Published January 9, 2015

In vitro fermentation characteristics of novel fibers, coconut endosperm fiber and chicory pulp, using canine fecal inoculum[1]

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ABSTRACT: The objective of this experiment was to determine the effects of in vitro fermentation of coconut endosperm fiber (CEF), chicory pulp (CHP), and selective blends of these substrates on SCFA production and changes in microbiota using canine fecal inocula. A total of 6 individual substrates, including short-chain fructooligosaccharide (scFOS; a well-established prebiotic source), pectin (PEC; used as a positive control), pelletized cellulose (PC; used as a negative control), beet pulp (BP; considered the gold standard fiber source in pet foods), CEF, and CHP, and 3 CEF:CHP blends (75:25% CEF:CHP [B1], 50:50% CEF:CHP [B2], and 25:75% CEF:CHP [B3]) were tested. Triplicate samples of each substrate were fermented for 0, 8, and 16 h after inoculation. A significant substrate × time interaction ($P < 0.05$) was observed for pH change and acetate, propionate, butyrate, and total SCFA concentrations. After 8 and 16 h, pH change was greatest for scFOS (–2.0 and –3.0, respectively) and smallest for PC (0.0 and –0.1, respectively). After 16 h, CEF had a greater butyrate concentration than CHP and all the CEF:CHP blends and it was not different than PEC. The substrate × time interaction was significant for bifidobacteria ($P < 0.05$) and lactobacilli ($P < 0.05$). After 8 h, bifidobacteria was greatest for BP and lowest for PC (12.7 and 10.0 log10 cfu/tube, respectively). After 16 h, PC had the lowest and scFOS had the greatest bifidobacteria (6.7 and 13.3 log10 cfu/tube, respectively). In general, CEF, CHP, and their blends had similar bifidobacteria populations after 8 and 16 h of fermentation when compared with BP and scFOS. After 16 h, lactobacilli populations were greatest for B1, B2, B3, BP, and scFOS, intermediate for PEC, and lowest for PC ($P < 0.05$). Overall, our data suggest that CEF had a butyrogenic effect and that CEF, CHP, and their blends had similar bifidobacteria and lactobacilli populations as popular prebiotic and fiber substrates. Future research should investigate the effects of CEF, CHP, and their blends on gastrointestinal health and fecal quality in dogs.

Key words: chicory pulp, coconut endosperm fiber, dog, fermentation, in vitro, microbiota

 J. Anim. Sci. 2015.93:370–376
doi:10.2527/jas2014-7962

INTRODUCTION

Dietary fibers are heterogeneous compounds, varying in their chemical composition and fermentative behavior. For this reason, the pet food industry is constantly searching for novel fiber sources and blends that have the potential to be moderately fermentable and to optimize the gastrointestinal health of pets.

Common sources of dietary fiber used in pet foods include beet pulp, a soluble and moderately fermentable fiber source, and cellulose, an insoluble, nonfermentable, and nonviscous fiber source. Short-chain fructooligosaccharide (**scFOS**) and inulin are also used as prebiotics in diets for dogs and cats. Chicory pulp (**CHP**) is the dried and ground product obtained after extraction of inulin by diffusion of the chicory root shreds. Chicory pulp is known by its high concentration of pectin and moderate concentration of inulin, with the latter being a well-documented prebiotic in domestic animals (Flickinger et al., 2003).

Coconut endosperm fiber (**CEF**) is the defatted coconut residue after milk extraction during the wet

[1]M. R. C. G., L. L. B., G. C. F., and K. S. S. have no conflict of interest. Y. M. and P. R. B. are employed by The Nutro Company.

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Received April 18, 2014.
Accepted November 11, 2014.

processing of fresh coconut (Trinidad et al., 2006), which contains approximately 28% ADF and 50% NDF. The outer skin and seed husks are first removed by a mechanical peeling process. Following the peeling process, the material is cold pressed and then heated before being pressed a second time.

