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LIST OF ABBREVIATIONS

Ace	Acetic acid
ADF	Acid detergent fibre
AIA	Acid insoluble ash
AID	Apparent ileal digestibility
Ala	Alanine
AOAC	Association of Official Analytical Chemists
Ara	Arabinose
Arg	Arginine
Asp	Asparagine
ATP	Adenosine triphosphate
ATTD	Apparent total tract digestibility
BCFA	Branched chain fatty acids
BG	Brewer’s grains
βG	β-glucan
BUN	Blood urea nitrogen
But	Butyric acid
Ca	Calcium
CHO	Carbohydrate
CHO-OH	Hydrolysable carbohydrates
CHO-R	Rapidly fermentable carbohydrate
CHO-S	Slowly fermentable carbohydrate
CP	Crude protein
Cu	Copper
Cys	Cysteine
DDG	Dried distillers grains
DDGS	Dried distillers grains with solubles
DE	Digestible energy
DF	Dietary fibre
DM	Dry matter
EE	Ether extract

Fe	Iron
Fru	Frucose
Fuc	Fucose
Gal	Galactose
GC	Gas chromatography
GE	Gross energy
GIT	Gastro-intestinal tract
Glc	Glucose
Glu	Glutathione
Gly	Glycine
HC	Hominy chop
HF	High fermentability
His	Histidine
^{1}H-NMRS	Proton nuclear magnetic resonance spectroscopy
HO-Pro	Hydroxy Proline
iNCP	Insoluble non-cellulosic polysaccharides
iNSP	Insoluble non-starch polysaccharides
Iso-But	Iso-Butyric acid
Iso-Leu	Iso-Leucine
Iso-Val	Iso-Valeric acid
$IVDMD_{PP}$	*In vitro* dry matter digestibility (Pepsin+Pancreatin)
$IVDMD_{RX}$	*In vitro* dry matter digestibility (Roxazyme)
$IVDMD_{RX+VZ}$	*In vitro* dry matter digestibility (Viscozyme+Roxazyme)
$IVDMD_{VZ}$	*In vitro* dry matter digestibility (Viscozyme)
Leu	Leucine
LF	Low fermentabilty
LH	Lucerne hay
Lys	Lysine
MC	Maize cobs
ME	Metabolisable energy
Met	Methione
Mn	Manganese
N	Nitrogen

NDF	Neutral detergent fibre
NDS	Neutral detergent soluble
NE	Net energy
NSC	Non-structural carbohydrates
NSP	Non-starch polysaccharides
NSPase	NSP degrading carbohydrase
OM	Organic matter
Phe	Phenyalanine
PP	Pepsin-Pancreatin
Pro	Propionic acid
Prol	Proline
RS	Resistant starch
RX	Roxazyme
SCFA	Short chain fatty acids
Ser	Serine
SH	Soy hulls
sNCP	Soluble non-cellulosic polysaccharides
sNSP	Soluble non-starch polysaccharides
TDF	Total dietary
Thr	Threonine
tNSP	Total non-starch polysaccharides
TRF	Terminal restriction fragment
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
Val-a	Valeric acid
VZ	Viscozyme
WB	Wheat Bran
WSC	Water-soluble carbohydrates
Xyl	Xylose
Zn	Zinc

ABSTRACT

The objectives of the research were to examine the effects of high dietary levels of fibrous feeds, and of supplementation with Roxazyme® G2 (RX), on the digestive metabolic and physiological responses of growing pigs fed maize-soybean diets.

The nutrient and dietary fibre (DF) composition, the swelling and water-binding capacities of maize (MM), its hominy chop (HC) and cobs (MC), dehulled soybean (dSBM) and the hulls (SH), brewer's grains (BG), lucerne hay (LH) and wheat bran (WB) were evaluated using standard procedures. Feed fibre fractions were isolated by simulating upper tract digestion in an Ankom® Daisy^{II} Incubator, whereby each feed was digested in pepsin (porcine, 200 FIP-U/g, Merck No, 7190), followed by pancreatin (porcine, grade IV, Sigma No P-1750), with recovery of the fibrous residues. In a third step to complete the simulated pig gastro-intestinal digestion, the pepsin-pancreatin fibre extracts were digested by RX or Viscozyme L ® V2010 (VZ). Enzyme activity was measured as the coefficients of partial degradability (solubilisation) of the washed fibre extracts. The kinetics and products of fermentation of the DF were evaluated in an Ankom^{RF} gas production system, using buffered faecal inoculum.

Among the feed ingredients, dissimilar, fibre source-dependent activities between RX (0.02 to 0.12) and VZ (0.04-0.33) were observed. The lowest RX activities were observed on the maize and soybean derived fibres, with similarly low VZ activity on MC fibre. Variation in the activity of faecal microbial enzymes was similarly indicated by the variable production of fermentation gas (51.8-299.4 mL g^{-1} DM) and short chain fatty acids (SCFA) (2.3-6.0 mMol g^{-1} DM). Soy hull, dSBH, MM and HC fibres were highly fermentable, with low fermentability of BG, MC and WB fibres. The fibres differed in the composition of fermentation SCFA, whereby SH, LH and MC shifted fermentation to Ace, and BG, dSBM, WB, MM, HC favoured Pro, while MM and HC favoured But production.

The same nutritional properties were similarly evaluated in complete diets which were formulated from the ingredients for growth, and metabolic trials. For the growth trial, a standard (STD) (control), 141 g total dietary fibre (TDF) kg^{-1} dry matter (DM) maize-soybean growing pig diet, and five iso-nutritive, 246 g TDF kg^{-1} DM nutritionally balanced diets were

formulated. The high DF was achieved by partial replacement of the MM and dSBM in the STD diet with MC, SH, BG, LH or WB.

The differences in RX and VZ activities and in the fermentation characteristics which were observed on the fibre extracts from the high fibre ingredients were reflected in the DF from the respective complete diets in which they were included. However, the fibre from the basal dietary ingredients reduced the absolute values and the variation in the activities of RX (0.03-0.06) and VZ (0.16-0.22), and similarly reduced the variation in gas (126.6-187.6 mL g^{-1} DM) and SCFA (4.1-5.4 mMol g^{-1} DM) production of the DF from the fibrous diets. Enzyme activities on the STD DF were low for RX (0.03) and high for VZ (0.25). The STD DF produced 205.3 mL gas g^{-1} DM, which was similar to SH DF, and higher than all the other diets. The STD DF produced 5.0-mMol SCFA g^{-1} DM, which was quantitatively, and not statistically higher than the other fibres. The composition of SCFA was similar across all diets, except for the high percent Ace, with low Pro by the SH DF. Compared to the STD, the high DF diets increased percent Ace, with reduced Pro and But.

The STD, MC, SH, BG, LH and WB diets were each prepared in duplicate mixes, one of which was fortified with 200 mg RX kg^{-1} feed (as fed). Seventy-two intact Large White X Landrace, male, 32.0 ± 5.6 kg live weight (LW) pigs were allocated to the diets in two completely randomised weight blocks in a 2 (fibre source) X 2 (enzyme) factorial arrangement. The pigs were fed a*d libitum* for 10 weeks. Cumulative LW gain and feed intake were measured at different stages of growth, and at slaughter. Apparent total tract digestibility (ATTD) of nutrients was estimated at 65-70 kg LW, using 0.2% (as fed) chromium oxide as the indigestible marker. Ileal tissue was sampled 50 cm above the ileo-caecal valve, on which *villi* height and area, and crypt depth were evaluated by computerised image analysis. Blood was sampled at slaughter from the severed *vena jugularis*, 16 hours after feeding. Serum urea, creatinine, triglycerides, glucose, and total cholesterol were analysed chemically. The serum metabolome was further explored using Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H -NMRS).

There was fibre X RX interaction for *villi* height, whereby the enzyme reduced the *villi* height in pigs on the SH, STD and WB diets, with an opposite effect on pigs on the MC, BG, LH

diets. The soluble fibre content was negatively correlated with crypt depth. Chemical analysis did not detect differences in metabolite concentration between the STD and the high fibre diets. However, more serum cholesterol was observed in pigs fed the WB compared to the LH and MC diets. ^{1}H-NMRS indicated that feeding pigs the WB diet increased serum Cys and His, while supplementation of RX increased serum formate, glucose, and urea. There was diet X enzyme interaction for fructose, glucose, Arg, Cys, Ser, and Trp, whereby RX increased the levels in pigs on MC and WB, with an opposite effect in pigs on the other diets.

