Journal homepage: http://www.sasas.co.za/journals

South African J. Anim. Sci. 2019, 49 (No. 2)

Co-products in maize-soybean growing-pig diets altered *in vitro* enzymatic insoluble fibre hydrolysis and fermentation in relation to botanical origin

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(Received 20 June 2018; Accepted 17 December 2018; First published online 2 April 2019)

Abstract

The study examined the effects of botanical factors and fermentation-based, high-level dilution of co-product feeds in maize–soybean growing-pig diets on enzymatic insoluble fibre hydrolysis and fermentation. Feed insoluble fibre residues that were recovered after pepsin-pancreatin digestion were subjected to Roxazyme® G2 (Roxazyme) versus Viscozyme L® V2010 (control) hydrolysis, and to 64-hour fermentation using pig faecal inoculum. The control diet was a 13 MJ metabolizable energy, 141 g total dietary fibre/kg dry matter maize-meal/hominy chop-soybean diet, which was diluted with maize cob, soybean hulls, barley brewer's grains, lucerne hay or wheat bran in 12 MJ metabolizable energy, 246 g total dietary fibre/kg dry matter iso-nutrient, single co-product test diets. Fermentable insoluble fibre was employed in a computerized iterative selection of ingredients in two iso-nutrient 11 MJ metabolizable energy 319 total dietary fibre/kg dry matter mixed fibre test diets for maximal contrast (high (HF) versus low (LF)) in fermentability. Insoluble fibre extractive pepsin-pancreatin digestibility differed between feed ingredients, and the single co-product test diets, and between the HF and LF mixed co-product diets. Fibre digestibility depended on both the origin and enzyme, with interaction, whereby carbohydrases expressed similar low (0.04 - 0.05) insoluble fibre digestibility for maize cob, moderate (0.12) digestibility for wheat bran and brewer's grain, with inferior Roxazyme G2 digestibility for maize hominy chop (0.02 vs 0.10) and meal (0.04 vs 0.16), dehulled soybean meal (0.02 vs 0.17), lucerne hay (0.08 vs 0.18), and soybean hulls (0.05 vs 0.33). Co-product-enzyme affinities were expressed in single fibre diets. Low Roxazyme-basal fibre affinity limited its comparative single co-product (0.03 - 0.07 vs 0.16 - 0.22) HF (0.07 vs 0.17) and LF (0.4 vs 0.20) dietary fibre digestibility. Screening for HF/LF did not affect enzymatic digestion, though enzyme combination increased HF, but not LF digestibility. Gas and short chain fatty acid production predicted fermentability proportionately in the declining order of dehulled soybean ≥ maize ≥ soy hulls ≥ maize hominy chop > wheat bran >lucerne hay ≥ brewer's grain = maize cob. Induced HF and LF contrast was significant. Co-product fibre enrichment decreased fermentability for all except the soy hull and HF diets. Cereal fibre yielded proportionately less acetate, with more propionate and butyrate, and a greater butyrate shift for maize fibre. The HF fibre induced more ACE and less butyrate. Biomarkers of deleterious proteolytic fermentation were high for lucerne (iso-butyrate) and soy hulls (iso-valerate). In conclusion, high-level and fermentation based co-product feed dilution into maize-soybean growing pig diets altered enzymatic insoluble fibre hydrolysis and fermentation in relation to botanical origin. Roxazyme expressed weak hydrolytic potency on maize and soybean insoluble fibre.

Keywords: fermentation gas, fermentation kinetics, fibre fermentability, insoluble non-starch polysaccharides, non-starch polysaccharide degrading enzymes, proteolytic fermentation, short-chain fatty acids

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URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v49i2.1

Introduction

The processing of food grains into flour, vegetable oil, alcohol and biofuels offloads quantitatively variable, chemically diverse non-starch polysaccharides (NSP) into co-products (Bach Knudsen, 2014). Rapidly increasing human demand and random adverse climate change-related events are disrupting global grain markets, forcing producers into greater dependency on co-products for sustainable, cost-effective pig feeding strategies. Chemical and quantitative heterogeneity in co-product fibre and the consequent variable impact on nutrient extraction (Bach Knudsen, 2014; Swiatkiewicz *et al*., 2016) disturb established precision-feeding systems, imposing risks on animal productivity and the environmental footprint (Zijlstra & Beltranena, 2013; Woyengo *et al*., 2014).

Fitting co-products into efficient modern pig feeding systems requires effective evaluative methods to facilitate processing, and selective and calibrated dietary inclusion for optimal biological, economic and environmental outcomes. In growing pigs, strategic co-product feeding primarily involves targeting maximal fermentable fibre (Anguita *et al*., 2006; Gutierrez *et al*., 2013; Iyayi & Odeola, 2015) to offset fibre-induced nutrient and digestive metabolic energy wastage (Noblet & Le Goff., 2001). Emerging evidence supports the consideration of dietary polysaccharides that promote gut health (Aumiller *et al.*, 2015; Agyekum & Nyachoti, 2017; Celi *et al*., 2018). Prebiotic fibre includes resistant starch, galacto and fructo oligosaccharides, mixed-linked β-glucans and soluble arabinoxylans (Aumiller *et al*., 2015). Beyond prebiosis, saccharolytic fermentation produces short chain fatty acids (SCFA), which beneficially modulate epithelial cell, and peripheral tissue metabolism (Byrne *et al*., 2015; Pieper *et al.*, 2016). Butyrate (BUT) is the most enterotrophic and primary colonocyte energy substrate (Hamer *et al.*, 2008; Tonel *et al*., 2010). In the intestinal epithelium, SCFA modulate nutrient transporter gene expression (Daly & Shirazi-Beechey, 2006) digestive enzyme secretion (Mangian & Tappenden, 2009) and mucosal immune homeostasis (Pieper *et al*., 2016). Peripherally, SCFA modulate satiety (Sleeth *et al*., 2010; Byrne *et al*., 2015), glucose (Theil *et al*., 2011) and lipid (Fushimi *et al*., 2006) metabolism to increase intermediary energy harvesting (Byrne *et al*., 2015). Saccharolysis spares proteolytic fermentation, which tends to intensify with depletion of fermentable NSP distal to the colon (Yao *et al*., 2016), thereby reducing ammonia (Zervas & Zijlstra, 2002; Nahm, 2003), odours (Rideout *et al*., 2004) and toxic metabolites (Bach Knudsen, 2015). Biomarkers of deleterious proteolytic fermentation are the branched chain fatty acids (BCFA) iso-BUT, 2-methyl-butyrate and iso-valerate (Ríos-Covián, *et al*., 2016; Celi *et al*., 2018), the metabolites of branched-chain amino acids, valine, leucine and isoleucine (Verbeke *et al*., 2015; Pieper *et al.*, 2016), respectively.