When incorporated up to 25% of baking goods, the dietary fiber of coconut residue has been shown to be moderately fermentable and butyrogenic and to reduce glycemic index, serum total cholesterol, low-density lipoprotein, and triglycerides of humans with moderately hyperlipidemia (Trinidad et al., 2006). The desirable prebiotic effect of CHP in conjunction with the potential butyrogenic behavior of CEF may be beneficial to the gastrointestinal health of companion animals. Therefore, the objective of this study was to determine the effects of in vitro fermentation of CEF, CHP, and selective blends of these substrates on SCFA concentrations and changes in microbiota using canine fecal inocula.

MATERIALS AND METHODS

Substrates

Chicory pulp (ADM Alliance Nutrition, Effingham, IL), CEF (Stance Equine, Kenmore, QLD, Australia), 3 CEF:CHP blends, the 3 fiber references, and 1 established prebiotic were tested. The specific proportions of CHP and CEF used to produce the tested fiber blends as well as the resulting percentage of soluble and insoluble fiber concentrations and the ratio of soluble to insoluble fiber are presented in Table 1. The fiber references included pelletized cellulose (**PC**; J. Rettenmaier, Schoolcraft, MI), citrus pectin (**PEC**; high methoxy rapid set from TIC Gums, Belcamp, MI), and beet pulp (**BP**; Michigan Sugar Company, Bay City, MI). The prebiotic tested was scFOS (GTC Nutrition, Westchester, IL).

Chemical Analyses

Substrate samples were ground through a 2-mm screen in a Wiley Mill (model 4; Thomas Scientific, Swedesboro, NJ) and analyzed according to procedures by the American Organization for Analytical Chemists for DM and OM (AOAC, 2006; methods 934.01 and 942.05). Crude protein was calculated from Leco total N values (AOAC, 2006; method 992.15). Total lipid concentration (acid hydrolyzed fat [**AHF**]) of substrates was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Dietary fiber concentrations were determined according to Prosky et al. (1992).

Table 1. The combination of chicory pulp and coconut endosperm fiber

Fiber blend[1]	Blend percent soluble fiber	Blend percent insoluble fiber	Sol:Insol[2] ratio	Coconut endosperm fiber, %	Chicory pulp, %
B1	6.0	45.5	1:8	75	25
B2	9.5	48.3	1:5	50	50
B3	13.0	51.1	1:4	25	75

[1]B1 = 75:25% coconut endosperm fiber:chicory pulp; B2 = 50:50% coconut endosperm fiber:chicory pulp; B3 = 25:75% coconut endosperm fiber:chicory pulp.

[2]Sol = soluble fiber; Insol = insoluble fiber.

Samples to be analyzed for SCFA (2 mL) were mixed with 0.5 mL of 25% metaphosphoric acid (Erwin et al., 1961). Concentrations of SCFA were determined via gas–liquid chromatography. Briefly, concentrations of acetate, propionate, and butyrate were determined in the supernatant of the tubes using a Hewlett-Packard 5890A Series II gas chromatograph and a glass column (180 cm by 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100 mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven temperature, detector temperature, and injector temperature were approximately 125, 175, and 180°C, respectively. Short-chain fatty acid concentrations were corrected for by the quantities of SCFA produced in blank tubes. All samples were analyzed in duplicate, and an error of 5% or less was considered acceptable.

In Vitro Fermentation Procedures

Fiber substrates were fermented in vitro for 0, 8, and 16 h with the fecal inoculum obtained from 3 purpose-bred female dogs (Butler Farms, Clyde, NY) with hound bloodlines, a mean initial body weight of 20 kg, and a mean age of 3.5 yr. Dogs were individually housed in 1.2 by 2.4 m clean floor pens in a climate-controlled room at the animal care facility of the Edward R. Madigan Laboratory on the University of Illinois campus, Urbana, IL. All dogs were fed the same commercial diet (Proplan Sport All Life Stages; Purina, St. Louis, MO). Freshly voided feces from each of the 3 donors were maintained at 39°C and used to inoculate all substrate × time combinations in triplicate. Triplicate tubes containing no substrate also were fermented with each inoculum source and time point to enable appropriate corrections for OM disappearance, SCFA, and microbial populations not arising from the substrates themselves.