There was large DF source-dependent variation among diets in ATTD of DM (0.80-0.85), organic matter (OM) (0.81-0.87), gross energy (GE) (079-0.85) and CP (0.81-0.85), whereby, relative to the STD diet, high DF reduced the ATTD of DM (all diets except SH), organic matter (OM) and energy and CP (all diets except the MC). Positive correlation was observed between fermentability and the ATTD digestibility of DM, OM, energy, ADF, NDF, and fat. Negative correlation was observed between the swelling capacity and the ATTD of DM, OM, energy and protein, between DF solubility and DM, OM, protein, ADF and NDF, and between water binding capacity and ATTD of DM and OM, energy and NDF.

At slaughter, there was similarly large, and DF source-dependent variation among the high fibre diets in feed intake (2.31-2.71 kg as fed day-1), live weight gain (0.75-0.86 kg day-1), and feed: gain ratio (2.73-3.00). Corresponding values for the STD diet were 2.44 kg day-1, 0.83 kg day-1and 2.86 kg day-1, respectively. Relative to the STD, LH reduced feed intake and live weight gain, and MC increased the feed: gain ratio. Predictions based on the *in vitro* fermentability of DF and feed intake suggested that due to poor fermentability, and or restriction of feed intake, relative to a standard fibre diet, high dietary levels of MC, WB and BG may reduce fermentation in the lower gut, while similar dietary levels of SH and LH may result in substantial increases in fermentation.

At 50 kg LW, the fermentability of DF was positively correlated with feed intake and with weight gain, while water binding capacity and solubility of DF were negatively correlated with feed intake. At slaughter, the solubility of DF was negatively correlated with feed intake and feed: gain ratio. Large variation among the high fibre diets was also observed in the

slaughter weight (89.2-96.8 kg), dressing % (68.6-76.4), meat colour (80.4-82.3), lean % (69.5-71.2), and fat % (10.1-12.6). In comparison, pigs on the STD diet scored 94.7 kg slaughter weight, 75.1% dressing, 81.6 cm carcass length, 82.5 meat colour, 68.4% lean, and 15.0% fat. Relative to the STD, LH reduced dressing and fat %. Lucerne hay and WB increased the lean%.

For the metabolic trial, two iso-nutritive, mixed high fibre (319 g TDF kg^{-1} DM), nutritionally balanced diets were formulated to contain DF of high (HF) versus low (LF) fermentability. The diets had similar content of soluble DF and similar swelling and water binding capacities. Viscozyme was more active than RX on both the HF (0.20 versus 0.04) and the LF (0.17 versus 0.07) DF. The combination of RX and VZ statistically increased the enzyme activity on the HF (0.25) and quantitatively increased enzyme activity on the LF (0.18) DF, suggesting additive or synergistic effects. More gas was produced by the HF (159.5 mL g^{-1} DM) compared to the LF DF (96.6 mL g^{-1} DM). More SCFA were produced by HF (5.0 mMol g^{-1} DM), compared to the LF DF (3.6 mMol g^{-1} DM). Compared to the STD, HF DF increased percent Ace, with reduced Pro and But. The LF DF increased percent Ace, with quantitative, and not statistical reduction of Pro and But.

In a metabolic trial, the HF and LF diets, and their duplicates containing 0.270 g RX kg^{-1} DM of feed (as fed) were fed *ad libitum* to eight ileum T-cannulised, intact Large White X Landrace male pigs weighing 65.0 ± 5.1 kg. The diets were allocated to the pigs in a duplicate 4 x 4 Latin Square design, in a 2 (enzyme) x 2 (fermentability) factorial arrangement. Each period consisted of two weeks of adaptation followed by five days of sampling. The ileal digesta was collected in each period and was similarly subjected to the fermentation test. Apparent ileal digestibility (AID) and ATTD were determined using 0.2% (as fed) chromium oxide as the indigestible marker. N excretion in faeces and urine were measured, and N retention was calculated. Blood was sampled by *vena jugularis* puncture on the last day of each period. Two blood samples were collected, the first 15 hours after removal from feed (15-hour serum), and the second 3 hours after re-introduction to feed (3-hour serum). Serum metabolites were evaluated by both chemical analyses and by ^{1}H-NMRS, as described for the growth trial.

Roxazyme did not affect the fermentation characteristics of the ileal digesta. In similar proportion to the fermentability of the PP digesta, the HF ileal digesta was more fermentable (65.4 mL gas g^{-1} DM and 6.1 mMol SCFA g^{-1} DM) than the LF ileal digesta (46.7 mL gas g^{-1} DM and 4.4 mMol SCFA g^{-1} DM SCFA). Prediction based on the *in vitro* fermentability of DF and feed intake suggested the HF diet could support one half times more fermentation in the lower gut compared to the LF diet.

The HF diet had higher AID of DM (62.5 vs. 58.6), OM (65.6 vs. 62.1), energy (64.4 vs. 61.0), fat (85.8 vs. 81.7) and ash (41.8 vs. 32.7). The AID of HO-Pro, Met and Val were higher for the LF diet. There was diet X enzyme interaction on the AID of Met, whereby the RX reduced the AID of met in the LF diet, and not that of the HF diet. The ATTD was higher for the HF diet for DM (74.2 vs. 68.4), NDF (64.7 vs. 57.4), and ADF (35.1 vs. 21.0).

There was positive correlation between the fermentability of DF and the AID DM, OM, ash, ash, fat and energy. The solubility of DF was negatively correlated with the AID of DM, OM, ash, fat, ADF and energy, and with the ATTD of DM, OM, ash, fat, energy, NDF, and ADF. Negative correlation was also observed between the swelling capacity of DF and the AID of protein, Trp and Lys. The solubility of DF was positively correlated with Ser, Ala, Val, Iso-Leu and His.

There was diet X enzyme interaction for urea in the 15- hour serum, whereby RX tended to reduce the urea in the LF diet, while it increased that of the HF diet. Fermentability negatively correlated with urea in the 15- hour serum, and positively correlated with serum glucose in the 3-hour serum. In the 3-hour sample, ^{1}H-NMRS indicated higher fucose, Pro and cholesterol in the LF diet. ^{1}H-NMRS also indicated fermentability x RX interaction for Ser, Tyr, Lys, creatine, and possibly, glucose or fructose, glycerol or Gly and His or Arg, whereby RX increased the levels in the LF diets, with opposite effect in the HF diet.

In conclusion, enzyme activities and fermentability were higly variable among different DF sources, and the effects were evident in the fibrous complete diets. The results of the *in vitro* studies supported the application of the methods to formulate fermentable insoluble fibre-rich, maize-soybean-mixed co-product diets. Correlation analyses suggested that DF fermentability, and solubility, swelling and water binding capacities explained significant

proportions of the variances of the metabolic and physiological responses of the pigs to different feeds. Predictions based on the *in vitro* fermentability of DF and feed intake suggested that a strategy whereby pig diets are enriched in DF after the feedstuffs are screened on DF fermentability could substantially increase fermentation in the lower gut. Overall, the results suggested that productivity can be maintained in growing pigs fed diets containing up to twice the standard levels of DF, provided producers target co-product feeds that contain highly fermentable DF. The use of RX to improve nutrient digestion and to stimulate gut fermentation was not justified.

Key words: *enzymes, fermentability, fibre, grain-processing co-products, growing pigs, ileal histo-morphology, maize-soybean diets, non-starch polysaccharides, proton nuclear magnetic resonance spectroscopy, Roxazyme G2*

CHAPTER 1

INTRODUCTION

1.1 Background

Economic and environmental pressures may force producers to substitute the cereal and oilseed grains in growing pig diets with their cheaper, but highly fibrous processing co-products (Zijlstra *et al.,* 2010). The high cost of feed will likely persist due to increasing global demand for the grains (Rosegrant *et al.,* 2001). Biophysical models predict global decline in crop production, if the extreme scenarios of the impact of climate change obtain (Parrya *et al.,* 1999). On the other hand, bio-fuel production is competing for the grains, while expanding the co-product market (Taheripour *et al.,* 2010). Greater and more efficient utilisation of the co-product feedstuffs is therefore likely to be an important economic and environmental response of commercialised agriculture to climate change (Adams *et al.,* 1998).