Beneficial fermentation is a concept conceived and still largely confined to studies on temperate cereal test diets that are uniquely endowed with native or purified readily fermentable NSP (Bach Knudsen, 2014). However, the merging evidence increasingly links beneficial microbiota (*Bifidobacterium* and *Lactobacilli* spp) and fermentation patterns to a broader spectrum of fibre types (e.g. reviews by Molist *et al*., 2014; Aumiller *et al*., 2015; Jha & Berrocoso, 2016; Simpson & Campbell, 2015), which might include fermentable insoluble NSP (iNSP) in maize and all secondary plant tissues (Bach Knudsen, 2015). Among feeds, the fermentation of iNSP occurs in complex fashion, being subject to the primary and secondary structures and lignification, attributes which depend on the species, cultivar and morphological origin (Bach Knudsen, 1997; 2014), and are subject to environmental influences (Collins *et al*., 2010; De Vries *et al*., 2012; Aumiller *et al*., 2015). Whereas hydrolytic and fermentative degradation rapidly deplete the soluble NSP within the upper digestive tract (Noblet & Le Goff, 2001; Back Knudsen, 2014), the iNSP are degraded to lesser and highly variable extents, mostly at the distal colon (Bach Knudsen, 1997; Noblet & Le Goff, 2001). In monogastrics, exogenous polysaccharide degrading enzymes potentially improve the nutritional efficacy of complexly fibrous diets through substrate-dependent iNSP depolymerization to randomly polydisperse oligosaccharides (Choct *et al*., 2006). However, in pigs, current enzymes seem impotent, particularly on maize-soybean diets (Willamil *et al*., 2012; Agyekum & Nyachoti, 2017), more so when the diets contain substantial co-product fibre (Kerr & Shurson, 2013).

Fibre-gut microbe-pig interactions are complex (Zijilstra *et al*., 2010; Pieper *et al*., 2016). The best insight into the underpinning mechanics might be through a modelling approach (Zijilstra *et al*., 2010). Modelling in turn requires potent methods to mimic porcine fibre digestion for robust prediction of the biogenic effects (Lee *at al.,* 2018), and to match enzymes to dietary NSP (Park *et al.*, 2016a). Effective methods should be standardised, low cost, rapid and broadly applicable across a range of substrates. Pig fibre nutrition research employs a three-step (gastric-ileal-colon) *in vitro* hydrolytic digestive procedure (Boisen & Fernandez, 1997). Mature sow rectal faecal microbiota inoculum are employed in batch fermentation of washed fibrous extracts from pepsin (gastric) + pancreatin (small intestinal) feed digestion (Bindelle *et al*., 2007), from which total SCFA and cumulative gas jointly predict insoluble fibre fermentability (Bindelle *et al*., 2007; Jonathan *et al*., 2012). Endpoint SCFA and microbiota (Verbeke *et al*., 2015; Agyekum & Nyachoti, 2017) and the gas production kinetics (France *et al.,* 1993; Groot *et al.*, 1996) provide important

insights into gut functionality. With wider testing, the approach might provide a practical evaluative tool for optimal co-product feeding.

The present study therefore employed the *in vitro* porcine digestion model to evaluate how the botanical origin, high-level and fermentation-based co-product dilution into maize-soybean soybean diets influence exogenous enzymatic and fermentative fibre degradation.

Materials and Methods

The procedures used for feed analyses, and enzymatic and fermentative digestion were approved by the Ethics Committees of the University of South Africa (Ref: 20111/CAES/024R1) and the Agricultural Research Council of South Africa (APIEC10/20).

Test feeds (Table 1) included maize *(Zea mays)*, its hominy chop and cob, dehulled soybean *(Glycine max)*, and the hulls, brewer's barley (*Hordeum vulgare* L) grains, lucerne *(Medicago sativa)* hay, and wheat *(Triticum aestivum)* bran. Representative feeds were sampled from coarse-milled bulk factory commercial feedstocks at OPTI Feeds (Pty) Ltd, Lichtenburg, South Africa, which were composites of co-product feeds sourced from grain produced in various agronomic systems. Feeds were milled through a 1-mm screen in an Ika analytical mill and oven-dried for 18 hours in a forced draught oven at 100 ºC.

A standard maize soybean mix (control) and two sets of test diets were formulated, in which treatments differed in the fibre types (Table 2a, 2b), but were equally balanced for essential nutrients according to the NRC (1998). Metabolizable energy was quantified from chemical components (Noblet & Perez, 1993) (equation 30 and 43). In single co-product test diets, fibrous co-product feeds were singly incorporated into a standard (control) maize grain-hominy chop-soybean growing pig diet. In a second set of mixed co-product diets, the ingredients were screened within least cost diet formulation based on fermentable fibre content (g pepsin-pancreatin digesta/kg dry matter feed x mL fermentation gas/kg dry matter of digesta) to maximize contrast (high (HF) versus low (LF)) in fibre fermentability.

