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Non Ruminant Nutrition

Exogenous enzymes, meal size, and meal frequency: effect on ileal and total tract digestibility of carbohydrates, and energy and fiber degradation in growing pigs fed a wheat-barley grain-based high-fiber diet

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Abstract

When conducting a digestibility trial, pigs are usually fed only twice a day with a restricted feed intake which is not representative of the feeding conditions in a commercial farm. This study aimed to determine the effects of meal size and frequency, and exogenous enzymes (xylanase and phytase) on the digestibility of a high-fiber diet using porcine in vivo and in vitro approaches. Pigs (n = 6) were fitted with a T cannula, and each received all treatments using a 6 × 6 Latin square experimental design. The diets were supplemented (Enz) or not with a combination of xylanase and phytase and distributed into three feeding programs: one received two meals per day that met three times the maintenance energy requirement (2M), one received the same quantity of feed in eight meals (8M), and another received an amount that met five times the maintenance energy requirements in eight meals (8M+). For in vitro experiment, the degradability of fiber with or without xylanase supplementation only was determined. Enzyme supplementation increased apparent ileal digestibility (AID) of dry matter, starch, and degradation of insoluble non-starch polysaccharides (I-NSP) in all in vivo treatments ($P < 0.05$). The 2M compared with 8M increased the AID of starch and total tract digestibility of organic matter and I-NSP ($P < 0.05$). Enzyme supplementation decreased the content of insoluble arabinoxylan ($P < 0.05$) and increased arabinoxylan oligosaccharides ($P < 0.05$) in the in vivo ileal digesta and in vitro incubation. The results of this study confirm degradation by xylanase of the fiber fraction at the ileal level, which resulted in less fermentation of fiber in the large intestine. However, number and size of meals had little influence on feed digestibility. The consequences of shifting fiber fermentation more towards the upper part of the gastrointestinal tract need further investigation. The in vitro model provided a confirmation of the action of xylanase on the degradation of non-starch polysaccharides.

Lay Summary

To reduce cost and also utilize locally produced ingredients, pig diets nowadays can include a large proportion of fiber-rich ingredients. Exogenous enzymes can be added to diets to improve their digestibility and limit negative effects of fiber. Usually, when conducting a digestibility trial, pigs are fed only twice a day with a restricted feed intake which is not representative of feeding conditions in a commercial farm. This study aimed to determine the effect of meal size and frequency, and enzyme supplementation on digestibility of a diet rich in fiber in growing pigs and in vitro. The diets were supplemented (Enz) or not with xylanase and phytase, and according to different size and frequency: one treatment was pig receiving two meals per day with five times the maintenance energy requirement (2M), another received the same quantity of feed in eight meals (8M), and the last received an amount close to *ad libitum* feeding in eight meals (8M+). An in vitro experiment was also conducted to look at degradability of fiber with and without xylanase. The results showed that xylanase allows degradation of fiber and increases digestibility of dry matter, starch, and energy. The number and size of meals have little influence on digestibility.

Key words: dietary fiber; digestibility; exogenous enzyme; growing pig, meal size; meal frequency

Abbreviations: AIA, acid insoluble ash; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; AX, arabinoxylans; A:X, arabinose to xylose ratio; AXOS, arabinoxylooligosaccharides; CHO, total carbohydrates; DE, digestible energy; DM, dry matter; GE, gross energy; GIT, gastrointestinal tract; I-AX, insoluble arabinoxylans; I-NSP, insoluble non-starch polysaccharides; NDC, non-digestable carbohydrates; NSP, non-starch polysaccharides; OM, organic matter

Introduction

To lower costs and also limit the environmental impact of swine production, optimization of the use of nutrients by pigs is essential. To better determine the nutritional value of diets, a good understanding of digestibility processes and absorption of nutrients in the digestive tract is necessary. Typically, animal experiments designed to assess the nutritional value of diets and their digestibility are conducted with pigs fed twice a day with a lower amount offered than recommended (NRC, 2012). However, these conditions do not represent the ones found in commercial farms where pigs have free access to feed without limitation of meals or intake. Moreover, the number

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and size of the meals are known to affect the digestibility of nutrients (Roth and Kirchgessner, 1985; Ruckebusch and Bueno, 2008) and their absorption.

Anti-nutritional factors such as phytate and fiber can also affect digestibility. To limit their effects, exogenous enzymes including phytase and xylanase are often supplemented in diets. In addition to their specific action on phytate and fiber polysaccharides, these enzymes can positively affect overall nutritional value by improving the digestibility of ash, amino acids, and energy (Selle et al., 2009). However, for xylanase, variable effects are observed. Part of this variation could come from the fact that its activity and efficacy depend on pH and retention time in different parts of the digestive tract (Morgan et al., 2017). Longer retention allows more time for exogenous enzymes to degrade their substrates while also increasing their exposure to endogenous enzymes which can reduce their activity (Strube et al., 2013). When meals are less frequent, an increased quantity of feed is held in the stomach because gastric emptying is slower (Svihus, 2010). Van Leeuwen and Jansman (2007) noticed that retention time in the stomach varied between 3 and 4 h when pigs received two meals per day. In contrast, Wilfart et al. (2007) observed that when animals were fed every 4 h, retention time in the stomach was 1 h.