Reviews of literature by Noblet and Le Goff (2001), Wenk (2001), Aarnink and Verstegen (2007), Bindelle *et al.* (2008) and Zijlstra *et al.* (2010) revealed more than three decades of research on the nutrition of fibre in growing pigs. The evidence showed that generally, depending on the type and level to which fibre is included in the diet, reduced nutrient digestibility and restriction on feed intake combine to limit nutrient, particularly energy intake, and therefore animal performance. Beneficial effects of dietary fibre (DF) in the metabolic and physiological function of the gastro-intestinal tract (GIT) were also reported, which similarly depend on the type and level of fibre.

In order to mitigate the negative impacts of including fibrous ingredients in growing pig diets, the focus of research is increasingly extending to maximising the beneficial effects of fibre, which largely derive from its fermentability. For instance, microbial synthesis in the ileum may reduce the requirement for some amino acids (Zhu *et al.,* 2007). Short chain fatty acids (SCFA) produced during fermentation may contribute substantially to the energy requirement (Anguita *et al.,* 2006). Highly fermentable fibre has prebiotic effects in the lower gut, and the

SCFA products have trophic effects on the absorptive epithelia of the intestines (Smiricky-Tjardes *et al.,* 2003; Kien *et al.,* 2007; Martins *et al.,* 2010; Reilly *et al.,* 2010; de Lange *et al.,* 2010). The supply of fermentable fibre in the colon reduced the production of toxigenic (Cone *et al.*, 2005) and odorous (Nahm, 2003; Bindelle *et al.,* 2008; Zervas and Zijlstra, 2002) compounds. Increased fermentation diverted N from excretion as volatile urea in urine, to excretion as stable bacterial protein in faeces (Bindelle *et al.,* 2009). Of interest to human health, SCFA acids are also known to control satiety (Sleeth *et al.,* 2010) and are involved in glucose (Theil *et al.,* 2011) and lipid (Fushimi *et al.,* 2006) homeostasis.

Zijlstra *et al.* (2010) evaluated the current scientific evidence and concluded that there is scope to expand the role of co-product feed ingredients in the nutrition of the growing pig, provided there is application of modern processing and enzyme technologies, and provided high precision energy and amino acid evaluation systems are used. The strategy therefore should be to maintain productivity on the high fibre diet, with minimal need for supplementary essential amino acids and energy, which can substantially increase the cost of the fibrous diet. Producers need to purposefully select the fibrous ingredients based on the properties of the fibre, process the feedstuffs, and match the non-starch polysaccharides (NSP) substrates to NSP polysaccharide degrading enzymes (NSPases), to formulate diets for optimum nutritional, physiological and environmental outcomes.

In order to fit into the high precision modern feeding systems, the interventions proposed above require clarity on the mechanisms underpinning the metabolic and physiological activity of DF. However, these are not yet fully understood, a consequence of the complex three-way interaction of the effects of source of fibre, the animal's homeostasis and the microbial response in the gut (Williams *et al.,* 2001). Among feedstuffs, the metabolic and physiological activity of DF in the digestive tract is mediated by the shared but source-dependent properties of fermentability, solubility, viscosity, swelling and water binding, and adsorption (Bach Knudsen, 2001). These properties derive primarily from the secondary and tertiary structures of the constituent NSP, and secondarily, from the plant cell wall architecture, both of which are highly heterogeneous among plants (Bach Knudsen, 1997). Zijlstra *et al.* (2010) argued that the complexity of the factors that determine the metabolic

activity of DF justify concerted research towards a modelling approach to facillitate the practical application of nutritional interventions that effectively target its beneficial effects.

The application of NSPase technology is intended to ameliorate the detrimental effects of DF, thereby enabling higher levels of inclusion, and expanding the range of fibrous feed ingredients (Barletta, 2011). Enzymes may also alter the fermentation processes in the GIT (Bindelle *et al.,* 2011; Jonathan *et al.,* 2012), and therefore present an opportunity to manipulate fermentation to enhance its beneficial effects.

It is noteworthy, however that historically, the development of enzyme technology targeted poultry on standard, European type diets in which the specific target are the gel forming glucans and arabinoxylans of wheat, oats, barley or triticale (Zijlstra *et al.,* 2010). Unlike in poultry, and perhaps except in very young pigs, or on diets that contain high levels of the viscous polysaccharides containing cereals, the digestive system of the pig seems not as prone to the negative effects of viscous gel forming fibre (Barletta, 2011). Not surprisingly therefore, in growing pigs, there is uncertainty on the efficacy of exogenous enzyme technology, particularly on maize-based diets enriched with high levels of typically highly chemically complex insoluble fibres (Barletta, 2011).

The inconsistent findings on the efficacy of enzymes are also attributed to the interactions of type, level of fibre, age of the experimental animals (Ji *et al.,* 2008) and may partly reflect the effect of processing (De Vries *et al.,* 2012). For comparability, good experimental design therefore requires proper definition and characterisation of the fibre for the critical physico-chemical properties, correct description of enzyme activities and matching the affinities to the NSP substrates, with optimisation of the enzyme dosage (Zijlstra *et al.,* 2010). Overall, there is greater effect on nutrient digestion and growth performance of young, compared to older pigs (Noblet and Le Goff, 2001), and greater effect in temperate cereal (wheat, barley, rye and oat) diets, as opposed to maize diets (Ji *et al.,* 2008). Synergistic effects of processing and enzyme technologies on nutrient digestibility have been reported (De Vries *et al.,* 2012).

Given the diversity of potentially usable fibrous co-product ingredients, there is need to develop rapid, inexpensive techniques to evaluate the degradation properties of DF, and to match the NSP substrates to the activities of different NSPases in commercial products. Boisen and Fernandez (1997) described an *in vitro,* three-step enzyme hydrolytic procedure to simulate the GIT digestion of fibrous feeds by pigs. Bindelle *et al.* (2007a) adapted the *in vitro* fermentation gas production technique originally described for ruminants (Menke, et. al., 1979), to simulate the fermentation of DF flowing into the pig's hindgut. They conveniently used pig faeces as inoculum, to ferment DF extracted by simulated upper tract digestion, according to Boisen and Fernandez (1997).

The development of modern high-technology feed evaluation equipment presents further opportunity to adapt the traditional *in vitro* assays to be more rapid, with greater replication, accuracy and precision. For instance, the Boisen and Fernandez (1997) *in vitro* gastric-ileal digestion procedure was recently adapted (Akinsola *et al. personal communication)* for use in an Ankom Daisy^{II} Incubator. The set-up accommodates multiple samples and replicates, which is convenient for subsequent, similarly rapid, sensitive, and automated ANKOM^{RF} fermentation gas production assays on the fibrous residues. The latter procedure also allows for follow-on SCFA and metagenomic analyses of the bacteria in the fermentation media.

Given the diversity of co-product feeds whose nutrient and anti-nutrient composition is often not defined, there is need for techniques that provide robust exploration of the impact of feeding high levels of such feeds on the overall intermediary metabolism of the animal. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) based metabonomics has recently been used to complement traditional chemical analytical methods in studying changes in intermediary metabolism that are caused by feeding fibrous diets to pigs (Yde *et al.,* 2011). Explorative ^{1}H-NMR spectroscopy conveniently allows for the detection of numerous metabolites in bio-fluids such as plasma, with little or no preparation and without pre-selection of the analytes. An important application of this approach is therefore to detect subtle dietary influences of DF on nutrient metabolism which may impact on metabolic efficiency and on meat quality, and to identify anti-nutrients and nutritionally beneficial

metabolic modifiers, which may be present in non-conventional fibrous feedstuffs (Yde *et al.,* 2011).

The research investigated the digestive metabolic and physiological effects on growing pigs of including high levels of different fibrous feedstuffs in maize-soybean based diets that are supplemented with Roxazyme® G2 (RX), in relation to the physico-chemical properties of the DF.