Aliquots (26 mL) of a semidefined medium, added to maintain microbial viability, were aseptically transferred into 50-mL tubes containing 300 mg of substrate or blend (Bourquin et al., 1993). Anaerobic

Table 2. Chemical composition of selected fiber substrates and blends

	Substrate[1]								
Item[2]	CEF	B1	B2	B3	CHP	BP	scFOS	PEC	PC
DM, %	92.9	91.9	91.2	90.6	89.6	90.2	95.6	92.0	94.5
	DM basis								
OM, %	93.4	92.9	92.2	91.3	97.9	92.8	100.0	97.9	99.6
CP, %	22.7	19.3	15.6	11.8	8.5	7.5	0.0	1.9	0.0
AHF, %	11.5	10.0	8.8	6.7	4.6	5.0	1.9	2.6	1.6
TDF, %	45.2	51.2	57.3	63.3	70.4	63.0	ND	59.4	98.3
Insoluble fiber, %	42.7	45.8	50.4	53.8	53.9	45.8	ND	0.0	96.2
Soluble fiber, %	2.5	5.4	6.9	9.5	16.5	17.2	ND	59.4	2.1
GE, kcal/g	4.8	4.6	4.4	4.2	4.1	4.1	4.0	3.9	4.2

[1]CEF = coconut endosperm fiber (100% coconut fiber); B1 = 75:25% coconut endosperm fiber:chicory pulp; B2 = 50:50% coconut endosperm fiber:chicory pulp; B3 = 25:75% coconut endosperm fiber:chicory pulp; CHP = chicory pulp (100%); BP = beet pulp (pelletized); scFOS = short-chain fructooligosaccharides; PEC = pectin (high methoxy from TIC Gums, Belcamp, MI); PC = pelletized cellulose.

[2]AHF = acid hydrolyzed fat; TDF = total dietary fiber.

conditions were maintained by sealing the tubes with rubber stoppers equipped with 1-way gas release valves. Fecal samples were pooled and then diluted 1:10 (wt/vol) in anaerobic dilution solution by blending it for 15 sec in a Waring blender (Waring Products, New Hartford, CT) under a stream of CO_2. Blended, diluted feces were filtered through 4 layers of cheesecloth and sealed in 125-mL serum bottles under CO_2.

Appropriate sample and blank tubes were aseptically inoculated with diluted feces (4 mL). Tubes were incubated at 39°C with periodic mixing for the respective fermentation times. At the appropriate time, tubes were removed from the 39°C incubation and immediately processed for analysis. A 2-mL subsample was taken from each 50 mL tube for SCFA analysis, and another 2 mL was frozen at –80°C for microbial analyses.

Quantitative Polymerase Chain Reaction

Escherichia coli, *Bifidobacterium* genus, *Lactobacillus* genus, and *Clostridium perfringens* were quantified via quantitative PCR (**qPCR**) using specific primers. Amplification was performed in a set of triplicate reactions for each bacterial group within each sample according to the procedures of DePlancke et al. (2002). For amplification, a 10-µL final volume containing 2x SYBR Green PCR Master Mix (Applied BioSystems, Foster City, CA), 15 pmol of each primer, and 10 ng of template DNA was used. Pure cultures of each bacterium were used to create a 5-fold dilution series (10×10^0 to 10×10^5) in triplicate from target species. Deoxyribonucleic acid from each serial dilution was extracted using a QIAamp DNA Stool Mini-kit (Qiagen, Valencia, CA) and amplified along with fecal DNA samples using a Taqman ABI PRISM 7900HT Sequence Detection System (Applied BioSystems). The number colony forming units of each standard curve serial dilution was determined by plating the *E. coli* grown on Luria-Bertani Medium (10 g/L tryptose, 5 g/L yeast extract, and 5 g/L NaCl [pH 7]), the *Lactobacillus* genus grown on Difco Lactobacilli MRS broth (Becton, Dickinson, and Company, Sparks, MD), and the *C. perfringens* and *Bifidobacterium* genus grown on Difco Reinforced Clostridial Medium (Becton, Dickinson, and Company). Cycle threshold values were plotted against standard curves for quantification (cfu/mL) of the target bacterial DNA from fermentation samples.

