Relationship between feed characteristics and histomorphometry of small intestines of growing pigs

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Abstract

The use of agricultural by-products has become the central focus in reducing feed costs in pig production. However, there is a need to determine how the gastrointestinal tract of pigs responds when subjected to fibrous ingredients. The objective of the study was to predict villi height and apparent villi surface area from physicochemical measurements of maize cob-based diets. Eighteen growing male pigs (initial bodyweight 14.3 ± 1.20 kg) were used in the experiment. The pigs were penned individually and subjected to diets containing 0 g, 80 g, 160 g, 240 g, 320 g, and 400 g maize cob meal/kg diet. Feed and water were provided *ad libitum*. Using stepwise regression, bulk density (BD) and neutral detergent fibre (NDF) were the best predictor variables influencing villi height (VH) and apparent villi surface area (AVSA). VH produced quadratic and linear responses with BD and NDF, respectively. The equations are VH = $211.3(BD)^2$ – 591.0(BD) + 442.4; and VH = 0.03(NDF) + 22.8. Conversely, AVSA produced quadratic and linear responses with NDF and BD, respectively. The equations are AVSA = $0.00036(NDF)^2$ – 0.012(NDF) +7.25 and AVSA = - 47.12(BD) + 45.03. In conclusion, the BD and NDF of a feed could be used to predict VH and AVSA of growing pigs fed maize cobs.

Keywords: maize cob meal, mucosa lining, physicochemical properties, visceral organs
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Introduction

Use of alternative resources to feed pigs reduces competition for grain with humans. Fibrous feed ingredients for example can be used as a cheap energy source for feeding pigs (Bakare *et al*., 2016). These feed ingredients have been reported to interact with the microflora and the mucosa of all sites of the gastrointestinal tract (GIT), which are major components of optimal gut health (Montagne *et al*., 2003). Optimum gut health describes aspects such as effective digestion and absorption of nutrients, the absence of GIT illness, normal and stable intestinal microbiota, effective immune status, and a state of wellbeing. The GIT, which is the first organ system that is affected directly by fibrous diets, displays acute and long-term adaptations (Ferraris & Carey, 2000). Such changes occur to the visceral organs and mucosal architecture of the intestines. Mucosal architecture relates to villi height (VH) and crypt depth (CD) and is influenced by feeding level and composition of diets.

There are conflicting reports on the influence of dietary fibre on the mucosal architecture. High fibre inclusion levels were reported to increase VH and CD (Ngoc *et al*., 2012), and to decrease VH (Agyekum *et al*., 2012). Some reports have argued that fibre does not influence mucosal architecture (Jin, 1992; Jin *et al*., 1994). Different fibre sources elicit varied impacts on the mucosa lining. Using physicochemical properties of fibrous diets, therefore, would increase the level of accuracy for predicting the mucosal architecture of the intestines. The relationship between diet characteristics and VH and apparent villi surface area (AVSA) provides a theoretical basis in the formulation of diets for weaner pigs. Feed compounders would formulate diets that allow optimum gut health in pigs from the fibrous feed sources.

Maize cobs were used in this study as a fibrous ingredient and are the remains after the kernels have been removed. About 180 kg to 200 kg maize cobs are produced per tonne of grains (Božović *et al*., 2004). This translates to significant quantities of maize cobs being potentially available as feed for livestock. Villi

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height and AVSA determine absorptive capacity of nutrients for use by the pigs, which consequently determines their overall performance (Naburrs *et al*., 1993; Vente Spreeuwenberg & Beynen, 2003). Visceral organs also contribute to the efficiency of converting dietary nutrients in pork products (De Lange *et al*., 2001). Hence, they were measured in this experiment. The main objective of the study, therefore, was to determine the relationship between feed characteristics (physicochemical properties) and histomorphometry (VH and AVSA) of small intestines of growing pigs.

Materials and methods

A total of 18 clinically healthy castrated male growing pigs, weighing 14.3 ± 1.20 kg were used in the experiment. The pigs were treated in accordance with the University of KwaZulu-Natal Guidelines for the Care and Use of Laboratory Animals (Reference number: 061/12/Animal). All pigs were penned individually in metabolism crates measuring 1.5 × 1 m and containing a plastic tube feeder (Big Dutchman Lean Machine®) and a low-pressure nipple drinker. The diets and drinking water were provided ad libitum throughout the experimental period. HOBO data loggers (Onset Computer Corporation, Pocasset, Mass., USA) were used to automatically record ambient temperatures and relative humidity during the trial. The average temperature and humidity during the study period were 21 ^{o}C and 42.6 %, respectively.