1.2 Problem statement

The current pressure on grain markets may increasingly force pig producers to substitute the cereal and legume grains in conventional growing pig diets with their cheaper, but typically fibrous processing co-products. The impact on productivity, and hence on the viability of the industry are major concerns. The application of NSPases is considered critical to ameliorate the detrimental effects of DF, thereby enabling higher levels of inclusion, and expanding the range of fibrous feed ingredients. This production scenario has stimulated research focussed on addressing the uncertainty on the efficacy of currently available commercial NSPase cocktails, particularly on maize-soybean diets. The possibility to feed growing pigs on high fibre diets has also stimulated interest to exploit the beneficial influences of fermentation on the pig's physiology. In addition to increased DF, possible strategies to increase fermentation in the gut include the screening of the fibre sources on the fermentability of DF, and, possibly, the application of NSPases. However, the mechanisms that underpin the metabolic and physiological activity of DF in growing pigs are not yet clarified sufficiently to realise these objectives. There is need to further examine the relationships between the critical physico-chemical properties of fibre and the host animal and gut microbial responses. Clarification of such relationships will assist producers to purposefully select fibrous ingredients, and to match them to fibrolytic enzymes, in order to formulate diets for optimum production, economic and environmental outcomes.

1.3 Hypotheses

1. There are differences in the digestive metabolic and physiological responses of growing pigs fed maize-soybean diets containing high levels of different fibre rich feedstuffs,

which are due to the heterogeneity in the hydration and fermentation characteristics of the DF.

2. The efficacy of RX in growing pigs fed maize-soybean based diets containing high levels of fibre rich feedstuffs depends on the composition of DF, its hydration and fermentation characteristics.

1.4 Objectives

1.4.1 Broad Objective

The goal was to evaluate the potential of partially substituting the conventional feed ingredients in growing pig diets with cheaper, but typically fibrous co-product feedstuffs, in diets formulated for optimum metabolic, physiological, production, economic and environmentally friendly outcomes.

1.4.2 Specific Objectives

1. To evaluate maize *(Zea Mays)* (MM), its hominy chop (HC) and cobs (MC), dehulled soybean (*Glycine max) (dSBM)* and its hulls (SH), brewer's (barley; *Hordeum vulgare L)* grains (BG), lucerne (*Medicago sativa)* hay (LH), wheat (*Triticum aestivum*) bran (WB) and their constitutive high fibre, maize-soybean based growing pig diets in terms of;

i. the nutrient and fibre composition
ii. the solubility of DF, and feed water binding and swelling capacity
iii. the fermentation properties of DF by pig faecal bacteria
iv. the degradation of DF by Roxazyme® G2 (RX)

2. To determine the effects of high fibre diets formulated by partial substitution of the basal ingredients of a standard maize-soybean diet with MC,LH,SH,BG, WB or their fermentability contrasted mixtures, and fortified with RX, on growing pigs in terms of;

i. animal performance; nutrient digestibility and N retention, feed intake, weight gain, efficiency of feed utilisation and carcass traits
ii. the morphology of the ileal epithelium

iii. blood metabolites

1.5 Delineation and limitations of the research

1.5.1 Scope of the research

The research was conducted in four phases:

a) Determination of the physicochemical and fermentation properties of feeds;
   i. Nutrient composition
   ii. Fibre fractions
   iii. Swelling capacity
   iv. Water binding capacity

b) *In vitro* assays:

   i. Simulation of gastric-ileal digestion using an Ankom Daisy^{II} Incubator digestion technique
   ii. Simulation of lower gut fermentation properties of DF using the Ankom ^{RF} gas production system
   iii. NSPase activity on DF extracted from different feed ingredients and complete diets

c) Growth trial:

A growth trial was conducted using pigs fed on maize-soybean diets in which the basal ingredients were partially substituted by different fibrous feed ingredients and which were fortified with RX. The following parameters were measured;

   i. Growth and slaughter performance
   ii. Histometry of the ileal epithelium using computerised image analyses
   iii. Blood metabolites using chemical and ^{1}H-NMRS analyses

d) Metabolic trial

A metabolic trial was conducted using ileal-cannulised growing pigs individually fed high fibre diets with contrasting fermentability and fortified with RX. The following analyses were performed:

i. Apparent ileal, total tract digestibility of nutrients
ii. N balance
iii. Chemical and ^{1}H-NMRS analyses of blood metabolites

1.5.2 Limitations of the research

Financial, logistical and procurement challenges during the planning and implementation of the research resulted in the following limitations;

i. The study had to exclude maize distiller's dried grains (DDG) or distiller's dried grains with solubles (DDGS). These are important biofuel co-products that have attracted the most of global research in fibre nutrition in pigs. Inclusion of the most researched feedstuffs was critical for comparability of findings with previous research.
ii. The viscosity of DF was not evaluated. The research used the solubility and swelling of DF as proxy-variables. Viscosity is a major variable controlling the physiological activity of DF.
iii. The *in vitro* gastric-ileal digestion procedure originally described by Boisen and Fernandez (1997) was adapted for application in the Ankom Daisy^{II} incubator. Although the set-up allows for greater replication and control of environmental variables, the sample sizes still require multiple runs to generate sufficient residue for subsequent fermentation tests, which is costly and time consuming. This limited the number of possible replications for the fermentation studies.
iv. For the fermentation tests, the Ankom^{RF} gas production system was adapted to ferment pig feeds using buffered pig faecal inoculum. During the mathematical modelling of the fermentation data, the relatively slow fermentation capacity of the pig faecal inoculum resulted in difficulty in fitting the gas data to different models, which further reduced the assay replicates. The available gas models were developed for ruminant fermentation

kinetics, which are determined using the more rapidly fermenting rumen liquor. The measurement of bacterial species and gas composition were also excluded due to logistical and cost constraints.

v. In the growth and balance trials, at the time a uniform crop of the animals was secured for the experiment, difficulty in timely procurement of the feed ingredients and in conducting the surgical interventions resulted in the evaluation of pig performance after the early growing phase. Pig responses to the DF are likely most pronounced and therefore most important during the early growth phase (weaning to 30 kg live weight). Time and logistical constraints also excluded phase feeding to align dietary composition to the changing nutritional requirements with age.

CHAPTER 2

DIETARY FIBRE IN GROWING PIG NUTRITION: A REVIEW

2.1 Introduction

The current pressure on grain markets may increasingly force pig producers to substitute the cereal and legume grains in growing pig diets with their cheaper, typically fibrous processing co-products (Zijlstra *et al.,* 2010). The impact on productivity, and hence on the viability of the industry are major concerns.

The digestive and metabolic effects of DF in growing pigs were comprehensively reviewed by Noblet and Le Goff (2001), Wenk (2001), Aarnink and Verstegen (2007) and Brownlee *et al.* (2011). Depending on the source and dietary level, the evidence generally revealed a negative impact of DF on nutrient digestion and animal performance, with a tendency for positive, fermentation-induced influences on gut health, odour compound production and emission. Consequently, the drive to increase the role of co-product feeds in growing pig nutrition has expanded research on the effects of fibre on gut health (Smith *et al.,* 2010; Bach Knudsen *et al.,* 2012) and on odour emissions (Nahm, 2003; Zervas and Zijlstra, 2002). Of interest to human nutrition are the beneficial effects of DF on metabolic health (Lattimer and Haub, 2010), which can be explored on the pig model. Zijlstra *et al.* (2010) argued there is scientific justification for a greater role for fibrous feed ingredients in growing pig diets, provided there is application of modern feed evaluation, processing and enzyme technologies, and provided high precision energy and amino acid evaluation systems are used.

The need for research on the possibility to use diet formulation (Bindelle *et al.,* 2011) and NSPases (Bedford and Cowieson, 2011) to control fermentation in the lower gut is increasingly recognised. Currently, there is renewed interest to address the uncertainty on the efficacy of enzymes in pigs, particularly on maize-soybean based diets (Barletta, 2011; Willamil *et al.,* 2012).

The mechanisms that control the metabolic and physiological activities of DF are complex and not yet understood sufficiently to enable producers to purposefully select the fibrous ingredients, process, and match the NSP substrates to NSPases, to formulate high fibre diets for optimum nutritional, physiological and environmental outcomes. They involve complex interactions of the animal homeostasis, the type of fibre and the microbial ecological responses in the gut (Zijlstra *et al.,* 2010). Among different sources, the metabolic and physiological activity of fibre is mediated by the shared but source-depended, highly variable properties of fermentability, viscosity, hydration and adsorption (Bindelle *et al.,* 2008). These properties derive primarily from the secondary and tertiary structures of the constituent NSP, and secondarily, from the plant cell wall architecture and molecular associations, which are highly heterogeneous among fibres (Mertens, 2003). Zijlstra *et al.* (2010) argued that the complexity of the factors that determine the metabolic activity of DF requires concerted research towards a modelling approach to facilitate the application of practical nutritional interventions that target its beneficial effects.