Statistical Analysis

Data were analyzed as a complete randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included substrate, time (length of fermentation), and substrate × time interaction. Differences among substrates were determined using least square means with Tukey adjustment to control for experiment-wise error. A probability of $P < 0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

Chemical Composition of Selected Fiber Substrates and Blends

All substrates were similar in DM and OM concentration (Table 2). Dry matter concentrations ranged from 89.6 (CHP) to 95.6% (scFOS). Short-chain fructooligosaccharide had the greatest OM concentration (100%) and 25:75% CEF:CHP [**B3**] the lowest (91.3%). Crude protein concentration varied among substrates, ranging from 22.7 (CEF) to 0.0% (scFOS and PC). Coconut endosperm fiber had the greatest AHF concentration (11.5%), whereas PC had the lowest (1.6%). Total dietary fiber (**TDF**) and its fractions also differed among substrates. Pelletized cellulose had the greatest TDF concentration (98.3%), predominantly comprising insoluble fiber (96.2%) and having the lowest soluble fiber (2.1%), whereas CEF had the lowest TDF concentration (45.2%), with 42.7% comprising insoluble fiber. Citrus PEC had the greatest soluble TDF fraction (59.4%). Total dietary fiber concentration of scFOS could not be determined because this substrate is completely solubilized in the TDF solution and therefore not captured in the filtration step of this procedure. All substrates had similar GE concentration, being

Table 3. Concentrations of SCFA (mmol/g DM) and pH change following 8 and 16 h of in vitro fermentation of selected fiber sources and fiber blends

	pH change		Acetate		Propionate		Butyrate		Total SCFA	
Substrate[1]	8 h	16 h	8 h	16 h	8 h	16 h	8 h	16 h	8 h	16 h
CEF	-0.5^{Ad}	-0.9^{Bd}	0.6^{Ad}	1.6^{Bd}	0.59^{Abcd}	1.12^{Bc}	0.09^{Aa}	0.27^{Ba}	0.41^{bc}	0.76^{b}
B1	-0.4^{Ade}	-0.9^{Bd}	0.8^{Acd}	1.5^{Bd}	0.60^{Abcd}	0.99^{Bcd}	0.08^{Aab}	0.16^{Bb}	0.37^{bc}	0.67^{b}
B2	-0.3^{Ae}	-0.9^{Bd}	0.8^{Acd}	2.0^{Bcd}	0.64^{Abc}	1.10^{Bc}	0.09^{Aa}	0.17^{Bb}	0.39^{bc}	0.97^{b}
B3	-0.4^{Ade}	-1.0^{Bcd}	0.6^{Ad}	1.8^{Bd}	0.47^{Ade}	0.91^{Bd}	0.06^{b}	0.12^{bc}	0.35^{bc}	0.84^{b}
CHP	-0.3^{Ae}	-1.0^{Bcd}	0.6^{Ad}	2.2^{Bbc}	0.44^{Ae}	0.96^{Bd}	0.05^{b}	0.11^{bc}	0.27^{Abc}	0.95^{Bb}
BP	-0.7^{Ac}	-1.1^{Bc}	1.1^{Ac}	2.3^{Bb}	0.55^{Ac}	1.04^{Bcd}	0.07^{ab}	0.12^{bc}	0.35^{bc}	0.91^{b}
scFOS	-2.0^{Aa}	-3.0^{Ba}	2.5^{Aa}	3.1^{Ba}	1.21^{Aa}	2.00^{Ba}	0.09^{a}	0.06^{c}	1.11^{a}	1.25^{ab}
PEC	-1.2^{Ab}	-2.1^{Bb}	1.6^{Ab}	3.4^{Ba}	0.71^{Ab}	1.72^{Bb}	0.08^{Aab}	0.27^{Ba}	0.69^{Aab}	1.58^{Ba}
PC	0.0^{f}	-0.1^{f}	0.0^{e}	0.0^{e}	0.01^{f}	0.01^{e}	0.00^{c}	0.00^{d}	0.02^{c}	0.00^{c}
SEM[2]	0.024		0.069		0.028		0.013		0.129	