Feeds were analysed using AOAC (2006) methods for dry matter (934.01), crude protein (990.03), ether extract (942.05), ash (920.39) and total dietary fibre (991.43). Neutral and acid detergent fibre were analysed according to Van Soest *et al*. (1991). Gross energy (GE) was determined by combustion in a DDS isothermal CP500 bomb calorimeter.

Feeds were enzymatically digested in a three-step porcine digestion model (Boisen & Fernandez, 1997), with modifications for maximal digestion in an ANKOM Daisy^{II} incubator. The incubator setup consisted of four digestion chambers, in one of which, for each enzyme digestive step, samples were sequentially incubated in separate batches of feed ingredients [(3 replicates x 8 feeds) + 1 blank (no sample)], single [4 replicates x (5 test diets + 1 control) + 1 blank] and mixed co-product diets (12 replicates x (HF + LF test diets) +1 blank]. The 39 ± 0.5 °C digestion of 0.5 ± 0.01 g dry matter ground feed samples in 99% acetone-washed ANKOM® F57 filter bags was performed as follows.

Pepsin digestion: 2-hour incubation in [600 mL 0.1 M, pH 6.0 phosphate buffer + 240 mL 0.2 M HCl (adjusted to 2.0 pH using 1 M solutions of HCl or NaOH)] + 0.6 g fresh pepsin solution (porcine, 2000 FIP-U/g, Merck no. 7190) + 12 mL of chloramphenicol (Sigma no. C-0378, 0.5 g/100 mL ethanol).

Pancreatin digestion: 5-hour incubation after topping up step 1 digestion medium with [240 mL of phosphate buffer (0.2 M, pH 6.8) + 120 mL 0.6 M NaOH) (pH adjusted to 6.8 using 1 M HCl or NaOH)] + 2.4 g pancreatin (porcine, grade IV, Sigma no. P-1750).

Fibrolysis: 24-hour incubation in 750 mL fresh phosphate buffer (0.1 M, pH 4.8) + either 12 mL Viscozyme (Viscozyme L® V2010 120 L, mixture including β-glucanase, xylanase, arabinase, cellulase (120 FBG/g)), Novo Nordisk, Bagsvaerd), or 0.17 g Roxazyme (Roxazyme ® G2 DSM Pvt Ltd, with endo-1,4-β-glucanase (8000 U/g), endo-1,3 (4)-β-glucanase (18000 U/g) and endo-1,3 (4)-β-xylanase (26000 U/g). For HF and LF fibrous residues, the cocktails were mixed in a third treatment to test synergistic/complementary enzyme effects.

Dry matter lost after the second and third digestion steps was estimated gravimetrically by washing off fat and soluble residue through sequential gentle rinsing in warm tap water, 95% ethanol and 99% acetone, before forced air oven-drying at 85 °C for 18 hours.

Table 1 Variation in dry matter (g/kg), chemical (g/kg dry matter) and energy (MJ/kg dry matter) composition among test pig feed ingredients of diverse botanical origin

Components	Feed ingredient							
	Maize grain	Maize hominy chop	Maize cobs	Brewer's grains	Wheat bran	Lucerne hay	Dehulled soybean	Soybean hulls
Dry matter	901	896	944	875	902	946	904	913
Organic matter	989	979	944	956	951	910	929	951
Gross energy	16	18	17	22	17	16	18	16
Crude protein	62	97	42	241	167	161	515	119
Ether extract	36	71	11	73	32	17	13	11
Crude fibre	13	59	352	212	90	381	43	421
Neutral detergent fibre	121	331	811	752	443	518	148	703
Acid detergent fibre	29	103	669	280	131	454	66	542
Hemi-cellulose[1]	92	228	142	472	312	64	82	161
Acid detergent lignin	20	18	47	57	41	94	13	36
Cellulose[2]	9	85	622	223	90	360	53	506
Soluble dietary fibre	4	9	5	18	17	26	7	14
Insoluble dietary fibre	113	249	871	627	363	600	152	773
Total dietary fibre	118	258	876	646	380	627	158	787

[1] Neutral detergent fibre − acid detergent fibre
[2] Acid detergent fibre − acid detergent lignin

Table 2a Feed ingredient (g/kg) composition of experimental growing pig diets

Ingredients	Diets							
	Standard	Added dietary insoluble fibre type						
		Soybean hulls	Maize cobs	Wheat bran	Lucerne hay	Brewer's grains	Low fermentability	High fermentability
Maize	560	390	394	209	383	401	273	282
Maize hominy chop	65	65	65	65	65	65	65	65
Maize cob	-		142				108	57
Dehulled soybean	300	329	350	262	303	267	280	322
Soybean hulls		169						203
Brewer's grains						219	202	
Wheat bran	25			423			25	25
Lucerne hay					204			
DL methionine	2	2	2	2	2	2	2	2
Limestone flour	26	23	24	24	21	25	25	22
L-lysine HCl	1	-						
Monocalcium phosphate	11	11	11	3	11	10	10	1.1
Sodium chloride	10	10	10	10	10	10	10	10
Vitamin & mineral premix	2	2	2	2	2	2	2	2

Table 2b Chemical (g/kg dry matter) and energy (MJ/kg dry matter) composition of experimental growing pig diets