Xylanase added to the diet can also increase the proportion of soluble xylans and decrease the viscosity of digesta because it simultaneously leads to a reduction in the molecular weight of arabinoxylan (Cowieson et al., 2007) which could improve digestibility. However, the results are variable, with some authors noting an improvement in digestibility with the inclusion of exogenous xylanase whereas others did not see significant effects (Nortey et al., 2007; Woyengo et al., 2008). This variation highly depends on the type of diet and fibre content and composition.

To assess the efficacy of enzymes on a particular substrate, the use of in vitro digestion models is an interesting approach (Aftab and Bedford, 2018; Vangsøe et al., 2020a; Vangsøe et al., 2020b). They can complement in vivo experiments in which the effects of enzymes are not always observed; in some cases due to higher variability in in vivo studies.

The present study was conducted to address the gaps in literature concerning meal size and frequency and enzyme inclusion in high-fiber diets. The hypothesis was that smaller and more frequent meals including enzymes would increase ileal and total tract digestibility by modifying retention time. The first objective of the present experiment was to determine the effects of size and frequency of meals on ileal and total tract digestibility of carbohydrates and energy and fiber degradation in growing pigs. A second objective was to determine the effect of enzyme inclusion on ileal and total tract digestibility of carbohydrates and energy and fiber degradation in growing pigs and also to determine the general action of xylanase on its substrate in vitro.

Materials and Methods

Animals and surgery

Animals were housed and used in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC 2009) . The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Laval University. Six barrows of 36 kg ± 1.5 kg ([Yorkshire x Landrace] × Duroc, Groupe Cérès, Lévis, QC, Canada) were used for the in vivo study. The pigs were housed in individual pens on slatted floors. Following a 10-d adaptation period, surgeries were performed to install ileal T-cannulas (Wubben et al., 2001), followed by a 2 wk recovery period. Pigs were fed a commercial diet based on corn and soybean meal during the adaptation period (Meunerie St-Bernard, St-Bernard, QC, Canada).

Experimental diets

The diets fed in the experiment were pelleted and formulated using wheat, barley, and soybean meal (Meunerie St-Bernard, Tables 1 and 2). This diet was offered to animals without (2M, 8M, and 8M+) and with (2M-Enz, 8M-Enz, and 8M+Enz) inclusion of xylanase (19,700 bxu/ kg; Econase XT, AB Vista, Marlborough, UK) and phytase (692 FTU/ kg, Quantum Blue, AB Vista). Both enzyme activities were measured after pelleting. Acid insoluble ash (AIA) (Natural DE Powder 50 lbs, Probiotech Inc., St-Hyacinthe, Qc, Canada) was used as an indigestible marker to calculate the digestibility of nutrients.

Experimental Plan

The animals were weighed at the start of each experimental period. Each pig received a different treatment per period according to a 6 × 6 Latin square (six pigs, six experimental periods, six treatments). Each experimental period lasted 14 d. The total allowance of feed per day was divided into two equal meals fed at 0800 and 1530 (2M) and eight equal meals distributed at 0800, 0930, 1100, 1230, 1400, 1530, 1700, and 1830 (8M and 8M+). The amount of feed distributed daily was equivalent to 3 times metabolizable energy (ME) requirement at maintenance (106 kcal ME/d/ $BW^{0.75}$; NRC, 2012) in the case of 2M and 8M treatments and was 5 times the ME requirements for 8M+ treatment. Daily feed allowance was calculated at the start of each period based on the animal's weight measured at that time. Digesta sampling took place on days 13 and 14 of each experimental period. Digesta was sampled for 12 h by attaching a bag to the cannula, which was changed every 30 min, and the content was put into a container per pig with formic acid. A pooled digesta sample was taken from the container at the end of the day. Feces were sampled by collecting fresh feces during the day on day 12. All samples were frozen at –20 °C immediately after collection and then freeze-dried before chemical analysis.

In vitro experiment

To assess the effect of xylanase on the degradation of arabinoxylan and other fiber components, the experimental diet was subjected to in vitro digestion. Three different treatments were tested: the diet without xylanase (Xyl-Without), the diet with xylanase and phytase added during the diet mixing at the feed mill (Xyl-Included) and finally, the diet without enzymes with the xylanase added directly in the in vitro device (Xyl-Added). The enzymatic activity of xylanase in Xyl-Added was the same as in Xyl-Included. In vitro digestion was performed according to the protocol of Vangsøe et al. (2020a). The incubation time during the simulated gastric phase was 90 min at pH 3.5 with the addition of pepsin (0.025 g porcine pepsin, 2,000 FIP U/g, Merck, Darmstadt, Germany). During the simulated intestinal phase, pH was then adjusted to 6.8, pancreatin (0.02 g porcine pancreatin, grade 8, Sigma–Aldrich) was added, and the incubation