[a–f]Within a column, means without a common letter differ (effect of substrate at specific time point; $P < 0.05$).

[A,B]Within a row, means without a common letter differ (effect of time within substrate; $P < 0.05$).

[1]CEF = coconut endosperm fiber (100% coconut fiber); B1 = 75:25% coconut endosperm fiber:chicory pulp; B2 = 50:50% coconut endosperm fiber:chicory pulp; B3 = 25:75% coconut endosperm fiber:chicory pulp; CHP = chicory pulp (100%); BP = beet pulp (pelletized); scFOS = short-chain fructooligosaccharides; PEC = pectin (high methoxy from TIC Gums, Belcamp, MI); PC = pelletized cellulose.

[2]The interaction of substrate and time of fermentation for pH change, acetate, propionate, butyrate, and total SCFA were significant ($P < 0.05$).

greatest for CEF (4.8 kcal/g) and lowest for PEC (3.9 kcal/g). As expected, blends of CEF and CHP (75:25% CEF:CHP [**B1**], 50:50% CEF:CHP [**B2**], and B3) presented a linear decrease or increase in the concentration of nutrients in relation to the inclusion percentage of either CEF or CHP in each blend. For example, B1 had greater concentrations of CP, AHF, and GE and lower concentration of TDF in comparison to B2 or B3.

Beet pulp, PEC, and PC were used as control substrates in this study as they are chemically well characterized and commonly used as fiber sources in pet foods. The chemical composition of the control substrates were similar to values previously reported in the literature (Kerr et al., 2013; Fischer et al., 2012; Bosch et al., 2008; Sunvold et al., 1995a,b,c). Usually, little variation in macronutrient composition is observed for purified cellulose and PEC, whereas BP can be more variable due to processing conditions.

Previous research examining the chemical composition of coconut flour has reported a TDF value of approximately 60%, with 56% insoluble and 4% soluble fiber (Trinidad et al., 2006). Another study reported TDF, CP, and crude fat concentrations of 38.0, 21.7, and 8.4%, respectively (Gunathilake et al., 2009). Even though coconut flour is also a byproduct of the coconut milk industry, differences in processing conditions could result in variation in the chemical composition of the byproducts. In the current study, CEF had an intermediate TDF concentration (45.2%) when compared to the previous studies but a similar insoluble to soluble fiber ratio. In addition, CP concentration was also comparable to previous research, while the fat concentration was slightly greater (11.5%), which could potentially be due to differences in the fat extraction method used in the current study (AHF) vs. the crude fat method used in the previous study (Gunathilake et al., 2009). Processing conditions in the manufacture of these byproducts may also contribute to nutrient variability.