This review explores the state of knowledge and trends in research on some key questions in the nutrition of fibre in growing pigs. The review focusses on (i) the heterogeneity among feedstuffs in the chemical, physical and the fermentation properties of DF, in relation to their variable perturbation of the normal digestive metabolic and physiological functions of the GIT(ii) the conundrum in defining and characterising DF with nutritional relevancy to the monogastric animal (iii) the uncertainty on the relevance of enzyme technology in growing pigs (iv) developments in *in vitro* methods for predicting the metabolic impacts of fibrous diets in growing pigs. (iv) developments in the application of advanced nuclear magnetic resonance based metabonomics in fibre nutrition in pigs.

2.2 The definition of DF

Since Hipsley (1953) proposed the term “dietary fibre”, its functional definition remains controversial, and dynamic. Unlike other dietary components, the definition of DF is dependent on the methods of its analyses, the nutritional relevance of which is still considered largely inadequate (Mertens, 2003). The most recent literature on pig nutrition defines DF in chemical or analytical terms as the NSP plus lignin (Bach Knudsen, 2001). In human nutrition, the definition is broadened to “the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the intestine, with complete or partial fermentation in the large intestine” (AACC, 2001). This definition includes polysaccharides, oligosaccharides and associated plant substances that promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation. A significant improvement is the inclusion of the unnatural complex carbohydrate compounds, such as the resistant starches.

In this study, DF is defined as total DF (TDF), based on the AOAC method 991.43 (AOAC, 2007). Total dietary fibre includes the compounds outlined in **Figure 2.1.** However, the method does not recover the oligosaccharides, and the fructans are only partially recovered (Geor, 2007).

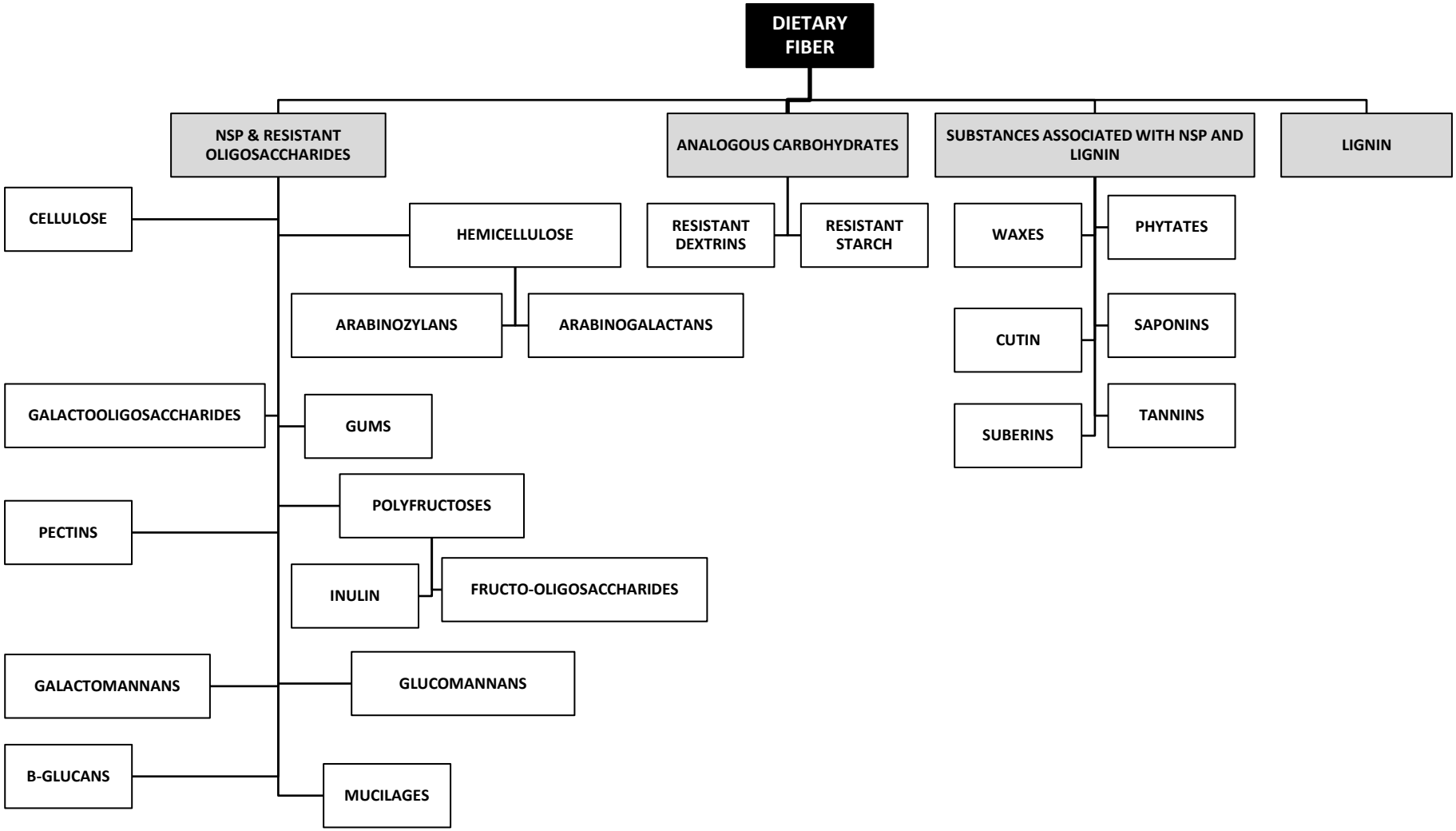


Figure 2.1: Major constituents of DF (From AACC, 2001)

2.3 The chemistry of fibre

Zijlstra *et al.* (2010) argued that a major limitation of previous research in the nutrition of fibre in growing pigs is that the test diets are not consistently analysed for the chemical moieties relevant to its nutritive or anti-nutritive value, such that the analyses and comparison of findings is not always possible. It is important therefore that the full range of compounds present in DF is clearly defined. Ideally, fibre fractions should be classified and analysed in entities relevant to the metabolic and physiological activity of DF.

2.3.1 Components of dietary fibre

The main components of DF are the NSP. These are classified based on five main structural properties (Sinha *et al.,* 2011):

- Monomeric composition
- Monosaccharide ring forms (6-membered pyranose (p) or 5-membered furanose (f))
- Positions of the glycosidic linkages on the ring structures (1,2,3,4,5, or 6)
- Steric configurations (α or β) of the glycosidic linkages
- Presence or absence of non-carbohydrate residues

The monosaccharides commonly present in NSP include the hexoses (D-glucose, D-galactose, D-mannose, L-rhamnose), pentoses (L-arabinose, D-xylose, L- Fucose) and acidic sugar derivatives (D-galacturonic acid, D-glucuronic acid and its 4-O-methyl ether).

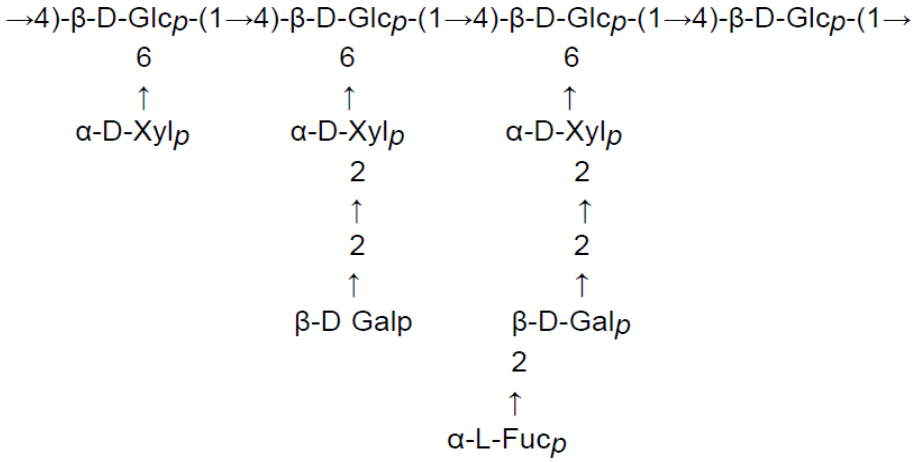
A unique feature of polysaccharides is their polydispersity. Thus, from the small complement of the monomers, physico-chemically highly heterogeneous NSP are synthesised (Izydorczyk *et al.,* 1992). Examples of structures of the NSP commonly present in the pig diets are indicated in **Figure 2.2.**