Components	Diets							
	Standard	Added dietary insoluble fibre type						
		Soybean hulls	Maize cobs	Wheat bran	Lucerne hay	Brewer's grains	Low fermentability	High fermentability
Dry matter	897	900	904	902	901	906	926	921
Organic matter	922	907	912	921	905	919	921	918
Ether extract	30	25	25	29	26	38	32	22
Crude protein	219	233	229	233	233	232	227	226
Starch							286	278
Gross energy	15	16	17	17	16	15	17.7	17.1
Metabolizable energy	13	12	12	12	12	12	11	11
Neutral detergent fibre	146	239	242	274	224	269	337	331
Acid detergent fibre	47	133	141	86	135	96	163	187
Hemicellulose[1]	99	106	101	188	89	173	174	144
Acid detergent lignin	17	20	21	26	33	25	27	22
Cellulose[2]	30	113	120	60	102	71	310	309
Soluble dietary fibre	6	7	5	10	10	8	8	7
Insoluble dietary fibre	135	243	243	234	234	235	312	310
Total dietary fibre	141	250	249	244	244	243	320	318

[1] Neutral detergent fibre - acid detergent fibre
[2] Acid detergent fibre - acid detergent lignin

Washed fibre isolates were fermented in pig faecal inoculum, according to Bindelle *et al*. (2007), with modifications for optimum fermentation in an ANKOM^{RF} automated gas measurement system. The setup had 10 x 200 mL fermentation modules, in which substrates were fermented separately in different runs, namely [2 blanks (no substrate) + 8 feed ingredients], [2 blanks + 1 control + 5 single fibre diets], 2 [1 blank + 1 control + 1 HF + 1 HF mixed fibre diets]. The blank contained no substrate to estimate particle attachment. Fermentation was optimized by standard 39 °C anaerobic procedures.

Buffer: Marten & Barnes (1980) phosphate buffer, according to the Ankom Gas production system manual.

Inoculum: Pooled rectal faecal aliquots from three healthy mature Large White-Landrace crossbred sows on a standard antibiotic-free dry sow ration, homogenized in batches of 60 g in 400 mL buffer by 60-second stomacher pummelling, filtered through four layers of mutton cloth.

Fermentation: 0.75 g ± 0.001 g dry matter substrate + 100 mL buffer + 50 mL inoculum, 64-hour cumulative SCFA and gas production, gas measured by pressure-volume transduction at five-minute intervals.

Fermentation was terminated by immersing the modules in iced water for 15 minutes. Representative fermentation residues were transferred into airtight vials, and frozen-stored at -20 °C. To determine the SCFA and BCFA, frozen residues were thawed and purified by passing through Cameo 30 (0.45 µm) filters. Aliquots of the filtrate were injected into a Varian 3300 FID detector gas chromatograph using a CP Wax 58 (FFAP) CB Cat no 7654 column (25 m, 0.53 mm, 2.0 µm) in which helium was the carrier gas. The column temperature programme started at 50 °C for 2 minutes, increasing at 15 °C per minute to a constant final temperature of 190 °C for 5 minutes.

The kinetics of fermentation gas production were described by fitting a nonlinear, monophasic algorithm (Groot *et al*., 1996) using the general linear models of SAS (2010):

$$\mathrm{GV} = \frac{A}{\left(1 + \frac{T½}{T}\right)^2}$$

where: GV: gas volume (mL/g dry matter) at time T
A: gas volume (mL/g dry matter) at T = ∞
T: time (hours)
$T_{½}$: time (hours), when GV = A/2

Factorial ANOVA of fermentation parameters (Model I) and enzymatic digestibility coefficients (Model II) were performed using the PROC MIXED procedures of SAS (2010):

$$Y_i = \mu + \alpha_i + e_i \qquad \text{Model I}$$
$$Y_{ij} = \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij} \qquad \text{Model II}$$

where: µ defined the overall mean
α_i the effect of the fibre type
β_j the enzyme effect
$(\alpha\beta)_{ij}$ the fibre-enzyme interactions
i and j the respective treatment levels
e_i, and e_{ij}, the random errors

Treatment means were separated using the Bonferroni *t*-test at *P* = 0.05.

Results

Feed pepsin-pancreatin digestibility (Table 3) differed among feed ingredients (*P* <0.001), the single co-product test diets (*P* <0.0001), and the HF and LF mixed co-product diets (*P* <0.001). Partial multi-enzymatic insoluble fibre digestibility (Table 4) depended on both the origin (*P* <0.001) and the enzyme (*P* <0.001), with interaction (*P* <0.001), whereby the enzymes had similarly (*P* >0.05) low digestibility of maize cob fibre and moderate digestibility of wheat bran and brewer's grain, with inferior (*P* <0.05) Roxazyme digestibility of maize hominy chop, maize meal, dehulled soybean meal, lucerne hay, and soybean hull fibre. The pattern of enzyme-feed fibre affinities was expressed in the single fibre co-product diets. Inferior (*P* <0.05) Roxazyme basal fibre digestibility limited (*P* <0.05) the digestibility of both the single and mixed co-product-maize-soybean dietary fibre. While the HF/LF feed screening resulted in similar

enzymatic digestibility, the combination of the enzyme products increased (P <05) the digestion of HF, but not of LF fibre.

Table 3 Pepsin-pancreatin feed digestibility for insoluble residue extraction (n = 36)

	Mean	SEM	*P*-values
Feed ingredients			
Brewer's grains	0.31[f]	0.014	<0.001
Maize cobs	0.16[g]		
Maize hominy chop	0.71[c]		
Maize	0.75[b]		
Dehulled soybean meal	0.80[a]		
Lucerne hay	0.35[e]		
Soybean hulls	0.29[f]		
Wheat bran	0.58[d]		
Standard[1] and single[2] fibre diets			
Standard	0.70[a]	0.003	<0.0001
Brewer's grains	0.64[c]		
Maize cobs	0.68[b]		
Lucerne hay	0.67[bc]		
Soybean hulls	0.65[c]		
Wheat bran	0.68[b]		
Mixed fibre diets[3]			
Low fermentability	0.55[b]	0.005	<0.001
High fermentability	0.60[a]		