A premium commercial feed (Meadow Feeds Ltd, Pietermaritzburg, South Africa) with a low level of DF was used as the basal feed. The basal diet had these ingredients; yellow maize (500 g/kg), soya bean (158 g/kg), soybean oil cake (20.2 g/kg), wheat bran (163 g/kg), sunflower oil cake (85 g/kg), molasses syrup (25 g/kg), and additives (48.8 g/kg). The maize cobs were first ground to pass through a 3-mm screen. Thereafter, the grounds were included at 0 g, 80 g, 160 g, 240 g, 320 g and 400 g maize cob meal/kg diet of pigs. Three pigs were randomly allocated to each treatment diet in the experiment. The number was sufficient for the study to provide data points for determining relationships. It was assumed that the pigs would increase their intake in proportion to the degree of dilution of the basal feed with the maize cob. They were allowed to adapt to the feed for seven days.

All feed samples were oven-dried and ground to pass through a 1-mm sieve before analysis. Ash (method 942.05), dry matter (DM) (method 2001.12), and crude protein (CP) (method 990.03) were determined according to the method of AOAC (2005). Gross energy was determined with a bomb calorimeter (Model C2000 basic IKA Co., Germany). NDF and ADF were analysed with filter bags by a fibre analyser (Ankom 220, Ankom Technology Corp) (Ferreira & Mertens 2007). NDF and ADF are considered a complete description of fibre in a diet and can be used as nutrients to improve feed intake and efficiency of growth in pigs. Hence, the nutrients were considered for analysis instead of total dietary fibre and non-starch polysaccharides. BD of the feeds was measured according to the water displacement method (Peterson & Baumgardt, 1971). Water-holding capacity (WHC) was determined by centrifugation (Robertson & Eastwood, 1981). Three replicates for each sample were analysed. Table 1 shows the chemical and physicochemical properties of diets.

Table 1 Chemical and physicochemical properties of diets

	Inclusion level (g/kg)					
	0	80	160	240	320	400
DM (g/kg)	989.3	989.3	990.4	990.5	990.2	991.1
[1]calc DE (kcal/kg)	3320.0	3152.8	2890.0	2627.3	2460.1	2221.3
CP (g/kg DM)	195.7	185.7	168.1	152.8	139.4	116.4
EE (g/kg DM)	52.9	51.2	45.9	45.2	41.3	39.9
Ash (g/kg DM)	61.1	59	54.9	53.2	52.1	46.7
NDF(g/kg DM)	192.3	230.6	294.4	355.3	401.3	457.4
ADF(g/kg DM)	88.4	111.5	147.4	181.2	218.4	250.5
Density (ml/g DM)	1.45	1.52	1.47	1.42	1.25	1.22
WHC (g water/g DM)	3.76	3.17	3.57	4.08	4.41	4.75

DM: dry matter; GE: gross energy; CP: crude protein; EE: ether extract
NDF: neutral detergent fibre; ADF: acid detergent fibre; WHC: water holding capacity
[1]calc DE = 949 + (0.789 × GE) - (43 × % Ash) - (41 × % NDF)(Noblet & Perez, 1993)

After four weeks on the experimental diet, pigs were euthanized with intravenous injections using sodium pentobarbitone (Kyron Company, C92/1.9/6). Then the pigs were slit open in the abdomen and all the intestines and organs (liver and pancreas) in the abdominal cavity were removed. The intestines were untwined and lengths of the small and large intestines were recorded. The weights of the liver and pancreas and the empty weights of the small and large intestines and stomachs were also recorded.

The small intestines of each pig were divided into duodenum, jejunum, and ileum segments. From each intestinal segment, 10 cm pieces were cut and fixed immediately in 10% neutral formalin prior to preparation and analysis. The intestinal samples were sectioned and stained with haematoxylin-eosin stain. The sections were observed at 100 × magnification using a light microscope equipped with an ocular micrometre. The VH, CD, villus to crypt ratio, villus apical width and AVSA were recorded. Between eight and 11 randomly chosen and well-oriented villi were selected for measurements. VH was measured from the tip to the base of the villus, and CD was measured from the tip of the crypt to the point where it meets the muscularis mucosa (Velayudhan *et al*., 2008).