Table 1. Composition of experimental diets (as-fed basis)

Item	Without enzymes	With enzymes
	2M, 8M, and 8M+	2M-Enz, 8M-Enz, and 8M+Enz
Ingredients, kg (as-fed basis)		
Wheat	456.0	461.9
Barley	250.0	250.0
Soyabean meal	146.7	145.7
Wheat bran	50.0	50.0
Oil mix	47.2	45.4
L-Lysine HCl	5.7	5.7
L-Threonine	1.4	1.4
DL-Methionine	0.7	0.7
L-Tryptophane	0.1	0.1
Limestone	8.8	11.4
Monocalcium phosphorus	6.9	1.0
Salt	4.2	4.2
Vitamins and trace minerals premix[1]	2.0	2.0
Copper sulfate	0.3	0.3
Celite[3]	20.0	20.0
Econase XT	-	0.1
Quantum Blue	-	0.15
Analyzed nutrient composition (dry matter basis)		
DM, g/kg	900	898
GE, MJ/kg DM	16.7	16.7
CP, g/kg	199	197
Starch, g/kg	483	488
Total NSP, g/kg	128	129
I-NSP, g/kg	93	96
Total AX, g/kg	65	65
I-AX, g/kg	51	53
Total A:X	0.686	0.639
Insoluble A:X	0.586	0.600
AIA, g/kg	15	16
Xylanase activity[2,4], bxu/kg	<2,000	19,700
Phytase activity[2,5], FTU/kg	<50	692

AIA, acid insoluble ash, AX, arabinoxylans, A:X, arabinose to xylose ratio, CP, crude protein, DM, dry matter, GE, gross energy, I-AX, insoluble arabinoxylans, I-NSP, insoluble non-starch polysaccharides, NSP, non-starch polysaccharides.
[1] Provided per kg of diet: vitamin A palmitate 6,000 IU, vitamin D_3 600 IU, vitamin E acetate 60 mg, menadione sodium bisulfite 3.75 mg, riboflavin 8 mg, niacin 44.0 mg, calcium pantothenate 25.0 mg, vitamin B_{12} 25.0 µg, Fe (ferrous sulphate) 150 mg, I (potassium iodate) 0.30 mg, Mn (manganous sulphate) 10 mg, Se (sodium selenite) 0.30 mg.
[2]Enzymes activities were measured after pelleting.
[3]Natural DE Powder 50 lbs, Probiotech Inc., St-Hyacinthe, QC, Canada.
[4]Econase XT, AB Vista, Marlborough, UK.
[5]Quantum Blue, AB Vista, Marlborough, UK.

continued for 4 h. Enzymes were inactivated in an 80 °C water bath for 20 min to end the digestion as they are not resistant to high temperatures. The samples were filtered (Foss Fibertec 1023, Hilleroed, Denmark) to collect the soluble fraction (filtrate). The non-digestible residue was washed with 96% ethanol and pure acetone to obtain the insoluble fraction and then dried at 103 °C for 20 h to determine the degradability of the dry matter.

Laboratory analysis

Freeze-dried diet, digesta, and feces samples were ground to pass a 1 mm screen using a CT 193 CyclotecTM mill (FOSS North America, Eden Prairie, MN, USA). All samples were analyzed for dry matter (DM, method 935.29, AOAC, 2007), gross energy (GE, Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL, USA), and ash (method 942.05, AOAC, 2007) to determine organic matter (OM). Starch was analyzed by the enzymatic colorimetric method described by Bach Knudsen (1997). In sum, samples were incubated with thermostable α-amylase (Thermamyl 120L, Novo Nordisk, Copenhagen, Denmark, 120 KNU g^{-1}, 100 µL) and then amyloglucosidase (EC, Boehringer Mannheim, Germany, 140 U mL^{-1}, 200 µL). Glucose was quantified with a glucose oxidase reagent (Megazyme, Altona Place, Australia) after centrifugation. Fiber and total non-starch polysaccharides (NSP) were analyzed by the enzymatic-chemical-gravimetric procedure described by Bach Knudsen (1997). In brief, the starch-free total NSP fraction was swelled with 12 M H_2SO_4, hydrolyzed to monomeric sugars with 2 M H_2SO_4 for 1 h, derivatized to alditol acetate and finally determined on a gas chromatograph. For total carbohydrates (CHO), the method was the same, except that samples were subjected to acid hydrolysis without removing starch and precipitation carried out in ethanol (direct method); for details see Lærke et al. (2015). The in vitro procedure separated digesta into a soluble filtrate and an insoluble residue (Vangsøe et al., 2020a). The soluble filtrate was treated according to the CHO procedure but with an H_2SO_4 concentration of 1 M for 30 min to minimize the destruction of the monomer of this fraction. The insoluble residue of the in vitro digestion was analyzed according to the NSP method described above. The acid insoluble ash (AIA) was analyzed following acid digestion of samples with 4N HCl, filtration with an ashless filter, and total combustion in the oven based on the procedure by Van Keulen and Young (1977).