[abcdf] For each set of means, values that do not share a common superscript are different at P <0.05
SEM: Standard error of the mean
[1] 13 MJ metabolisable energy, 141 g total dietary fibre/kg dry matter
[2] 12 MJ metabolisable energy, 246 g total dietary fibre/kg dry matter, added fibre = brewer's grains, lucerne hay, maize cobs, soy hulls or wheat bran
[3] 11 MJ metabolisable energy, 319 ± 1.4 g total dietary fibre/kg dry matter, ingredients screened for maximal contrast in fibre fermentability = mL fermentation gas/kg dry matter

Individual feed insoluble fibre fermentation gas and SCFA production (Table 5) decreased (P <0.05) proportionately in the order of dehulled soybean ≥ maize ≥ soy hulls ≥ maize hominy chop > wheat bran > lucerne hay ≥ brewer's grain = maize cob. The botanical influence on dietary insoluble fibre fermentation was reflected in the single standard, single coproduct diets (Table 6). Screening dietary ingredients for HF and LF fibre imposed contrasting fermentability (P <0.05) (Table 7), due largely to differentiated allotment of soy hulls to the HF and brewer's grain to the LF diets. Dietary dilution with coproduct insoluble fibre decreased (P <0.05) fermentability for all except the soy hull and HF fibre. In the individual feed ingredients and in the single product diets, cereal fibre induced proportionately more (P <0.05) propionate and butyrate, and less (P <0.05) acetate, with a greater butyrate shift (P <0.05) for maize fibre. High levels (P <0.05) of proteolytic markers were produced from lucerne (iso-butyrate) and soy hull (iso-valerate) fibre, signifying protein fermentation. Consistent with the differentiated allotment of soy hulls to HF and brewer's grain to LF diets, the HF fibre increased acetate more than the LF fibre, with less butyrate in the HF diet (P <0.05).

Table 4 Partial multi-enzyme cocktail insoluble fibre digestibility (n = 36)

Feedstuffs	Roxazyme	Viscozyme	Roxazyme+Viscozyme	SEM	*P*-values		
					Feed	Enzyme	Feed x Enzyme
Ingredients							
Brewer's grains	0.12^{c}	0.12^{c}					
Maize cobs	0.05^{ef}	0.04^{fg}					
Maize hominy chop	0.02^{g}	0.10^{cd}					
Maize meal	0.04^{fg}	0.16^{b}		0.010	<0.001	<0.001	<0.001
Dehulled soybean meal	0.02^{fg}	0.17^{b}					
Lucerne hay	0.08^{de}	0.18^{b}					
Soybean hulls	0.06^{e}	0.33^{a}					
Wheat bran	0.12^{c}	0.12^{c}					
Standard[1] and single[2] fibre diets							
Standard	0.03^{d}	0.21^{a}					
Brewer's grains	0.04^{d}	0.17^{b}					
Maize cobs	0.03^{d}	0.16^{b}		0.009	<0.001	<0.001	<0.001
Lucerne hay	0.06^{c}	0.22^{a}					
Soybean hulls	0.04^{cd}	0.21^{a}					
Wheat bran	0.06^{c}	0.17^{b}					
Mixed fibre diets[3]							
Low fermentability	0.07^{c}	0.17^{b}	0.18^{b}	0.013	<0.01	<0.001	<0.001
High fermentability	0.04^{c}	0.20^{b}	0.25^{a}				

^{abcdfg} For each set of means, values that do not share a common superscript are different at P <0.05
SEM: Standard error of the mean
[1] 13 MJ metabolisable energy, 141 g total dietary fibre/kg dry matter
[2] 12 MJ metabolisable energy, 246 g total dietary fibre/kg dry matter, added fibre = brewer's grains, lucerne hay, maize cobs, soy hulls or wheat bran
[3] 11 MJ metabolisable energy, 319 ± 1.4 g total dietary fibre/kg dry matter, ingredients screened for maximal contrast in fibre fermentability = ml fermentation gas^{-1} kg dry matter

Table 5 Fermentation characteristics (n = 6) of individual feed insoluble fibre in pig faecal inoculum

Diet	Short chain fatty acid production							Gas production	
	%					Total[1] (mMol/g dry matter)	Ratio (acetate:propionate:butyrate)		
	Acetate	Propionate	Butyrate	Iso-butyrate	Iso-valerate			*A*[2]	$T_{½}$[3]
Brewer's grains	51.4^{d}	39.7^{a}	7.3^{c}	0.4^{c}	1.2^{b}	2.3^{e}	52:40:08	61.3^{c}	13.4^{b}
Maize cobs	59.6^{b}	32.0^{c}	7.9^{c}	0.2^{c}	0.3^{e}	2.3^{e}	60:32:08	51.8^{c}	30.1^{ab}
Maize hominy chop	51.3^{d}	35.8^{b}	12.1^{b}	0.3^{c}	0.7^{cd}	4.3^{c}	52:36:12	173.5^{b}	29.8^{ab}
Maize meal	48.8^{e}	35.4^{b}	14.8^{a}	0.3^{c}	0.7^{cd}	5.3^{b}	45:39:16	245.2^{ab}	29.1^{ab}
Dehulled soybean meal	53.9^{c}	37.4^{ab}	7.0^{cd}	0.7^{ab}	1.0^{bc}	6.0^{a}	55:38:07	299.4^{a}	32.3^{a}
Lucerne hay	61.0^{b}	32.0^{c}	5.4^{e}	0.8^{a}	0.8^{cd}	2.4^{de}	62:33:05	65.2^{c}	18.5^{b}
Soy hulls	66.7^{a}	26.1^{d}	5.3^{e}	0.5^{abc}	1.5^{a}	4.5^{c}	72:23:05	184.4^{ab}	20.6^{b}
Wheat bran	51.7^{d}	38.8^{a}	8.5^{c}	0.4^{c}	0.6^{de}	2.9^{d}	52:39:09	67.6^{c}	12.7^{b}
SEM	1.21	0.88	0.65	0.04	0.08	0.29		18.11	2.95
P-values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001