Fermentation Metabolites and Microbiota after In Vitro Fermentation of Selected Fiber Substrates and Blends

A significant interaction ($P < 0.05$) of substrate and time of fermentation was observed for pH change and acetate, propionate, butyrate, and total SCFA (Table 3). Change in pH was significant ($P < 0.05$) over time within substrate, except for PC. At 8 and 16 h of fermentation, pH change was greatest ($P < 0.05$) for scFOS (–2.0 and –3.0, respectively) and lowest for PC (0.0 and –0.1, respectively). At 16 h, B3 and CHP had similar pH changes when compared to BP ($P > 0.05$). Similar to the changes in pH, acetate and propionate concentrations were greatest ($P < 0.05$) for scFOS (2.53 and 1.21 mmol/g DM, respectively) at 8 h of fermentation. Citrus PEC and scFOS had the greatest ($P < 0.05$) acetate concentrations (3.43 and 3.14 mmol/g DM, respectively), while scFOS had the greatest ($P < 0.05$) propionate concentration (2.0 mmol/g DM) at 16 h of fermentation. In addition, all substrates had greater ($P < 0.05$) production of SCFA at 16 h than at 8 h, except for PC. The latter had the lowest concentration of all SCFA (acetate, propionate, and butyrate) when compared to all other fiber substrates and blends tested ($P < 0.05$). At 8 h postfermentation, CEF, B1, B2, BP, scFOS, and PEC had similar butyrate concentrations, varying between 0.07 and 0.09 mmol/g DM. In contrast, butyrate concentration was greatest for CEF and PEC at 16 h, with both having

Table 4. Microbiota populations (log_{10} cfu/tube) following 8 and 16 h of in vitro fermentation of selected fiber sources and fiber blends[1]

	Bifidobacteria		Lactobacilli		*Escherichia coli**	
Substrate[2]	8 h	16 h	8 h	16 h	8 h	16 h
CEF	10.09^{ab}	10.42^{bc}	13.21^{c}	13.81^{ab}	10.07	10.06
B1	10.35^{ab}	12.34^{ab}	13.30^{bc}	14.16^{a}	10.21	10.49
B2	11.85^{ab}	12.37^{ab}	13.94^{abc}	14.26^{a}	10.51	10.66
B3	11.95^{ab}	12.47^{ab}	14.34^{a}	14.22^{a}	10.81	10.67
CHP	12.38^{ab}	12.67^{ab}	14.31^{a}	14.06^{ab}	10.73	10.54
BP	12.73^{a}	11.94^{ab}	14.47^{a}	14.17^{a}	10.66	10.32
scFOS	12.44^{ab}	13.26^{a}	14.05^{ab}	14.31^{a}	10.48	10.43
PEC	10.55^{ab}	9.09^{cd}	13.69^{abc}	13.26^{b}	10.11	9.80
PC	9.99^{Ab}	6.68^{Bd}	13.50^{Abc}	12.33^{Bc}	10.05	6.01
SEM[3]	0.561		0.149		0.677	

$^{a-c}$Within a column, means without a common letter differ (effect of substrate at specific time point; $P < 0.05$).

^{A,B}Within a row, means without a common letter differ (effect of time within substrate; $P < 0.05$).

[1]Mean values ± SD for 0 h tubes are 9.07 ± 0.41 log_{10} cfu/tube for bifidobacteria, 12.89 ± 0.29 log_{10} cfu/tube for lactobacilli, and 8.96 ± 0.56 log_{10} cfu/tube for *E. coli*. Concentration of *Clostridium perfringens* was below minimal detectable level.

[2]CEF = coconut endosperm fiber (100% coconut fiber); B1 = 75:25% coconut endosperm fiber:chicory pulp; B2 = 50:50% coconut endosperm fiber:chicory pulp; B3 = 25:75% coconut endosperm fiber:chicory pulp; CHP = chicory pulp (100%); BP = beet pulp (pelletized); scFOS = short-chain fructooligosaccharides; PEC = pectin (high methoxy from TIC Gums, Belcamp, MI); PC = pelletized cellulose.

[3]The interaction of substrate and time of fermentation for bifidobacteria and lactobacilli was significant ($P < 0.05$).