Calculations and statistical analysis

The AID of nutrients was calculated using AIA as an indigestible marker according to the following equation:

$$\text{Apparent ileal digestibility (AID), \%} = \{1 - [(\text{AIA}_{\text{diet}} \times \text{Nutrient}_{\text{Digesta}}) \div (\text{AIA}_{\text{Digesta}} \times \text{Nutrient}_{\text{diet}})]\} \times 100$$

where AIA_{diet} is the concentration of undigestible marker in the diet, $\text{Nutrient}_{\text{digesta}}$ is the concentration of the nutrient measured at the ileum, $\text{AIA}_{\text{Digesta}}$ is the concentration of undigestible marker recovered at the ileum, and $\text{Nutrient}_{\text{diet}}$ is the concentration of nutrient in the diet.

Similarly, ATTD of nutrients was calculated using AIA as an indigestible marker since partial sampling was used instead of the total collection. The equation is:

$$\text{Apparent total tract digestibility (ATTD), \%} = \{1 - [(\%\ \text{AIA}_{\text{diet}} \times \%\ \text{Nutrient}_{\text{feces}}) \div (\%\ \text{AIA}_{\text{feces}} \times \%\ \text{Nutrient}_{\text{diet}})]\} \times 100$$

where AIA_{diet} is the concentration of undigestible marker in the diet, $\text{Nutrient}_{\text{feces}}$ is the concentration of the nutrient measured in the feces, $\text{AIA}_{\text{feces}}$ is the concentration of undigestible

Table 2. Effect of meal size and frequency and enzyme supplementation on apparent ileal digestibility (AID) of nutrients and energy and carbohydrates and ileal content of carbohydrates

Item	2M		8M		8M+		SEM	*P*-values			Contrasts	
	Without	With	Without	With	Without	With		Enzyme	Meal	Interaction	2M vs. 8M	8M vs.. 8M+
AID, %												
DM	76.6	77.6	76.5	78.7	75.6	77.7	1.65	0.024	0.511	0.744	0.559	0.254
OM	77.8	78.1	77.7	79.7	76.4	78.4	1.63	0.065	0.333	0.560	0.414	0.144
Energy	80.9	81.5	80.9	82.5	80.4	81.9	1.54	0.058	0.660	0.758	0.491	0.403
Starch	98.7	98.6	98.1	98.5	97.9	98.2	0.23	0.181	0.006	0.213	0.049	0.092
Total NSP	41.0	43.2	40.0	47.8	38.5	44.9	4.46	0.009	0.578	0.503	0.447	0.327
I-NSP	41.0	47.0	41.0	50.1	38.7	46.0	4.48	<0.001	0.356	0.789	0.523	0.157
Total AX	41.3	44.1	40.7	48.4	38.3	45.4	4.35	0.006	0.488	0.561	0.451	0.246
I-AX	43.5	50.5	44.1	53.6	41.2	49.8	4.32	<0.001	0.281	0.856	0.413	0.117
Ileal content, %												
Total												
NDC	35.0	36.1	35.1	36.3	34.8	35.8	0.72	0.036	0.758	0.981	0.811	0.468
NSP	32.3	32.8	32.6	31.6	32.3	32.1	0.56	0.643	0.640	0.365	0.376	0.876
AX	16.4	16.3	16.4	15.8	16.5	16.0	0.26	0.059	0.743	0.591	0.450	0.642
A:X	0.674	0.686	0.691	0.687	0.672	0.686	0.0127	0.424	0.599	0.662	0.426	0.360
Insoluble												
NSP	23.6	22.8	23.5	22.5	23.4	23.2	0.52	0.090	0.804	0.707	0.702	0.518
AX	12.3	11.7	12.1	11.5	12.3	11.9	0.34	0.041	0.618	0.906	0.486	0.356
A:X	0.621	0.595	0.648	0.605	0.615	0.594	0.0140	0.004	0.131	0.628	0.128	0.061
Soluble												
NSP	8.8	10.2	9.1	9.1	8.9	8.9	0.54	0.252	0.429	0.283	0.427	0.590
AX	4.1	4.5	4.3	4.2	4.2	4.0	0.33	0.774	0.826	0.520	0.906	0.635
A:X	0.855	0.983	0.831	0.963	0.866	0.976	0.0516	0.005	0.856	0.970	0.656	0.619
AXOS	0.8	2.0	0.7	2.3	0.8	2.0	0.23	<0.001	0.829	0.631	0.569	0.651

2M, two meals per day providing three times the metabolizable energy requirement at maintenance, 8M, eight meals per day providing three times the metabolizable energy requirement at maintenance, 8M+, eight meals per day providing five times the metabolizable energy requirement at maintenance, AID, apparent ileal digestibility, AX, arabinoxylan, A:X, arabinose to xylose ratio, AXOS, arabinoxylooligoaccharide, DM, dry matter, I-AX, insoluble arabinoxylans, I-NSP, insoluble non-starch polysaccharides, NDC, non-digested carbohydrates, NSP, non-starch polysaccharides, OM, organic matter, S-AX, soluble arabinoxylans, SEM, standard error of the mean, S-NSP, soluble non-starch polysaccharides, With, diet supplemented with enzymes, Without, diet not supplemented with enzymes.

marker recovered in the feces, and $Nutrient_{diet}$ is the concentration of nutrient in the diet.