^{abcde} Means within a column with different superscripts are different at $P < 0.05$
SEM: Standard error of the mean
[1] Acetate, propionate, butyrate, iso-butyrate, iso-valerate
[2] A- mL/g dry matter at t = ∞
[3] Time (hours), when gas production = A/2

Table 6 Fermentation characteristics (n = 6) of standard[1], and single co-product[2] dietary insoluble fibre in pig faecal inoculum

Diet	Short chain fatty acid production							Gas production	
	%					Total[3] (mMol/g dry matter)	Ratio (acetate:propionate:butyrate)		
	Acetate	Propionate	Butyrate	Iso-butyrate	Iso-valerate			*A*[4]	$T_{½}$[5]
[1]Standard	45.4[c]	39.7[a]	13.5[a]	0.4[b]	1.5	5.0	46:41:13	205.3[a]	25.2
[2]Brewer's grain	48.6[b]	38.3[ab]	11.6[ab]	0.4[ab]	1.1	4.3	49:39:12	126.6[c]	14.2
[2]Maize cobs	47.5[b]	37.5[ab]	12.7[ab]	0.5[ab]	0.9	4.1	49:38:13	139.8[c]	17.8
[2]Lucerne hay	51.0[b]	36.1[bc]	11.3[b]	0.6[a]	1.1	5.4	52:37:11	155.2[bc]	19.3
[2]Soybean hulls	53.0[a]	34.8[c]	10.7[b]	0.4[ab]	1.1	5.2	54:35:11	187.6[ab]	23.4
[2]Wheat bran	50.0[b]	37.8[b]	10.9[ab]	0.5[ab]	0.9	4.4	51:38:11	141.0[c]	21.8
SEM	0.63	0.41	0.28	0.03	0.09	0.19		6.36	1.03
P-values	<0.0001	<0.0001	<0.01	0.22	0.35	<0.05		<0.001	<0.05

[abc] For each substrate, means within a column with different superscripts are different at $P < 0.05$

SEM: Standard error of the mean

[1] 13 MJ metabolisable energy, 141 g total dietary fibre/kg dry matter maize-soybean diet

[2] 12 MJ metabolisable energy, 246 g total dietary fibre/kg dry matter added fibre = brewer's grains, lucerne hay, maize cobs, soy hulls or wheat bran

[3] Acetate, propionate, butyrate, iso-butyrate, iso-valerate

[4] Gas production (mL/g dry matter) at $t = \infty$

[5] Time (hours), when gas production = A/2

Table 7 Fermentation characteristics (n = 8) of standard[1], and high versus low fermentability insoluble dietary fibre in pig faecal inoculum

Diet	Short chain fatty acid production							Gas production	
	%					Total[3] (mMol/g dry matter)	Ratio (Acetate:propionate:butyrate)		
	Acetate	Propionate	Butyrate	Iso-butyrate	Iso-valerate			*A*[4]	$T_{½}$[5]
[1]Standard	47.5[c]	38.2[a]	12.6[a]	0.5	1.3	5.4[a]	56:34:10	173.8[a]	23.2
[2]High fermentability	55.2[a]	33.8[b]	9.5[b]	0.5	1.1	5.0[a]	51:38:11	159.5[a]	27.4
[2]Low fermentability	50.5[b]	37.2[ab]	10.9[ab]	0.5	1.0	3.6	48:39:13	96.6[b]	29.8
SEM	0.96	0.67	0.42	0.03	0.10	0.3		9.57	1.99
P-values	<0.0001	0.0031	0.0066	0.8213	0.4382	0.0243		<0.0001	0.3558

[ab] For each set of means, values within a column with different superscripts are different at $P < 0.05$
SEM: Standard error of the mean
[1] 13 MJ metabolisable energy, 141 g total dietary fibre/kg dry matter
[2] 11 MJ metabolisable energy, 319 ± 1.4 g total dietary fibre/kg dry matter, feed ingredients screened for maximal contrast in fibre fermentability (mL fermentation gas^{-1} kg dry matter)
[3] Acetate, propionate, butyrate, iso-butyrate, iso-valerate
[3] Gas production (mL/g dry matter) at t = ∞
[4] Time (hours), when gas production = A/2

Discussion

The study examined how botanical factors and fermentation-based dilution of co-product feeds into high fibre maize-soybean growing-pig diets affect exogenous enzymatic and fermentative insoluble dietary fibre degradation, with the goal to improve the nutritional efficacy of such complex diets. Differentiated occurrence of NSP in plant tissues among feed-grade grain-processing co-products is well documented (Bach Knudsen, 2014). Cellulose is ubiquitous with chemical homogeneity, but high quantitative variance across plant genera (Bach Knudsen, 2014). Fibre in the gramineae is dominated by arabinoxylans (rye, wheat, maize and sorghum) and β-glucans (oats and barley) (Bach Knudsen, 2014). Legume fibre is similarly highly heterogenic, consisting mostly of pectins, xyloglucans and mannans (Jha & Leterme, 2012; Bach Knudsen, 2014; Aumiller *et al*., 2015). Plant iNSP are structurally differentiated moieties at the primary, secondary and tertiary domains of the cell wall architecture (e.g. reviews by McDougall *et al*., 1996, Collins *et al*., 2010, Bach Knudsen, 1997, 2014, Pedersen *et al*., 2015; Bach Knudsen *et al*., 2016). In the study test feed range, chemical variation in NSP was generically indicated by different neutral and acid detergent fibre, hemi-cellulose, ADL, cellulose, soluble versus insoluble fractions, with the quantitative fibre occurrence reflected in the total dietary fibre (Tables 1 & 2b). The variable fibre content was implicated indirectly in the different enzymatic (pepsin-pancreatin) indigestibility (Table 3).