Apparent villus surface area was estimated using the formula (Iji *et al*., 2001):

$$\mathrm{AVSA} = \frac{(\mathrm{a} + \mathrm{b})}{2} \times \mathrm{c}$$

Where:
a is villus apical width; b is villus basal width;and c is villus height

The data collected for visceral organ and mucosal architecture measurements were divided by the metabolic bodyweight to give relative measurements. This would take account of differences in mean weight between treatment diets (Bailey *et al*., 2004). Metabolic body weight of each animal was calculated using the formula:

Metabolic body weight (MBW) = $BW^{0.75}$

Where BW is bodyweight of the pig

Effects of feeding fibrous diets on visceral organs size and length, and mucosal architecture measurements were analysed using the general linear models procedure (SAS, 2008). These models were used:

For visceral organ measurements:

$Y_{ij} = \mu + TRT_i + e_{ij}$,

where:
Y_{ij} = response variable (visceral organ measurements)
μ = the overall mean response common to all observations
TRT_i = the effect of the fibre inclusion level
e_{ij} = the residual error

For mucosal architecture measurements:

$Y_{ijk} = \mu + TRT_i + Seg\ (TRT)_{ij} + e_{ijk}$

where:
Y_{ijk} = response variable (AVSA, CD, VH and VH : CD ratio)
μ =is the overall mean response common to all observations
TRT_i = the effect of the fibre inclusion level
$Seg(TRT)_{ij}$= intestinal segment nested in treatment
e_{ijk} = the residual error

Mean separation was done with the PDIFF procedure of SAS (2008) for factors that were significant (P <0.05). Stepwise regression was used to identify the feed characteristic (DM, GE, CP, BD, EE, NDF, ADF and WHC), which best influenced intestinal VH and AVSA. Thereafter, the quadratic response surface model was used to determine the relationships between feed characteristics selected from stepwise regression and VH and AVSA of the small intestines. Pearson's correlation coefficients between VH and AVSA were also determined.

Results

All pigs were clinically healthy at the beginning of the experiment and at the end. Pigs that received diets high in fibre content had low average daily weight gains (ADG) compared to those receiving low fibre diets (*P* <0.05) (Figure 1).