Arabinoxylan (AX) content of diet and digesta was calculated as the sum of arabinose and xylose analyzed in the NSP analysis.

$$AX_{Insol} = Arabinose_{Insol} + Xylose_{Insol}$$

$$AX_{Sol} = Arabinose_{Sol} + Xylose_{Sol}$$

where AX_{insol} is the ileal concentration of insoluble AX, $Arabinose_{insol}$ is the ileal concentration of insoluble arabinose, $Xylose_{insol}$ is the ileal concentration of insoluble xylose, AX_{sol} is the ileal concentration of insoluble AX, $Arabinose_{sol}$ is the ileal concentration of insoluble arabinose, and $Xylose_{sol}$ is the ileal concentration of insoluble xylose.

Assuming that arabinoxylan oligosaccharides (AXOS) do not precipitate in ethanol, they were therefore calculated as follows:

$$AXOS_{in\ vivo}, \% = AX_{Direct} - (AX_{Insol} + AX_{Sol})$$

$$AXOS_{in\ vitro}, \% = AX_{Direct} - AX_{Sol}$$

where $AXOS_{in\ vivo}$ is the ileal concentration in AXOS in vivo, $AXOS_{in\ vitro}$ is the concentration in AXOS in vitro in the filtrate, and AX_{Direct} is the ileal concentration of AX from the CHO procedure.

The non-digested carbohydrates (NDC) were calculated by removing ileal starch content from total carbohydrates (CHO) in vivo. Soluble NDC in vitro was calculated as the sum of rhamnose, fucose, arabinose, xylose, mannose, galactose, and uronic acids. Glucose cannot be included as we could not discriminate glucose originating from starch and digested β-glucan and cellulose.

$$NDC_{in\ vivo}, \% = CHO - ileal\ starch$$

$$\begin{aligned} \text{Soluble } NDC_{in\ vitro}, \% &= Rhamnose_{Sol} + Fucose_{Sol} \\ &+ Arabinose_{Sol} + Xylose_{Sol} + Mannose_{Sol} \\ &+ Galactose_{Sol} + Uronic\ acids_{Sol} \end{aligned}$$

where Soluble $NDC_{in\ vitro}$ is the concentration of soluble NDC in the filtrate in vitro, $Rhamnose_{sol}$ is the concentration of soluble rhamnose in the filtrate, $Fucose_{sol}$ is the concentration of soluble fucose in the filtrate, $Arabinose_{sol}$ is the concentration of soluble arabinose in the filtrate, $Xylose_{sol}$ is the concentration of soluble xylose in the filtrate, $Mannose_{sol}$ is the concentration of soluble mannose in the filtrate, $Galactose_{sol}$ is the concentration of soluble galactose in the filtrate, and $Uronic\ acids_{sol}$ is the concentration of soluble uronic acids in the filtrate.

The degradation of dry matter in vitro ($AID_{DM\ in\ vitro}$) was calculated using the starting weight of the sample ($Sample\ weight_{Start}$) and the weight of the indigestible residue ($Residu_{End}$):

$$AID_{DM_{in\ vitro}} = \left(1 - \frac{Residu_{End}}{Sample\ weight_{Start}}\right) \times 100$$

The in vitro carbohydrate degradation ($AID_{NSP\ in\ vitro}$) was calculated according to the concentrations in the soluble filtrate or indigestible residue ($NSP_{Filtrate\ or\ residue}$) and the diet ($NSP_{diet}$):

$$AID_{NSP_{in\ vitro}} = \left(1 - \frac{NSP_{Filtrate\ or\ Residue}}{NSP_{Diet}}\right) \times 100$$

Fermentation in the large intestine was determined by the difference between ATTD and AID of DM, OM, DE, total-NSP and I-NSP according to the following equation:

$$Fermentation_{Large\ intestine} = CATTD_{Feces} - CAID_{ileum}$$

Data were n = 3) and normally distributed. The experimental unit was the pig. For the digestibility trial in vivo, a 2 × 3 factorial analysis was used to evaluate the effect of enzyme inclusion (n = 2), meal size and frequency (n = 3), and their interaction. The model included the meal size and frequency and enzyme supplementation as fixed effects and the experimental period and pig as random effects. Contrasts were used to test the main effects of meal size and frequency (2M vs. 8M, 8M vs. 8M+) in the in vivo experiment and to test the effect of xylanase inclusion (Xyl-Added vs. Xyl-Included) in the in vitro experiment when no interaction was observed. Data were analyzed using the SAS GLIMMIX Procedure with a normal distribution of data (version 9.4, SAS Institute Inc., Cary, NC, USA). The differences were considered significant with $P < 0.05$.