In line with previous studies (Willamil *et al.*, 2012; Jha *et al*., 2015; Kong *et al*., 2015; Kwon *et al*., 2015; Bach Knudsen, 2015), in the present study, there were strong influences of the fibre source on both enzymatic and fermentative degradation of dietary fibre. Overall, enzymatic insoluble fibre degradation was highly variable, and limited (0.02 - 0.33). Enzyme-co-product interaction on fibre digestibility in feed ingredients, which was expressed in complete single co-product diets, confirmed expected differentiated enzyme-substrate specificity. Roxazyme expressed notably low affinity for the basal maize and soybean fibre types, which suggested limited efficacy on such diets owing to lack of substrate, which was in line with previous studies (e.g. Willamil *et al*., 2012; Pedersen *et al*., 2015; Park *et al*., 2016b). Though enzyme fibrolytic efficacy seems generally higher on temperate cereal fibre, for which most exogenous enzymes were developed, inconsistent pig responses are attributed partly to undefined interactions of animal and dietary factors in various experiments (Swiatkiewicz *et al*., 2016). Of an array of commercial fibrolytic enzyme cocktails, none improved the utilization of co-product-enriched maize-based diets (Kerr & Shurson, 2013). In contrast to the glycoside 10 xylanases, the glycoside 11 family of xylanases expressed greater specificity (Biely *et al*., 1997; Paes *et al*., 2012), which probably accounted for the low efficacy of xylanases in Roxazyme in cleaving the highly substituted maize arabinoxylans. Maize arabinoxylans exhibit greater frequency of random arabinose, D-glucuronic acid and acetyl group substitution into the β-linked D-xylose chains with feruloylated arabinose residues forming cross-links to create heterogeneous recalcitrant complexes (Pedersen *et al.*, 2014). The analytical markers for such arabinoxylans are high arabinose/xylose and uronic acid/xylose ratios (Pedersen *et al.*, 2014). The bulk of any *in vitro* enzymatic digestibility of maize fibre could be starch, and not the hull iNSP (Park *et al.*, 2016a). Unlike the extremely diverse pig gut microbial enzyme activities, only limited activities are expressed in manufactured enzyme cocktails. Therefore, greater diversity of gut microbial enzyme activities theoretically affords better digestion owing to synergistic/complementary degradation (Ghimire *et al*., 2016), which was demonstrated by the combination of the carbohydrase enzyme cocktails in degradation of HF, but not of LF. It is against this background that the efficacy of current enzymes, particularly for the iNSP-rich maize diet, remains equivocal (Zijlstra *et al*., 2010; Bedford & Cowieson, 2012; Willamil *et al*., 2012, Rho *et al*., 2018). Futurist enzymes could be tailored (Bindelle *et al*., 2011; Bedford & Cowieson, 2012) to diet specific or broad potency.

Fermentation gas and total SCFA jointly predict fermentability (Jonathan *et al*., 2012). In the present study, they similarly differentiated feed fibre fermentability, and both detected significantly induced control-HF-LF fermentability contrasts. However, the prediction was dissimilar for the single fibre diets, which had similar SCFA, but different gas yields. Jonathan *et al*. (2012) reported significant correlation of the gas and SCFA in the early stages of fermentation, and not by the end of fermentation. The anomaly was attributed to fermentation pathways that differently partition carbon into SCFA versus gases. Gas formation is not universal to anaerobic bacteria, least gas producing of which are the probiotic *Bifidobacterium* and *Lactobacilli* spp (Rowland *et al*., 2018). For example, lactate, which is metabolized early during fermentation to yield propionate, butyrate and gases was detected after 72 hours of fermentation of oat β-glucans and inulin, and not in other substrates. Further, the CO_2 produced from bicarbonate buffering is subject to both quantitative and qualitative SCFA production (Bindelle *et al*., 2011). Ideally, gas and SCFA prediction of fermentability should be qualitatively supported by measurement of intermediate and endpoint metabolites, and the residual substrate (Jonathan *et al*., 2012). Continuous fermentation models (Tanner *et al.*, 2014) might provide better simulation of the dynamics of porcine colon fermentation. In the present study,

considering both measures of fermentability, similar to the enzymatic digestion, the fibre type strongly influenced its fermentation characteristics, which was consisted with previous studies (Jha *et al*., 2011; Jha & Leterme, 2012; Jonathan *et al*., 2012; Jha & Berrocoso, 2015). The basal (maize grain, dehulled soybean) dietary fibre were the most fermentable, which ensured that, despite co-product addition, all test diets, which were maize-soybean diets remained highly fermentable. In maize grain, endosperm arabinoxylans are chemically simplest (Pedersen *et al.*, 2015) and therefore highly fermentable (Bach Knudsen, 2014). Soy iNSP are highly fermentable (McDougall *et al*., 1996) owing to extensive branching, which provides abundant cleaving sites for microbial enzymes (Jonathan *et al*., 2012). The least fermented co-products are those in which the secondary plant tissue is dominated by cellulose and the most complex non-cellulosic iNSP (Bach Knudsen *et al*., 2016), and the most lignified (Tables 1 and 2).