*Treatment effect ($P < 0.05$).

a concentration of 0.27 mmol/g DM. After 8 and 16 h of fermentation, total SCFA production was greatest for scFOS and PEC (1.11 and 1.58 mmol/g DM, respectively) and lowest for PC (0.02 and 0.00 mmol/g DM, respectively). In general, CEF, CHP, and their blends (B1, B2, and B3) had ($P > 0.05$) total SCFA production similar to BP and PEC. The observed increase in fecal SCFA concentration, especially butyrate, is a positive finding as butyrate is used by the colonocytes as a primary source of energy and has also been associated with gut health and positive shifts in hindgut microbiota.

In this experiment, an in vitro fermentation assay was performed to mimic the in vivo fermentative process of the selected fiber sources and blends in dogs. The in vitro assay is an effective method to estimate the fermentative behavior of fiber sources, and it is also a faster, cheaper, and safer method to assess the efficacy and adequacy of novel fibers as potential ingredients to be incorporated in pet foods. After 8 and 16 h of in vitro fermentation, scFOS and PEC resulted in the greatest pH change and greatest SCFA concentrations. This outcome was not surprising because both substrates consist of predominantly soluble and highly fermentable fibers. Coconut endosperm fiber, CHP, and their blends were moderately fermentable, resulting in intermediate SCFA concentrations and pH change. After 16 h of in vitro fermentation, CEF resulted in the greatest butyrate concentration, along with PEC. Augmented butyrate concentration also was observed in blends containing greater proportion of CEF (B1 and B2) when compared to the scFOS and PC. Little research is available on the fermentation characteristics of coconut fiber sources. Trinidad et al. (2006), using human fecal inoculum, investigated the in vitro fermentative profile of coconut flour fiber isolate. In that study, an altered molar ratio among acetate:propionate:butyrate (39:13:48%) was observed in contrast to the normal acetate:propionate:butyrate molar ratio observed for most of the fiber sources (60:20:20%). While an increased production of butyrate from CEF was observed when compared to the other fiber sources tested in the current study, the increase was not robust enough to change the pattern of the SCFA molar ratio acetate:propionate:butyrate (53:38:9%). The differences observed between these studies could potentially be explained by different inoculum sources and fecal microbiota (human vs. canine) and differences in the coconut fiber products tested.

Citrus PEC 12 and 24 h postfermentation with canine fecal inoculum had similar acetate (3.29 and 3.31 mmol/g OM, respectively) and butyrate (0.35 and 0.37 mmol/g OM, respectively) concentrations to the current study at 16 h postfermentation. However, propionate concentration differed (0.79 and 1.30 mmol/g OM; Sunvold et al., 1995a,c). Those studies reported negligible fermentation of cellulose, which is in agreement with our results. Compared to our results, Bosch et al. (2008) reported similar acetate concentration, after 8 h of in vitro fermentation, for PEC (1.59 mmol/g OM) but greater for BP (1.99 mmol/g OM). The same authors also reported similar propionate concentrations for BP (0.56 mmol/g OM) but lower propionate concentrations for PEC (0.38 mmol/g OM). However, butyrate concentrations were greater for PEC (0.26 mmol/g OM) and BP (0.33 mmol/g OM; Bosch et al., 2008) when compared to the current study.

For scFOS, similar acetate (2.34 mmol/g OM) and propionate (0.92 mmol/g OM) concentrations were observed 12 h postfermentation by Vickers et al. (2001). Faber et al. (2011) reported lower acetate (1.43 mmol/g DM), propionate (1.02 mmol/g DM), butyrate (0.00 mmol/g DM), and total SCFA (0.73 mmol/g DM) concentrations and pH change (–1.22) after 12 h of in vitro fermentation using canine inoculum. The discrepancy observed between that study and the current one could be due to differences in time allowed for in vitro fermentation (12 vs. 16 h) and source of scFOS. The source of scFOS used in the study by Faber et al. (2011) was of

low purity, resulting in partial in vitro hydrolytic digestion of this substrate that led to less substrate present for in vitro fermentation. In contrast, Bosch et al. (2008) reported greater concentrations of acetate (4.57 mmol/g OM), propionate (3.07 mmol/g OM), and butyrate (0.49 mmol/g DM) after 8 h of in vitro fermentation of scFOS (degree of polymerization [**DP**] = 4), using canine inoculum. The small DP of this substrate could have resulted in faster rate of fermentation, in contrast to the scFOS source tested in the current study (DP < 10).