Results

In Vivo

All pigs ate their daily feed allowance during the trial. For the 8M+ treatment, there was about 10–15% of diet refusal for the first 3 d of each period as pigs were adapting to an increased feed intake, but the full amount was consumed thereafter.

Apparent ileal digestibility

There was no Enzyme × Meal size and frequency interaction observed for any variable (Table 2). The results indicate an improvement in the AID of DM with enzyme inclusion (Enzyme effect, $P < 0.05$; Table 2). Also, AID total NSP, I-NSP, total AX and I-AX was improved (Enzyme effect, $P < 0.001$) with enzyme inclusion. A meal effect was observed for AID of starch (Meal size and frequency effect, $P = 0.006$), with digestibility 0.3% greater with 2M compared to 8M (Contrast 2M vs. 8M, $P = 0.049$).

Ileal content of non-starch polysaccharides

Enzyme supplementation increased NDC content (Enzyme effect, $P = 0.036$; Table 2). The I-AX content was decreased when enzymes were added to diet (Enzyme effect, $P = 0.041$). The A:X ratio of insoluble AX was decreased for all meal size and frequency with enzyme inclusion (Enzyme effect, $P < 0.004$). On the other hand, A:X ratio of soluble AX was increased with enzyme inclusion (Enzyme effect, $P = 0.005$). Enzyme inclusion increased the ileal content of AXOS (Enzyme effect, $P < 0.001$).

Apparent total tract digestibility and fermentation in the large intestine

An Enzyme × Meal size and frequency interaction were observed for ATTD of OM and I-NSP. Digestibility was increased by 1.4% with enzyme inclusion in 2M but

Table 3. Effect of meal size and frequency and enzyme supplementation on apparent total tract digestibility (ATTD) of nutrients and energy and carbohydrates and fermentation in the large intestine

Item	2M		8M		8M+		SEM	*P*-values			Contrasts	
	Without	With	Without	With	Without	With		Enzyme	Meal	Interaction	2M vs. 8M	8M vs. 8M+
ATTD, %												
DM	86.4	87.4	87.4	86.5	86.6	85.8	1.15	0.517	0.215	0.133	0.928	0.114
OM	88.2	89.5	89.4	88.4	88.6	88.2	1.00	0.849	0.402	0.048	0.841	0.208
Energy	88.1	88.8	89.2	88.5	88.6	87.7	1.22	0.494	0.355	0.220	0.435	0.158
Total NSP	67.6	71.6	71.0	70.5	70.7	68.8	2.50	0.666	0.664	0.137	0.434	0.462
I-NSP	61.8	69.0	68.4	66.8	67.8	64.8	3.01	0.586	0.498	0.036	0.253	0.466
Fermentation in large intestine, %												
DM	9.8	9.8	10.8	7.8	10.9	8.0	1.32	0.028	0.892	0.294	0.640	0.868
OM	10.5	11.6	11.8	8.7	12.2	9.8	1.23	0.086	0.657	0.132	0.434	0.440
DE	7.1	7.3	8.3	6.7	8.2	5.8	1.11	0.079	0.844	0.306	0.751	0.566
Total NSP	27.1	29.1	31.8	23.5	30.5	22.7	3.92	0.071	0.875	0.184	0.888	0.716
I-NSP	21.6	23.2	28.0	16.6	28.1	16.5	4.14	0.016	0.999	0.108	0.979	0.998

2M, two meals per day providing three times the metabolizable energy requirement at maintenance, 8M, eight meals per day providing three times the metabolizable energy requirement at maintenance, 8M+, eight meals per day providing five times the metabolizable energy requirement at maintenance, ATTD, apparent total tract digestibility, DM, dry matter, I-NSP, insoluble non-starch polysaccharides, OM, organic matter, NSP, non starch polysaccharides, SEM, standard error of the mean, With, diet supplemented with enzymes, Without, diet not supplemented with enzymes.

decreased by 1.1% and 0.5% in 8M and 8M+ respectively (Interaction Enzyme × Meal size and frequency, $P < 0.05$; Table 3). Results show a decrease in fermentation in the large intestine of DM and I-NSP as a consequence of enzyme inclusion ($P < 0.05$).

In vitro degradation of non-starch polysaccharides

An increase in the in vitro digestibility of I-NSP and I-AX was observed with xylanase inclusion ($P < 0.05$; Table 4). The AXOS and NDC content of the filtrate was also higher with xylanase inclusion (Xylanase effect, $P < 0.05$) and more markedly in Xyl-Included compared to Xyl-Added for AXOS (Added vs.. Included, $P = 0.003$). The A:X ratio soluble AX of the filtrate was decreased by adding xylanase ($P < 0.001$).