The butyrate-type fermentation of cereal fibre is attributed to its arabinoxylans (Chen *et al*., 2014; Ivarsson *et al*., 2014). The acetate-type fermentation of legume fibre is linked to polyuronides (Jonathan *et al*., 2012; Chen *et al*., 2014; Ivarsson *et al*., 2014). Intense fermentation with substantial BUT shift for maize and its hominy chop could be attributed to a concentration of resistant starch (Jonathan *et al*., 2012; Giuberti *et al*., 2013; He *et al.*, 2017). Resistant starch is fully recovered in the filtered pepsin-pancreatin residues (Giuberti *et al*., 2013). Its fermentation is beneficially bifidogenic (Fouhse *et al*., 2015). In the distal colon, an energy-protein imbalance may occur owing to depletion of fermentable NSP or excessive undigested exogenous and endogenous protein, which forces bacteria into amino acid fermentation, with deleterious effects on the intestinal barrier function (Pieper *et al*., 2016). Colon microbiota exhibit preference for few of the natural amino acids, including the branched-chain amino acids (Dai *et al*., 2011), which uniquely produce BCFA. BCFA also modulate epithelial cell metabolism and the mucosal immune system (Blachier *et al*., 2007; Neis *et al.*, 2015). In this study, proteolytic colon fermentation was evident in the legume co-products, which yielded more I-BUT (lucerne) or iso-valerate (soy hulls). Proteolytic fermentation probably occurred in the terminal stages of fermentation, consistent with distal colonic fermentation (Rowland *et al.*, 2018), after depletion of the fermentable iNSP. In addition to toxic metabolites (Cone *et al*., 2005; De Lange *et al*., 2010; Jha & Leterme, 2012), proteolytic fermentation favours pathogenic microbiota (Pieper *et al.*, 2016; Yao *et al*., 2016). *In vivo*, the dietary protein level, viscosity, particle size, bulking, anti-nutritional compounds, upper gut passage rate, feed intake and stress are among key factors that are associated with dietary protein wastage, and subsequent deleterious colon protein fermentation (Pieper *et al.*, 2016). In fibre extracts from the complete diets, concentration of maize resistant starch probably excluded amino acid fermentation (He *et al.*, 2017).

In vitro co-product screening is necessarily subject to biologic and cost-benefit validation in response-based experimentation. The control diet of the present study was the typical maize-soybean growing pig diet in which these basal ingredients were the only and therefore exclusive sources of dietary energy. Apart from lucerne hay, these co-products were largely of insignificant protein value, such that the primary benefit of their dilution into the standard diet was to substitute fermentable iNSP for expensive starch and fat. Starch and fat were theoretically most displaced by targeting feed fermentable fibre, which was demonstrated in the HF/LF diets. Given the high fibre, dietary energy should ideally be quantified in net energy terms to accommodate fibre-specific energy wastage (Noblet & Le Goff., 2001; De Lange *et al.*, 2010; Gao *et al.*, 2015; Velayudhan *et al*., 2015). Similarly, a truly digestible essential amino acid approach accounts for fibre-specific endogenous amino acid wastage (Zhu *et al*., 2005; Libao-Mercado *et al*., 2006; 2007), and for the trade-off between fermentative loss (Columbus *et al*. 2010), and microbial synthesized digestible amino acids (Torrallardona *et al*., 2003; Zhu *et al*., 2005; 2007; Libao-Mercado *et al*., 2009). The HF-LF approach to diet formulation could theoretically incorporate iNSP, whose properties induce beneficial probiotic bacteria and SCFA (Giuberti *et al*., 2013). Considering the strong influence of co-product botany on the dietary SCFA pattern, the objective to screen the ingredients to influence SCFA ratios may therefore be practical. However, *in vivo*, the stoichiometry of SCFA production is more complex. While generically related to the feed NSP composition (Awati *et al*., 2005), the SCFA pattern is further specifically controlled by the constitutive neutral sugars (Ivarsson *et al*., 2014). Fermentation stoichiometry should depend on the net fermentative redox gradient toward maximal microbial energy extraction (Macfarlane & Macfarlane, 1993; 2003), though gut fermentation pathways are complexly fluid, subject to substrate availability, in relation to interspecies competition and cross feeding (Macfarlane & Macfarlane, 2003). *In vivo*, additional complicating variables include endogenous secretions into the gut, and differential SCFA clearance by absorption (Den Besten *et al*., 2013).

In principle, the sensitivity of both fibre enzymatic hydrolysis and fermentation assays to botanical factors supported the application of the *in vitro* model to strategic co-product feeding. Subject to testing across a wider range of substrates for empirical clarity to the underpinning monomeric NSP chemistry-fermentation relationships, the study presents conceptual and procedural frameworks for targeting maximal fermentable energy, physiologically beneficial fermentation, and for matching enzymes to dietary NSP,

integral to the least-cost strategic formulation of co-product, iNSP-rich diets. Co-product and enzyme screening should be guided by prevailing dietary, animal and economic factors.

Conclusion

High-level fermentation-based dietary inclusion of co-product feeds in maize-soybean growing pig diets altered fibrolytic enzymatic efficacy, fermentability and the SCFA pattern according to the botanical origin, with important biogenic implications. Roxazyme expressed low potency in degrading maize and soybean insoluble fibre, which casts doubt on its potency to improve the nutritional efficacy in such diets. The *in vitro* approach could be a practical tool to match enzyme products to specific diets, and to screen co-products for calibrated dietary incorporation to promote maximal fermentative fibre digestion, and induce beneficial fermentation in low-cost, fibrous maize-soybean-co-product diets.

Acknowledgements

The authors express gratitude to the South African Pork Producers' Organisation (SAPPO) and to the National Research Foundation of South Africa, which funded the project jointly through the Technology and Human Resources for Industry Programme (THRIP) Project (Grant number TP2010072700024). Acknowledgement is also extended to the Animal Production Institute of the Agricultural Research Council of South Africa, and to Tshwane University of Technology, for providing the equipment and facilities for the experiments.

Authors' Contributions

The research reported was jointly designed, conducted, and interpreted by all the cited authors, who have read and approved the manuscript.

Conflict of Interest Declaration

None of the authors declare any competing interests.

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