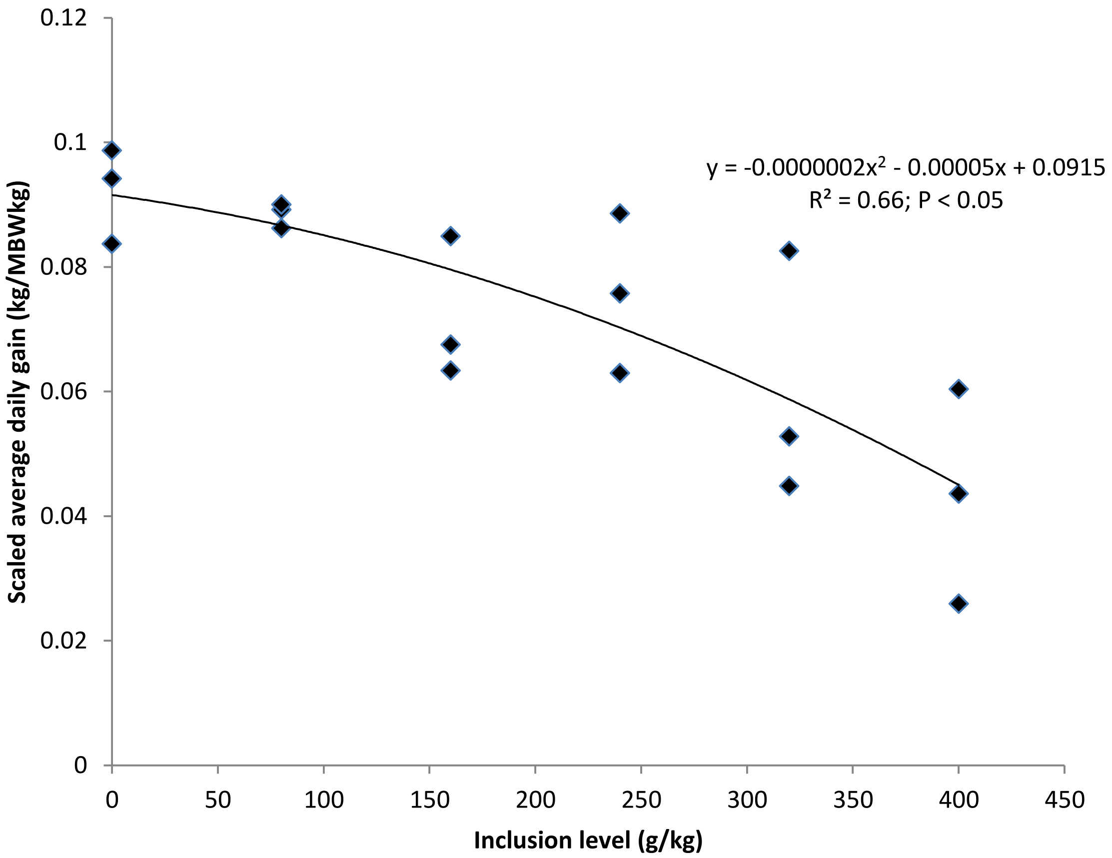

Figure 1 Relationship between scaled average daily gain and inclusion level of maize cob meal

Table 2 shows the means and relationships of organ weights and lengths of intestines of pigs fed diets with varying inclusion levels of maize cob meal. Weights of the livers of pig decreased with inclusion level of maize cob meal (*P* <0.05). Conversely, pancreas and stomach weights increased with inclusion level of maize cob meal. Weights of the small and large intestines of pigs fed diets with varying inclusion levels of maize cob meal were not different (*P* >0.05). Lengths of large intestines increased with inclusion level of maize cob meal. Means and relationships of the histological measurements of the mucosa of the intestines of pigs fed diets varying inclusion levels of maize cob meal are shown in Table 3. Villi height increased with inclusion level of maize cob meal in the duodenum, jejunum and ileum segments of the small intestines (*P* <0.05). The same trend was also observed for AVSA, which increased with inclusion level of maize cob meal for duodenum and ileum (*P* <0.05). CD was observed to increase with inclusion level of maize cob meal in all the segments of the small intestines (*P* <0.05). There were no differences in villi height to crypt depth ratio (VCR) of pigs fed incremental levels of fibre in the jejunum and ileum. VCR decreased with inclusion level of maize cob meal in the duodenum segment of the small intestine (*P* <0.05).

Table 2 Visceral organ weights and lengths of growing pigs fed diets with incremental levels of fibre

Organs	Inclusion level (g/kg)						SE	Linear		Quadratic	
	0	80	160	240	320	400		Regression coefficient	SE	Regression coefficient	SE
Weights, g/ MBW kg											
Liver	96.05	94.76	84.53	81.90	78.46	72.97	6.592	-0.66*	0.638	NS	-
Pancreas	5.02	5.74	5.32	6.95	7.30	6.94	0.404	0.08*	0.047	NS	-
Stomach	18.80	19.37	19.78	21.24	24.18	24.15	1.708	0.07 *	0.168	NS	-
Small intestine	102.21	84.88	95.65	86.83	95.70	100.48	5.403	NS	-	NS	-
Large intestine	52.33	49.26	56.94	49.70	48.62	67.26	6.390	NS	-	NS	-
Lengths, cm/ MBW kg											
Small intestine	126.14	105.10	107.49	103.23	128.15	137.31	8.117	-2.26 *	0.838	0.07 *	0.020
Large intestine	22.63	25.30	28.05	25.08	26.45	30.27	2.101	0.14 *	0.124	NS	-

MBW: metabolic body weight
NS: not significant (*P* >0.05); * *P* <0.05
SE: standard error

Table 3 Histological measurement (µm /Metabolic body weight kg) of mucosa of the intestinal segments in growing pigs fed diets with incremental levels of maize cobs