Discussion

Meal size and frequency had minimal effect on the ileal digestibility of nutrients as only AID of starch was marginally higher when pigs ate two meals per day instead of eight meals per day. These effects can be explained by a modification of retention time. Indeed, a bulky meal causes faster gastric emptying during the first 30 min (Auffray et al., 1967) but the rapid arrival of hypertonic digesta in the duodenum leads to distension of the latter. Starch is degraded to a large extent in the duodenum by pancreatic α-amylase (Gray, 1992). Mechanoreceptors and osmotic receptors send signals to stop gastric emptying following the arrival of digesta in the duodenum (Auffray et al., 1967). Therefore, retention time is increased due to the bulking caused by a large meal, such as for 2M. Thus, longer transit time caused by limited volume and frequency of 2M treatment allows a longer contact time of starch with α-amylase and would explain the increase in starch digestibility observed. Apparent ileal digestibility of starch also tended to be higher when the animal consumed less feed per day. This effect can probably be explained by a modification in retention time according to the size of the meals. Roth and Kirchgessner (1985) observed that total tract retention time was 52.2 h with a daily intake intended to cover 1 time the maintenance energy requirements. However, in the same study, total tract retention time was reduced to 35.3 h when the animal was fed 2.5 times the maintenance requirement.

No other effect of meal distribution (2M vs. 8M or 8M vs. 8M+) on AID of carbohydrates or energy was observed in this study, which is consistent with the studies by Mroz et al. (1994) and Chastanet et al. (2007) who did not observe an effect on ileal digestibility of nutrients depending on the number of meals offered per day. Chastanet et al. (2007) also mentioned that at a high feed intake level (above 3 times the ME requirements), no differences in AID were observed among different levels of feed intake or meal frequency. Therefore, even though AID of starch was higher in 2M, the improvement was numerically low compared to 8M (+0.3%) and 8M+ (+0.6%) and can explain why no effect was seen for AID of DM, OM, or energy. In addition, it should be noted that meal size and frequency had no effect on the effect of enzymes on the AID of carbohydrates and energy are given that no interaction was observed.

Regardless of meal size and frequency, enzyme inclusion (xylanase and phytase) improved the AID of DM, OM, and energy. These improvements were associated with degradation of I-NSP and I-AX in the upper gastrointestinal tract (Owusu-Asiedu et al., 2010). The changes observed in A:X ratios support degradation of AX by xylanase. The decrease in insoluble A:X ratio with enzyme inclusion indicated that

Table 4. Effect of xylanase added in vitro or included in diet on ileal carbohydrates evaluated in vitro

Item	Enzymatic treatment			SEM	*P*-values (contrast)	
	Xyl-Without	Xyl-Added	Xyl-Included		Xylanase addition	Xyl-Added vs. Xyl-Included
Digestibility, %						
DM	81.0	81.6	81.6	0.31	0.205	0.879
I-NSP	–13.3	-6.7	-10.4	1.08	0.023	0.073
I-AX	–16.5	-7.5	-11.7	1.51	0.020	0.122
Residue and filtrate content, %						
Insoluble						
NSP	11.8	11.1	10.9	0.11	0.005	0.383
AX	6.4	5.9	5.7	0.08	0.004	0.140
A:X	0.600	0.595	0.610	0.0131	0.621	0.063
Soluble						
CHO	45.0	45.9	46.2	0.36	0.054	0.637
NDC	2.1	2.5	2.5	0.14	0.007	0.796
NSP	3.9	4.6	4.5	0.08	0.002	0.795
AX	0.7	1.1	1.1	0.02	<0.001	0.061
A:X	0.970	0.839	0.865	0.0130	<0.001	0.097
AXOS	–0.05	–0.02	0.10	0.022	0.004	0.003

AX, arabinoxylan, A:X, arabinose to xylose ratio, AXOS, arabinoxylan oligoaccharides, CHO, total carbohydrates, DM, dry matter, I-AX, insoluble arabinoxylans, I-NSP, insoluble non-starch polysaccharides, NDC, non-digested carbohydrates, NSP, non-starch polysaccharides, S-AX, soluble arabinoxylans, SEM, standard error of the mean, S-NSP, soluble non-starch polysaccharides, Xyl-Added, diet with xylanase added directly in the in vitro system, Xyl-Included, diet with xylanase added during mixing at the feed mill, Xyl-Without, diet without xylanase.

xylanase's is acting and releasing fragments of insoluble AX, which have a high A:X ratio. Presumably, given xylanase preference to cleave at unsubstituted stretches of the AX, the highly substituted regions that are released are flanked by poorly substituted cleavage sites (Vangsøe et al., 2019; Vangsøe et al., 2020b). The soluble A:X ratio changed in the inverse direction as a consequence of enzyme activity, but this is not the only possibility as the same result could have occurred if there was some degree of arabinose removal from the soluble AX and subsequent absorption or fermentation. An increase in the content of soluble AXOS by xylanase inclusion also suggests that xylanase may have promoted fiber solubilization and depolymerization (Bedford, 2018; Vangsøe et al., 2020b). Fiber solubilization is interesting given that it supplies the intestinal microbiota with rapidly fermentable material, which may allow the energy value of the diet to be increased. Indeed, soluble fiber and, better still, oligosaccharides are easier to ferment by the microbiota than insoluble fiber, which require a longer time for degradation (Bach Knudsen, 2005). The increase in AID of DM with the inclusion of xylanase and phytase can be explained by an increased release of nutrients. Indeed, without exogenous enzyme inclusion, nutrients can end up encapsulated in the fiber matrix and become unavailable for absorption (De Lange et al., 2010).