Chicory pulp contains inulin, characterized as a moderately soluble and fermentable fiber. Previous research using canine fecal inoculum reported similar acetate (2.65 mmol/g OM) and propionate (1.10 mmol/g OM) concentrations but greater butyrate (0.37 mmol/g OM) concentration after 12 h of in vitro fermentation (Vickers et al., 2001).

The interaction of substrate and time was significant ($P > 0.05$) for bifidobacteria and lactobacilli ($P < 0.05$) but not for *E. coli*. Bifidobacteria and lactobacilli populations changed over time only for PC ($P < 0.05$), whereas all other fiber substrates and blends had similar microbiota populations after 8 and 16 h of in vitro fermentation (Table 4). At 8 h, bifidobacteria was greatest for BP (12.73 $\log_{10}$ cfu/tube) and lowest for PC. At 16 h, PC still had the lowest bifidobacteria concentrations; however, scFOS had the greatest. In general, CEF, CHP, and their blends had similar bifidobacteria populations after 8 and 16 h of fermentation when compared to BP and scFOS. Citrus PEC, at 16 h postfermentation, had lower ($P < 0.05$) bifidobacteria concentrations when compared to most of the blends but was similar to CEF. Lactobacilli counts over time within substrate differed only for PC ($P < 0.05$). At 8 h, lactobacilli counts were greatest for B3, CHP, and BP (14.34, 14.31, and 14.47 log10 cfu/tube, respectively) and did not differ from scFOS, B3, and PEC. Lactobacilli counts were lowest for CEF (13.21 $\log_{10}$ cfu/tube), which did not differ from B1, B2, PEC, and PC. Lactobacilli populations after 16 h of fermentation were greatest for B1, B2, B3, BP, and scFOS; intermediate for PEC; and lowest for PC ($P < 0.05$). A treatment effect was observed for *E. coli* ($P < 0.05$), as it was lower for PC and greater for B1, B2, B3, CHP, BP, and scFOS. The observed decrease in microbial populations over time for PC was likely due to the absence of substrate to be fermented by the bacteria because of the insoluble and nonfermentable characteristics of this fiber source.

To our knowledge, the effects of coconut fiber on the gut microbiota of dogs have not been studied. Few studies have examined the effect of BP, cellulose, and fructooligosaccharide on the canine fecal microbial population. Middelbos et al. (2007) noted a beneficial effect fecal bifidobacteria population, determined by qPCR, of dogs fed a diet containing 2.5% of BP, whereas no difference was observed between a control (no fiber supplementation) and a 2.5% cellulose diet. Swanson et al. (2002) did not observe any beneficial effects on the fecal microbial populations (bifidobacteria, lactobacilli, *C. perfringens*, and *E. coli*, determined by using sterile agar gels) of dogs fed a scFOS diet when compared with a control diet in which cellulose was the predominant fiber source. In the current study, the bacterial populations were, generally, slightly greater than the 2 aforementioned studies.

Overall, our data suggest that CEF had a butyrogenic effect and that CEF, CHP, and their blends resulted in similar total SCFA concentrations and bifidobacteria and lactobacilli populations as popular prebiotic and fiber substrates used by the pet food industry. Future research should investigate the effects of processing on CEF, CHP, and their blends as the harsh conditions of extrusion or retorting could alter their chemical and fermentative characteristics. Further studies are also warranted to examine the effect of these novel fiber sources and blends on gastrointestinal health and tolerance and fecal quality in dogs.

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