89.98	95.77
Ash	2.40	5.39	4.31
Crude protein	9.15	8.11	3.25
Acid-hydrolyzed fat	2.88	0.55	0.04
Total dietary fiber	62.14	66.96	-[2]
Insoluble fiber	29.74	54.23	-
Soluble fiber	32.40	12.73	-
Starch	4.57	2.94	-

[1] Orange fiber: Citrosuco Paulista, Matão, SP, Brasil. Beet pulp: TECTRON – Nutrição e Saúde Animal, Av. Maripá, 895. Toledo/PR. Inulin: ORAFTISIPX, Tienen, Belgium

[2] The inulin used is purified and partially enzymatically hydrolyzed to enhance solubility, therefore it is 100% soluble and no dietary fiber could be quantified.

Table 2: Ingredient composition of the experimental formulations with different fiber sources to dogs

Item	Experimental diets[1]				
	Control	OF1	OF3	BP	IN
Ingredient composition, % as-fed					
Polished rice	59.65	58.60	56.51	56.61	58.47
Orange Fiber	0.00	1.00	3.00	0.00	0.00
Beet pulp	0.00	0.00	0.00	3.00	0.00
Inulin	0.00	0.00	0.00	0.00	1.00
Poultry meal	27.32	27.39	27.55	27.43	27.51
Poultry fat	9.00	8.98	8.95	8.96	8.99
Palatant enhancer[2]	2.00	2.00	2.00	2.00	2.00
Potassium chloride	0.53	0.52	0.49	0.49	0.53
Vit-min premix[3]	0.50	0.50	0.50	0.50	0.50
Common salt	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.32	0.32	0.32	0.32	0.32
Mold inhibitor[4]	0.10	0.10	0.10	0.10	0.10
Methionine	0.05	0.05	0.05	0.05	0.05
Antioxidant[5]	0.04	0.04	0.04	0.04	0.04

[1] Control – without fiber inclusion; OF1 – addition of 1% of orange fiber; OF3 – addition of 3% of orange fiber; BP – addition of 3% of beet pulp; IN – addition of 1% of inulin.

[2] D'TECH 10L, Palatabilizante Líquido, SPF do Brasil Indústria e Comércio Ltda., Descalvado, Brazil.

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

[4] Mold-Zap Citrus, Alltech do Brasil Agroindustrial Ltda., Araucária, Brazil.

[5] Banox, Alltech do Br

asil Agroindustrial Ltda., Araucária, Brazil.

Table 3. Analyzed chemical composition of experimental diets with different fiber sources to dogs (values on DM-basis).

Item	Experimental diets[1]				
	Control	OF1	OF3	BP	IN
Dry matter (%)	91.05	90.03	91.47	90.06	90.41
Organic matter (%)	92.88	92.79	92.47	93.13	93.51
Crude protein (%)	25.84	26.57	25.31	26.39	24.33
Fat (%)	18.42	16.83	16.95	18.88	17.70
Starch (%)	47.24	46.78	46.58	46.18	49.28
Total dietary fiber (%)	4.51	4.88	5.84	4.91	3.10
Gross energy (kcal/kg)	5203	5060	5054	5050	4953
Ash (%)	7.02	7.21	7.53	6.87	7.18
Ca (%)	18.80	17.64	18.73	18.34	18.60
P (%)	12.56	11.62	12.17	11.92	12.43
Starch gelatinization degree (%)	98.83	99.32	97.30	91.04	94.70

[1] Control – without fiber inclusion; OF1 – addition of 1% of orange fiber; OF3 – addition of 3% of orange fiber; BP – addition of 3% of beet pulp; IN – addition of 1% of inulin.

Table 4: Nutrient intake and coefficient of total tract apparent digestibility of experimental diets with different fiber sources to dogs

Item	Experimental diets[1]					SEM	p value
	Contrl	OF1	OF3	BP	IN		
Nutrient intake during the digestibility study (g/dog/d)							
Dry matter	183.23	176.40	185.92	176.00	178.42	3.734	0.925
Organic matter	170.37	163.68	171.93	163.91	165.60	3.463	0.939
Crude protein	47.34	46.87	47.05	46.45	43.41	0.963	0.735
Fat	15.51	15.20	15.77	14.33	15.45	0.321	0.716
Total dietary fiber	8.09b	8.61b	10.86a	8.64b	5.53c	0.335	<0.001
Starch	86.56	81.41	86.61	81.27	87.92	1.818	0.685
Coefficients of total tract apparent digestibility (%)							
Dry matter	89.94^{ab}	91.17^{a}	88.5^{b}	88.89^{b}	89.75^{ab}	0.277	0.018
Organic matter	94.43^{a}	95.02^{a}	93.14^{b}	93.27^{b}	94.28^{a}	0.185	0.001
Crude protein	91.27^{a}	92.16^{a}	89.09^{b}	89.96^{b}	89.72^{b}	0.350	<0.001
Fat	97.13	96.53	95.94	95.99	96.25	0.157	0.076
Total dietary fiber	58.03^{ab}	65.02^{a}	59.58^{ab}	46.30^{bc}	42.31^{c}	2.1853	0.008
Starch	99.64	99.59	99.55	99.59	99.60	0.030	0.758
Gross Energy	94.38^{a}	94.93^{a}	93.13^{b}	93.27^{b}	94.18^{ab}	0.201	0.015