The AID of total NSP in vivo found in this study is in agreement with that reported in the literature, but they are at the higher end, with variation from 38.5% to 47.8% in this study in comparison with an average of 21% and values varying from 10% to 62% elsewhere (Bach Knudsen et al., 2012). The higher AID of total NSP observed in the study could be due to the indigestible marker used. Wang et al. (2017) found that the AID of GE and N was higher when AIA was used as the marker in a diet containing 10% oat bran, which supplies a soluble NSP similar to the current study (3.5% vs. 3.3%). Sales and Janssens (2003) also observed variations in digestibility when using AIA and attributed that to lower intake of the marker during feeding due to separation. However, in our case the diet was pelleted which limited segregation. Laerke et al. (2012) observed more variation in AID when using AIA compared to Cr_2O_3.

We observed negative AID values in vitro for I-NSP, I-AX and AXOS content. Negative values for AID of fiber in vivo have been reported in the literature and attributed to non-dietary substances present in the GIT such as mucins or bacteria that interfere with fiber analysis (Montoya et al., 2016). However, those non-dietary substances are not present in the in vitro system. In the calculations of AID in vitro, we compensated for the loss of material from the crucibles (undigested residue). Therefore, digestibilities may be overcompensated, which results in negative values.

The intention of the in vitro model was to confirm that the effects observed on AID and ileal content of NSP were indeed due to the inclusion of xylanase. The in vitro model allowed a confirmation of the xylanase effect, although the increase in AID of NSP in vitro is slightly higher (22%) than the improvement in AID in vivo (18%). This difference may be due to more controlled conditions observed in vitro such as pH and retention time. In vivo, pH varies after meals and with meal frequency and fiber intake, as feed and fiber can act as buffers in the stomach (Wenk, 2001). For xylanase, the activity window is between pH 4 and 6 (Svihus and Hervik, 2019). The retention time can also influence the action of xylanase. A longer retention time gives more time for degradation and exposes exogenous xylanase to endogenous proteases, leading to decreased xylanase activity (Strube et al., 2013). The in vitro retention time was 90 min in the simulated gastric phase and 4 h in the simulated intestinal phase. These values were comparable with the rare transit time data such as those published by Wilfart et al. (2007), who observed retention time in the stomach and the small intestine between 60–78 min and 3.6–4.4 h, respectively.

This higher AID of carbohydrates and energy in pigs offered a diet including enzymes led to reduced availability of substrate for microbial fermentation in the cecum and colon and then a lower fermentation in the large intestine of DM and I-NSP (the tendency for energy and OM), indicating that less degradation had occurred in the lower part of the digestive tract. The decrease in fermentation in the large intestine supports the hypothesis that xylanase inclusion shifts the digestion process of dietary nutrients and some fiber aborally in the gastrointestinal tract, thereby supporting a better energy digestibility at the ileal level while reducing substrates available for fermentation in the large intestine.

Unlike AID of energy and DM, the inclusion of enzymes did not have an effect on their ATTD. However, ATTD of OM and I-NSP were increased with enzyme supplementation in 2M but reduced in 8M and 8M+. When fermentation results were analyzed, we observed that 8M and 8M+ treatments supplemented with enzymes had a reduced fermentation of OM by 34% and 20% and by 42% and 41% for I-NSP. In contrast, the supplemented 2M treatment weakly increased the fermentation of OM and I-NSP. Therefore, a greater proportion of fermentable NSP had been fermented in the ileum for 8M and 8M+, leaving less for the hindgut, which would explain why ATTD of OM and I-NSP was not improved with enzyme inclusion like it was for the 2M treatment. The reduction in fermentation suggests that the diet should include more slowly fermentable fibers when supplementing with enzymes to ensure that the hindgut gets sufficient substrate to promote gut health.

In conclusion, enzyme supplementation can improve the digestibility of NSP in high-fiber diets. On the other hand, size and frequency of the meals had little effect on fiber digestibility and enzyme efficacy on fiber and energy utilization in growing pigs. An in vitro model can predict the effect of xylanase in vivo. However, evaluation of pH and retention time in vivo should be performed to better understand the limited effect of meal size and frequency and to develop an in vitro model more representative of physiological conditions.

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Conflict of Interest Statement

The authors declare there are no competing interests.

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