Item	Inclusion level (g/kg)						*P-value*			
							Linear		Quadratic	
	0	80	160	240	320	400	Reg. coeff	SE	Reg. coeffi	SE
Duodenum										
VH	25.4 ± 1.68	32.6 ± 1.63	33.6 ± 1.40	28.3 ± 1.73	29.5 ± 1.79	37.0 ±1.54	0.18*	0.089	NS	-
CD	26.0 ± 2.25	33.4 ± 2.19	35.8 ± 1.88	35.0 ± 2.33	37.8 ± 2.41	55.9 ± 2.07	-0.018*	0.012	NS	-
VCR	0.97 ± 0.08	0.8 ± 0.07	1.0 ± 0.06	0.8 ± 0.08	0.8 ± 0.07	0.7 ± 0.068	-0.0088*	0.0081	NS	-
AVSA	5.9 ± 0.40	6.6 ± 0.39	8.0 ± 0.33	6.7 ± 0.41	7.3 ± 0.43	9.2 ± 0.36	0.018*	0.0460	NS	-
Jejunum										
VH	24.3 ± 2.27	30.0 ± 1.80	33.0 ± 2.10	32.2 ± 1.51	29.6 ± 1.85	38.5 ± 1.71	0.22*	0.216	NS	-
CD	30.6 ± 1.99	28.9 ± 1.58	32.9 ± 1.84	32.9 ± 1.32	33.9 ± 1.62	39.6 ± 1.50	-0.071*	0.182	NS	-
VCR	0.80 ± 0.08	1.0 ± 0.07	1.1 ± 0.08	1.0 ± 0.06	0.9 ± 0.07	1.0 ± 0.06	NS	-	NS	-
AVSA	6.5 ± 0.50	5.7 ± 0.40	6.7 ± 0.46	7.5 ± 0.33	7.6 ± 0.41	9.5 ± 0.38	-0.014*	0.0464	0.0024*	0.0011
Ileum										
VH	26.2 ± 1.30	27.7 ± 1.48	35.7 ± 1.07	26.3 ± 1.21	34.7 ± 1.17	38.0 ± 1.56	-0.16*	0.069	NS	-
CD	24.2 ± 1.90	24.2 ± 2.16	26.2 ± 1.57	25.9 ± 1.77	33.1 ± 1.71	36.9 ± 2.28	-0.12*	0.097	0.011*	0.0049
VCR	1.3 ± 0.10	1.2 ± 0.11	1. 5 ± 0.08	1.0 ± 0.09	1.1 ± 0.09	1.1 ± 0.12	NS	-	NS	-
AVSA	6.2 ± 0.43	5.3 ± 0.50	7.4 ± 0.36	7.4 ± 0.40	7.5 ± 0.39	10.7 ± 0.52	0.015*	0.005	NS	-

VH: villi height; CD: crypt depth; VCR: VH : CD ratio; AVSA: apparent villi surface area
Reg. coeff: regression coefficient; NS: not significant (*P* >0.05); * *P* <0.05

Table 4 shows correlations of the various histological measurements of mucosa of the small intestines. There was a positive correlation between VH and AVSA (*P* <0.001). Moderate negative correlations were observed for VH and VCR, and CD and VCR (*P* <0.01). AVSA and CD showed a weak negative correlation (*P* <0.001).

Table 4 Correlation coefficients for histological measurements

	VCR	CD	AVSA	VH
VCR	-	-0.67*	-0.097NS	-0.41*
CD		-	-0.25*	-0.29*
AVSA			-	0.44*
VH				-

* *P* <0.001; NS: not significant (*P* >0.05);
VCR: VH to CD ratio; CD: crypt depth; AVSA: apparent villi surface area; VH: villi height

Using stepwise regression, BD and NDF were the best predictor variables influencing VH and AVSA (*P* <0.05). VH produced quadratic and linear responses with BD and NDF, respectively (*P* <0.05; Table 5). Conversely, AVSA produced quadratic and linear responses with NDF and BD, respectively (*P* <0.05; Table 5).

Table 5 Relationship between physicochemical properties and, villi height and apparent villi surface area

	Components of regression equation			P-value	
Item	**ax^2**	**bx**	**c**	**Linear**	**Quadratic**
VH	211.3 ± 58.49 $(NDF)^2$	-591.0 ± 159.06 (NDF)	442.4 ± 107.42	*	*
VH		0.03 ± 0.0047 (BD)	22.8 ± 1.59	*	NS
AVSA	0.000036 ± 0.0000148 $(NDF)^2$	-0.012 ± 0.00969 (NDF)	7.25 ± 1.493	*	*
AVSA		-47.12 ± 39.957 (BD)	45.03 ± 26.985	*	NS

* P < 0.05; NS: not significant (*P* >0.05)
a: regression coefficient of x^2; **b:** regression coefficient of x; **c:** intercept
VH: villi height; NDF: neutral detergent fibre; BD: bulk density