[1] Control – without fiber inclusion; OF1 – addition of 1% of orange fiber; OF3 – addition of 3% of orange fiber;

Table 5: Fecal production and characteristics, and digesta mean retention time of dogs fed experimental diets with different fiber sources.

Item	Experimental diets[1]					SEM	p value
	Contol	OF1	OF3	BP	IN		
Fecal traits							
Dry matter (%)	49.68[a]	47.75[ab]	43.46[c]	43.96[bc]	45.99[abc]	0.727	0.021
g feces, as-is basis (g/kg$^{0.75}$/d)	5.52[bc]	4.93[c]	7.40[a]	7.00[a]	6.05[b]	0.214	<.0001
g feces, DM-basis (g/kg$^{0.75}$/d)	1.49[b]	1.45[b]	1.32[b]	1.41[b]	2.77[a]	0.099	<.0001
Fecal Score	3.98	4.00	3.90	4.00	4.00	0.020	0.559
Mean weight of each defecation (g)	30.09	28.78	30.17	32.77	28.19	1.203	0.839
Number of defecations per day	1.30[b]	1.15 [b]	1.72 [a]	1.38 [b]	1.45 [ab]	0.054	0.031
Digesta mean retention time (h)	52.79	47.69	44.86	45.37	45.66	1.698	0.695

[1] Control – without fiber inclusion; OF1 – addition of 1% of orange fiber; OF3 – addition of 3% of orange fiber; BP – addition of 3% of beet pulp; IN – addition of 1% of inulin.

[a, b, c] – means in a row without a common superscript differ (P<0.05)

Table 6: Fermentation products concentrations on the feces of dogs fed experimental diets with different fiber sources.

Item	Experimental diets[1]					SEM	p value
	Control	OF1	OF3	BP	IN		
Fecal pH	6.98[a]	6.92[ab]	6.88[ab]	6.78[b]	6.83[ab]	0.029	0.068
Lactic acid (mmol/kg of fecal DM)	3.69[bc]	3.66[c]	5.86[a]	5.62[a]	4.59[b]	0.221	<0.001
Volatile Fatty Acids (mmol/g of fecal DM)							
Acetic	138.16[b]	177.00[ab]	216.74[a]	217.79[a]	174.41[ab]	8.590	0.008
Propionic	61.34[c]	74.87[bc]	79.87[ab]	90.29[a]	80.17[ab]	2.962	0.008
Butyric	38.31[c]	52.26[a]	51.64[ab]	45.03[bc]	40.61[c]	1.653	0.039
Total short-chain fatty acids	237.82[b]	302.50[a]	346.15[a]	353.12[a]	295.19[ab]	12.302	0.007
Isobutyric	9.50	10.42	7.86	7.16	8.59	0.384	0.052
Isovaleric	16.16	17.08	13.62	12.51	15.05	0.653	0.127
Valeric	1.51	1.85	1.76	1.63	1.54	0.078	0.374
Total branched-chain fatty acids	27.18	29.36	23.26	21.31	25.19	1.069	0.099
Total volatile fatty acids	265.00[b]	331.87[a]	369.42[a]	374.43[a]	320.39[ab]	12.464	0.012
Short Chain Fatty Acids (% of total)							
Acetic	57.84	58.47	62.26	61.40	58.87	0.538	0.066
Propionic	25.75[ab]	24.92[bc]	23.21[c]	25.58[ab]	27.17[a]	0.384	0.016
Butyric	16.41[a]	16.61[a]	14.53[ab]	13.02[b]	13.96[b]	0.395	0.028

[1] Control – without fiber inclusion; OF1 – addition of 1% of orange fiber; OF3 – addition of 3% of orange fiber;

BP – addition of 3% of beet pulp; IN – addition of 1% of inulin.

[a, b, c, d] – means in a row without a common superscript differ (P<0.05)