→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→

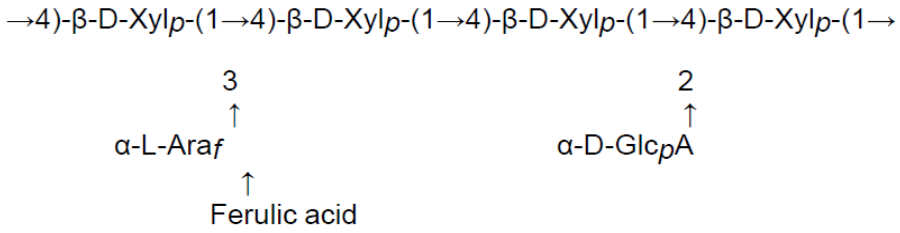
Cellulose

→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→

(1→3), (1→4)-β-D B-glucan



Xyloglucans (Fucogalactoxyloglucan)



Heteroxylans (Glucuronoarabinoxylans)

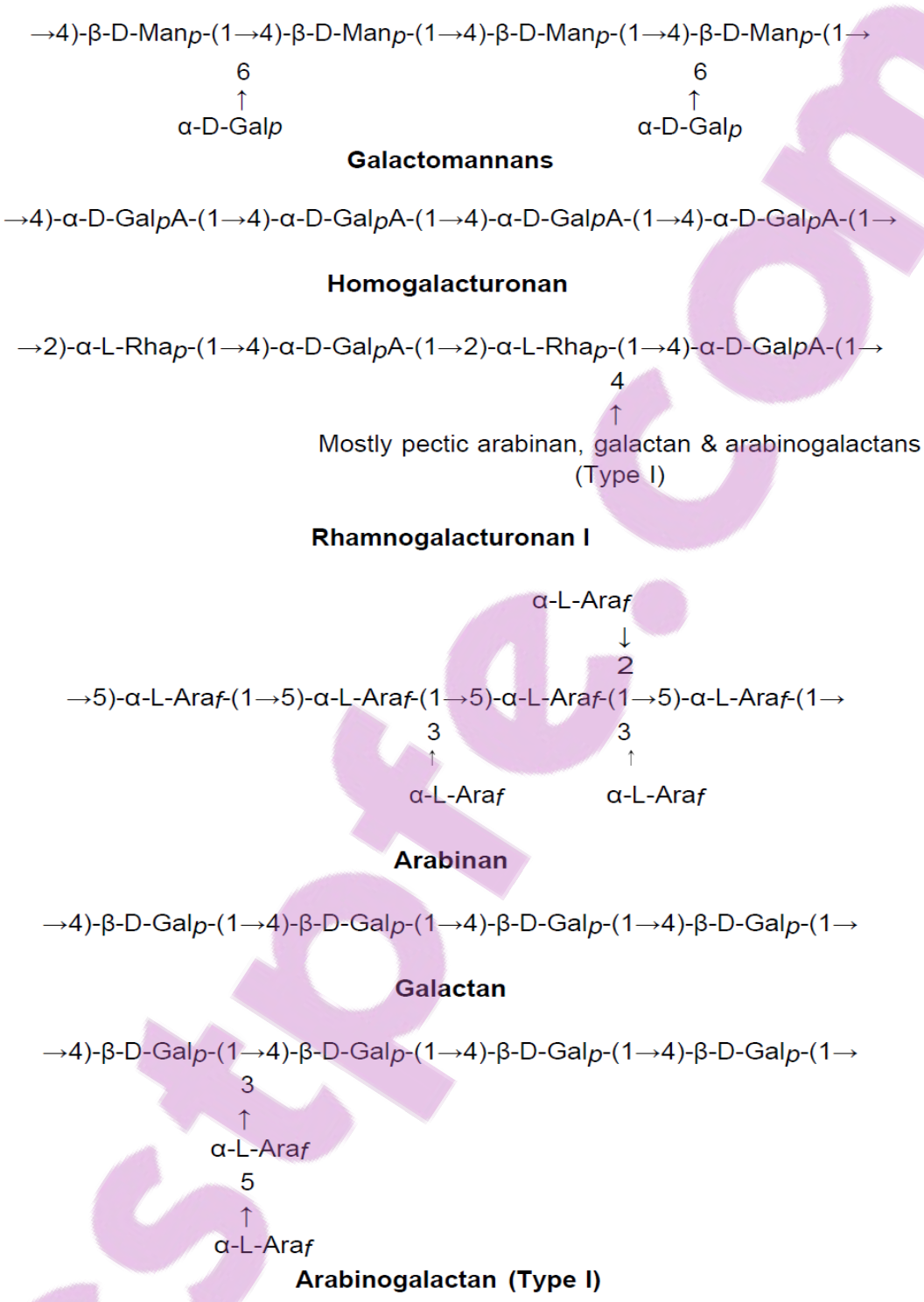


Figure 2.2: Structures of some of the naturally occurring NSP (From Harris and Smith, 2006)

2.3.1.1 Cellulose

Cellulose is a high molecular weight (7,000-10,000 units) linear homopolymer of (1-4) -ß-glucose units (Goring and Timell, 1962, cited by Choct, 1997). The secondary structure consists of parallel chains arranged in bundles that are linked through hydrogen bonds in a rigid helix that is resistant to degradation (Choct, 1997). Cellulose is believed to be identical in chemical composition regardless of the source.

2.3.1.2 Arabinoxylans

The arabinoxylans are the major heteroxylan in plants. Arabinoxylans are concentrated in the cell walls of the endosperm, the aleurone and in the outer layers of the cereal seed and husk (Collins *et al.,* 2010). The polymeric structure of arabinoxylans is a linear (1-4)-ß-xylan backbone to which substituent arabinose sugars are attached through oxygen atoms on positions 2 or 3 of the xylosyl residues (Perlin, 1951). Substituents in the chain may include other hexose sugars or hexuronic acids, phenolic compounds and proteins (Sinha *et al*., 2011). Variable molecular masses (650000-5000000) are reported (Fincher and Stone, 2004).

2.3.1.3 Mixed-linked- ß -Glucans

The β-glucans (βGs) are linear chains containing glucose monomers in ß-(1-3) and ß-(1-4) linkages (Bengtsson *et al.,* 1990). Among the cereal grains, the molecular weights vary widely (48000-300000) (Fincher and Stone, 2004), with lower values reported for wheat, compared to oats and barley (Collins *et al.,* 2010). The βG content of cereals is variable (e.g. 3–7% in oats and 5-11% in barley) (Skendi *et al.,* 2003). The proportions of ß-(1-3) and ß-(1-4) linkages depend on the plant species (Wood and Beer, 1998; Collins *et al.,* 2010). For example, oat βGs are comprised of 30% β-(1-3) linkages and 70% β-(1-4) linkages while barley has a higher (70%) proportion of the β-(1-4) linkages than insoluble β-(1-3) (30%) (Högberg and Lindberg, 2004).

2.3.1.4 Mannans

Mannans contain a mannan backbone in which mannose can be substituted by other sugars (Choct, 1997).

Galactomannans consist of a (1-4)-ß-mannan backbone substituted with single units of (1-6)-β-galactose (Choct, 1997). The mannose-to-galactose ratio varies from one to five (Reid, 1985). Galactomannans are reserve polysaccharides in the seed endosperm of leguminous plants (Sinha *et al.,* 2011). There is no evidence of the occurrence of galactomannans in cereal grains (Choct, 1997).

Glucomannans are similarly composed of a (1-4)-ß-mannan backbone, with interspersed glucose residues in the main chain, and may be acetylated (Sinha *et al.,* 2011). The mannose-to-glucose ratio ranges from 4:1 to less than 1:1 (Meier and Reid, 1982). Glucomannans are present as a minor component in cereal grains (Mares and Stone, 1973; Fincher and Stone, 1986). They are also present in underground storage organs (bulbs, roots and tubers) of some plants (Sinha *et al.,* 2011).

2.3.1.5 Xyloglucans

Xyloglucans have a (1-4)-ß-linked glucan backbone with single units of α-xylose attached to the sixth oxygen of the of the hexose sugar (Choct, 1997). Fuco-galactoxyloglucans are present in the cell walls of dicots (Harris and Smith, 2006). Xyloglucans have also been reported in rice (Shibuya and Misaki, 1978, cited by Choct, 1997).