Discussion

In the current study, three pigs were subjected to each treatment diet. The number was sufficient to answer satisfactorily the scientific questions posed by the study. The bodyweight gains of pigs decreased with inclusion level of maize cob meal. The results are in agreement with previous studies (Le Goff *et al*., 2002; Ndou *et al*., 2013), which reported a decrease in ADG in growing pigs fed diets high in fibre levels. The observation that feeding diets with incremental levels of maize cob meal produce variable effects on individual visceral organ mass was expected. The increase in relative weight of the pancreas and stomachs agrees with a previous study (Agyekum *et al*., 2012). The increase in weight of the pancreas might be attributed to hyper-secretion of pancreatic enzymes to compensate for digestion and absorption inefficiency (Pond *et al*., 1988). At high inclusion levels of fibre, more enzymes are produced to break down coarse-textured feed compared with lower fibre diets. A decrease in the weight of the liver, on the other hand, was observed in pigs in the current study. When pigs were fed diets containing lucerne, an increase in liver weight was reported (Pond *et al*., 1988; Jørgensen *et al*., 1996; Nyachoti *et al*., 2000). On the contrary, a previous study (Ma *et al*., 2002) reported decreases in liver weights in growing pigs fed on diets with wheat straw. Such conflicting findings suggest that organ weights are influenced by the source of the insoluble fibre.

The weights of the small and large intestines relative to MBW of pigs were not affected by the fibrous diets. This is consistent with a previous study by Jin *et al*. (1994). An explanation might lie in the chemical composition of the feed offered to the pigs. At higher maize cob inclusion levels, where energy would be low, the pigs would not obtain enough energy for protein accretion (Pond *et al*., 1988; Jørgensen *et al*., 1996).

Weights of the intestines, therefore, are maintained or to some extent decreased, depending on the amount of energy the pigs are able to utilise from the fibrous feed (Quiniou & Noblet, 1995; Van Milgen *et al*., 2001). The observed increase in length of large intestines with inclusion level of maize cob might be attributed to the prolonged presence of fibre in the gut.

VH and AVSA were positively correlated. The observation that VH and AVSA increase along the gut agrees with a previous study by Jin *et al*. (1994). A possible explanation for the reduced VH and AVSA in the duodenum segment of the small intestines might be the abrasive effects of fibrous diets offered to the pigs on the most apical part of the villi (Agyekum *et al*., 2012). The abrasive effects of these fibrous diets, however, were not measured in the study and therefore warrant further investigation.

An increase in VH and AVSA is regarded as an adaptive mechanism to improve the absorption of nutrients from the gut (Caspary, 1992; Langhout, 1998; Yasar & Forbes, 1999). In growing pigs, the inclusion of 10% wheat straw has been found to increase VH and deeper crypts in the jejunum and ileum, and increased cell division and CD in the large intestine (Jin *et al*., 1994). In the current study, VH and AVSA increased with inclusion level of fibre in the diets. High fibrous diets have reduced density of nutrients. Hence, VH and AVSA increase absorptive functions to capture as many nutrients as possible for nutritional requirements to be met. The crypt region in the intestines is a site of cell regeneration. A plethora of studies have reported increased depth of crypt to in response to degeneration of cells of the villi.

The current study showed that diet quality influences villi morphology. BD of a feed can be described in terms of particle size of feed ingredients. If a feed has high bulk density, it means it has finely ground feed particles compared with a diet with low bulk density. Conversely, a diet low in bulk density has coarse feed particles. Feed with larger particle size has a lower rate of passage through the gastrointestinal tract (Warner, 1981), which results in greater contact between the food and the intestinal mucosa, thereby increasing villus height (Dahlke *et al*., 2003). This results in greater absorption of available nutrients from the digesta. This conforms with the findings in the current study on growing pigs in which BD influenced VH.

NGF consists of hemi-cellulose, cellulose and lignin, and is regarded as a complete description of fibre in a diet. An earlier report (Bindelle *et al*., 2005) showed that increasing the level of NDF in the diet generally decreases the digestibility of nutrients. This might account for increased VH and AVSA as NDF increased. NDF might have triggered a natural increase in absorption and utilisation of nutrients by increasing VH and AVSA as nutrients became more limiting.

Conclusion

High levels of fibre in diets are associated with an increase in VH and AVSA of small intestines. BD and NDF were the best predictor variables influencing VH and AVSA in segments of the small intestines. BD and NDF had linear and quadratic relationships with VH and AVSA of growing pigs, respectively. Further studies are required to investigate the abrasive effects of fibrous diets on the gastro-intestinal tract of pigs.

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Authors' contributions

AGB designed the research project and wrote the manuscript (as part of his PhD programme). Critical revisions of the manuscript were performed by MC.

Conflict of interest

None of the authors of this work has a financial or other relationship with people or organisations that could influence inappropriately or bias the contents of this paper.

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