2.3.1.6 Pectins

Pectins are present in high concentrations in the cell wall of dicotyledonous plants, and, to a lesser extent, in the cell walls of stems and leaves of cereals, and are typically not present in the cereal seed (Choct, 1997).

Two main structural domains are defined. The most common and distinctive domain of pectins is the homo-galacturonan, made up of α-(1-4) linked D-galacturonic acid units

(Ridley *et al.,* 2001). A second domain are the rhamnogalacturonans in which β-(1-4)-α-D-galacturonan chains are interrupted at intervals by insertion of β-(1-2) -β-L-rhamnose residues (Choct, 1997). Rhamnogalacturonan I have galactan, arabinan and type 1 arabinoglycan side chains on the rhamnosyl residues (Collins *et al.,* 2010). In the Type I, arabinogalactans have a ß (1-4) galactan backbone substituted with 5-linked and terminal arabinose residues (Cheetham *et al.,* 1993). Type II arabinogalactans have a β-(1-3,5) linked galactan backbone. Unlike type I arabinogalactans, type II are not a structural component of the cell wall but are thought to be associated with extracellular space and plasmalemma (Sinha *et al.,* 2011). In wheat, a type II, a low molecular weight arabinogalactan associated with a hydroxyproline -rich peptide was reported (Fincher and Stone, 1974). Rhamnogalacturonan II has galacturonan segments with xylosyl side-units (Nakamura *et al.,* 2002; Willats *et al.,* 2004). L-fucose and D-glucuronic acid side chains have also been reported (Choct, 1997). Pure arabinans and galactans are also present in low concentrations in plants (Collins *et al.,* 2010).

2.3.1.7 Oligosaccharides

Oligosaccharides, mostly from soybeans, are present in significant quantities in the swine diet matrix (Smiricky-Tjardes *et al.,* 2003). They include the galacto-oligosaccharides; raffinose [α-Gal (1-6)α –Glc (1-2) β-Fru)], stachyose [$(Gal)_2$ 1:6 Glc 1:2 Fru)] and verbascose [$(Gal)_3$ 1:6 Glc 1:2 Fru)], and the fructans; inulin and fructo-oligosaccharides [(α-Glc (1-2) β-Fru (2-1) β -Fru (N)] or [β -Fru (2-1) β -Fru (N))] (Cummings and Stephen, 2007). The degree of polymerisation of fructo-oligosaccharides can range from two to seventy fructose residues (Gibson *et al.,* 2004).

2.3.1.8 Lignin

Lignin is not a NSP, but a complex polymer of oxygenated phenyl propane units with coniferyl, sinapyl and p-coumaryl alcohols that have undergone a complex dehydrogenative polymerization (Theander and Aman, 1979). Due to strong intramolecular bonding, lignin is extremely resistant to degradation (Dhingra *et al.,* 2012).

2.3.2 Classification of fibre components

Chemically, the carbohydrates can be classified based on the degree of polymerisation into sugars (1-2 units), oligosaccharides (3-9 units) and polysaccharides (≥ 10 units) (Cummings and Stephen, 2007). The challenge in the evolution of our understanding of the concept of fibre lies in how to classify its NSP and other components into nutritionally relevant categories. Early classification methods **(Table 2.1**) were based on extraction in alkali, which divided the NSP into cellulose, non-cellulosic polymers and pectic polysaccharides (Bailey, 1973). An alternative classification separated insoluble cellulose, from the soluble "hemicelluloses" (Neukom, 1976). Neither of these classification systems adequately accounted for the critical nutritional or physiologically active properties of fibre.

Table 2.1: Classification of the main components of fibre

Class	Monomers	Linkage
Cellulose	Glucose	β-(1-4)
Non-cellulosic polysaccharides		
Arabinoxylans	Arabinose and xylose	β-(1→4) xylose units
Mixed-linked β-glucans	Glucose	β-(1→3) and β-(1→4)
Mannans	Mannose	β-(1→4)
Galactomannans	Galactose and mannans	β-(1→4)-linking mannan chains with a- β-(1→6))-linked galactosyl side groups
Glucomannans	Glucose and mannans	β-(1→4) -linked mannan chain with interspersed glucose residues in the main chain
Pectic polysaccharide structural domains		
Homogalacturonan	Galacturonic acid	β-(1→4)
Rhamnogalacturonan I	Galacturonic acid Rhamnose Xylose	β-(1-4)-α-D-galacturonan chains interrupted at intervals by insertion of β-(1-2) -α-L-rhamnose (some of the carboxyl groups are methyl esterified))
Arabinans	Arabinose	α-(1→5)
Galactans	Galactose	β-(1→4)
Arabinogalactans Type I	Arabinose Galactose	β-(1→4) galactan backbone substituted with 5-linked and terminal arabinose residues
Arabinogalactans Type II	Arabinose Galactose	β-(1→3,6) galactan backbone substituted with 5-linked and terminal arabinose residues
Rhamnogalacturonan II	Arabinose Galactose Unusual sugar apiose, aceric acid, fucose	β-(1-3)- and β-(1-6)-D-galactopyranose
Xylogalacturonan	Galacturonic acid Xylose	β-(1-4)-α-D-galacturonan
Mucilage	Galactose-mannose Galactose-mannose arabinose-xylose Galacturonic acid-	Galactose

Class	Monomers	Linkage
	rhamnose	
Gums	Galactose	Xylose
	Glucuronic acid- mannose	Fucose
	Galacturonic acid- rhamnose	Galactose
Algal polysaccharides	Mannose	Galactose
	Xylose glucuronic acid glucose	
Lignin	Sinapyl alcohol	3-D structure
	Coniferyl alcohol	
	p-Coumaryl alcohol	

Data from Caprita *et al.* (2010), Sinha *et al.* (2011) and Elleuch *et al.* (2011)

2.4 Composition of DF in common pig feed ingredients

Cereal grains, soybean and typically low and variable fractions of their processing co-products are the main contributors to the fibre in a standard growing pig diet. Non-starch polysaccharides constitute approximately 14-22% of the standard growing pig diet (Canibe and Bach Knudsen, 2001). The generalised distribution of DF in different pig feedstuffs is summarised in **Table 2.2.** There is wide variation in the composition and concentration of NSP among species, cultivars and within plant tissues, which is also subject to the environment (Collins *et al.,* 2010; de Vries *et al.*, 2012). The growth conditions and the method of grain processing will therefore influence the composition of DF in the whole grain, and in its processed co-products.

Table 2.2: Distribution and composition of DF components in feedstuffs

Feedstuff	Fibrous tissue types	Typical compositions of DF
Fruits and vegetables	Mainly parenchymatous with some lignified and cutinised tissues	Cellulose, hemicelluloses (e.g. xyloglucans), pectic substances, and some glycoproteins; Cellulose, hemicelluloses (e.g. glucuronoxylans), lignin and some glycoproteins Cutin and waxes
Cereals	Parenchymatous and lignified tissues	Hemicelluloses (e.g. arabinoxylans and β-glucans) cellulose, proteins and phenolic esters; Hemicelluloses (e.g. glucuronoarabinoxylans) cellulose, lignin phenolic esters
Legume seeds	Parenchymatous and cells with thickened endosperm walls	Cellulose, hemicelluloses (e.g. xyloglucans), pectic substances, and some glycoproteins Hemicelluloses (mainly galactomannans) some cellulose, pectic substances and glycol Cellulose
Seed husks	Mucilage of epidermal cells	Mainly arabino galacturonosyl-rhamno-xylan

Data from Selvendran *et al.* (1987)

Figure 2.3 shows the morphology of a typical cereal grain, represented by the oat seed. The grains of monocotyledonous plants contain high concentrations of heteroxylans (mostly arabinoxylans), βGs, glucomannans, xyloglucans, pectin and cellulose (Sevendran *et al.,* 1987; McDougall *et al.,* 1996). The dominant polysaccharides are the arabinoxylans and the βGs (Collins *et al.,* 2010). Non-starch polysaccharide concentration is high in hulled oats and barley, and relatively low in rye, wheat and maize (Susenbeth *et al.,* 2011). Barley and oats contain the highest levels of βGs, while wheat and barley have higher content of the arabinoxylans (Collins *et al.,* 2010). The husk and pericarp or testa typically contain the insoluble DF (IDF), mainly cellulose and lignin, while the endosperm contains soluble DF (SDF), mainly the mixed linked βGs and arabinozylans (de Vries *et al*., 2012). The distribution of the different types of NSP among the aleurone, subaleurone, endosperm and the secondary cell walls (**Table 2.3 and Table 2.4**) depends on the species (Collins *et al.,* 2010).

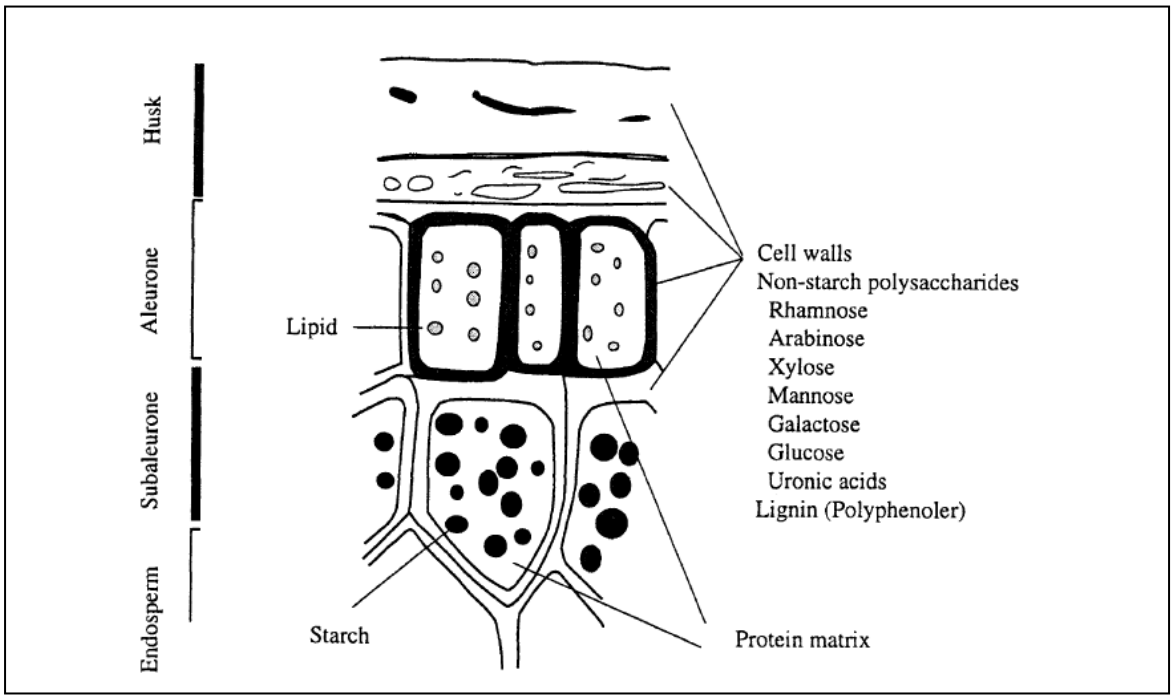


Figure 2.3: The morphology of oat grain (From Bach Knudsen, 2001)

Table 2.3: Carbohydrate and lignin content of whole cereal and soybean grains (g kg-1 DM)

	Maize	Wheat	Rye	Barley	Oats	Soybean
Monosaccharides	4.0	3.0	6.0	4.0	2.0	7.0
Sucrose	13.0	11.0	19.0	12.0	11.0	70.0
Raffinose	2.0	4.0	4.0	5.0	3.0	10.0
Stachyose	1.0	2.0	3.0	1.0	2.0	47.0
Verbascose	-	-	-	-	-	3.0
Total sugars	20.0	19.0	32.0	21.0	17.0	137.0
Starch	690.0	651.0	613.0	587.0	486.0	-
Fructan	6.0	15.0	31.0	4.0	3.0	-
β-glucan	1.0	8.0	16.0	42.0	28.0	-
Soluble non-cellulose polysaccharides	9.0	25.0	42.0	56.0	40.0	63.0
Rhamnose	0.0	0.0	0.0	0.0	0.0	1.0
Arabinose	3.0	7.0	12.0	6.0	3.0	9.0
Xylose	2.0	9.0	20.0	6.0	2.0	2.0
Mannose	2.0	2.0	2.0	2.0	2.0	5.0
Galactose	1.0	2.0	1.0	1.0	2.0	16.0
Glucose	1.0	4.0	6.0	39.0	28.0	6.0
Uronic acids	1.0	1.0	1.0	2.0	3.0	25.0
Insoluble non-cellulose polysaccharides	66.0	74.0	94.0	88.0	110.0	92.0
Rhamnose	0.0	0.0	0.0	0.0	0.0	2.0
Arabinose	19.0	22.0	24.0	22.0	15.0	17.0
Xylose	28.0	38.0	41.0	50.0	78.0	17.0
Mannose	1.0	1.0	3.0	2.0	1.0	8.0
Galactose	4.0	2.0	4.0	2.0	5.0	25.0
Glucose	9.0	7.0	20.0	8.0	5.0	1.0
Uronic acids	6.0	4.0	3.0	4.0	7.0	23.0

	Maize	Wheat	Rye	Barley	Oats	Soybean
Cellulose	22.0	20.0	16.0	43.0	82.0	62.0
Total NSPs	97.0	119.0	152.0	186.0	232.0	217.0
Klason lignin	11.0	19.0	21.0	35.0	66.0	16.0
DF (NSP + Lignin)	108.0	138.0	174.0	221.0	298.0	233.0

Data from Bach Knudsen (1997)

Table 2.4: Major NSP and lignin composition of cell walls of cereal grains (% DM)

Cereal	Cellulose	Glucomannan	B-glucan	Heteroxylan	Pectin	Lignin
Wheat						
Aleurone	2	-	29	65	-	-
Endosperm	2	-	20	70	-	-
Bran	29	-	6	64	-	8.3
Barley						
Aleurone	2	2	26	71	-	-
Endosperm	2	2	75	20	-	-
Rice						
Endosperm	29	2	20	27	3	-
Bran	29	2	6	60	8	12
Maize						
Bran	2	15	-	68	-	-
Oats						
Endosperm	-	3	85	-	-	-
Rye						
Aleurone	8	-	13	68	-	-
Endosperm	6	-	12	65	-	-

Data from Collins *et al.* (2010)

On the other hand, the grains of dicotyledonous plants contain mainly pectins, cellulose and xyloglucan (Sevendran *et al.,* 1987; McDougall *et al.,* 1996). Pectin is typically the major NSP in the parenchymatous tissues (Sevendran *et al.,* 1987; McDougall *et al.,* 1996). Cellulose and the xylans are concentrated in the hulls or husks of most legumes (de Vries *et al.*, 2012). Soybean meal contains approximately 35% carbohydrates, of which 5% are the galacto-oligosaccharides (stachyose, raffinose and verbascose), 20-30% are NSP, consisting of approximately 8% cellulose, and the remainder are largely the pectins (Choct *et al*., 2010).

2.5 Methods of analysis for DF

Consistency in defining DF and the accuracy and precision of its analysis underpin the comparability of the findings on research on fibre nutrition (Mertens, 2003). The latter defined the key terms relevant to describing the validity of methods of fibre analyses, and highlighted the difficulty in meeting these criteria. Relevancy defines the extent to which a method provides a means to evaluate feeds to formulate rations. Reproducibility refers to the accuracy and comparability of results among research, regulatory, and feed-testing laboratories. Accuracy defines the ability to measure the "true" value of a primary standard with known composition or of the consensus concentration of a reference material that is determined by a group of analysts exactly following a defined method. Precision defines the ability to repeat a measurement, measured as the variation among repeated results.

Analytical methods for DF have evolved from the non-enzymatic-gravimetric to the more advanced enzymatic methods, with gravimetric or chemical quantification of the fibre fractions. Non–enzymatic methods are based on pH-dependent solubility of the NSP in buffered solutions. Enzymatic degradation mimics the monogastric gut digestion to remove contaminating non-NSP fractions. Chemical quantification is a direct measurement achieved by either colorimetric and or chromatographic methods.

The choice of a method for the analysis of fibre depends on the objective of an investigation (Noblet and Le Goff, 2001). Mertens (2003) argued that the relevance of a method should be measured against how well the analytically measured fibre matches its nutritional definition, and the extent to which it relates to the metabolic and physiological activities of DF. A general criticism of the current methods is the lack of relevance to the digestive metabolic and physiological activity of the fibre extracts in the GIT (Geor, 2007; Sinha *et al.,* 2011).

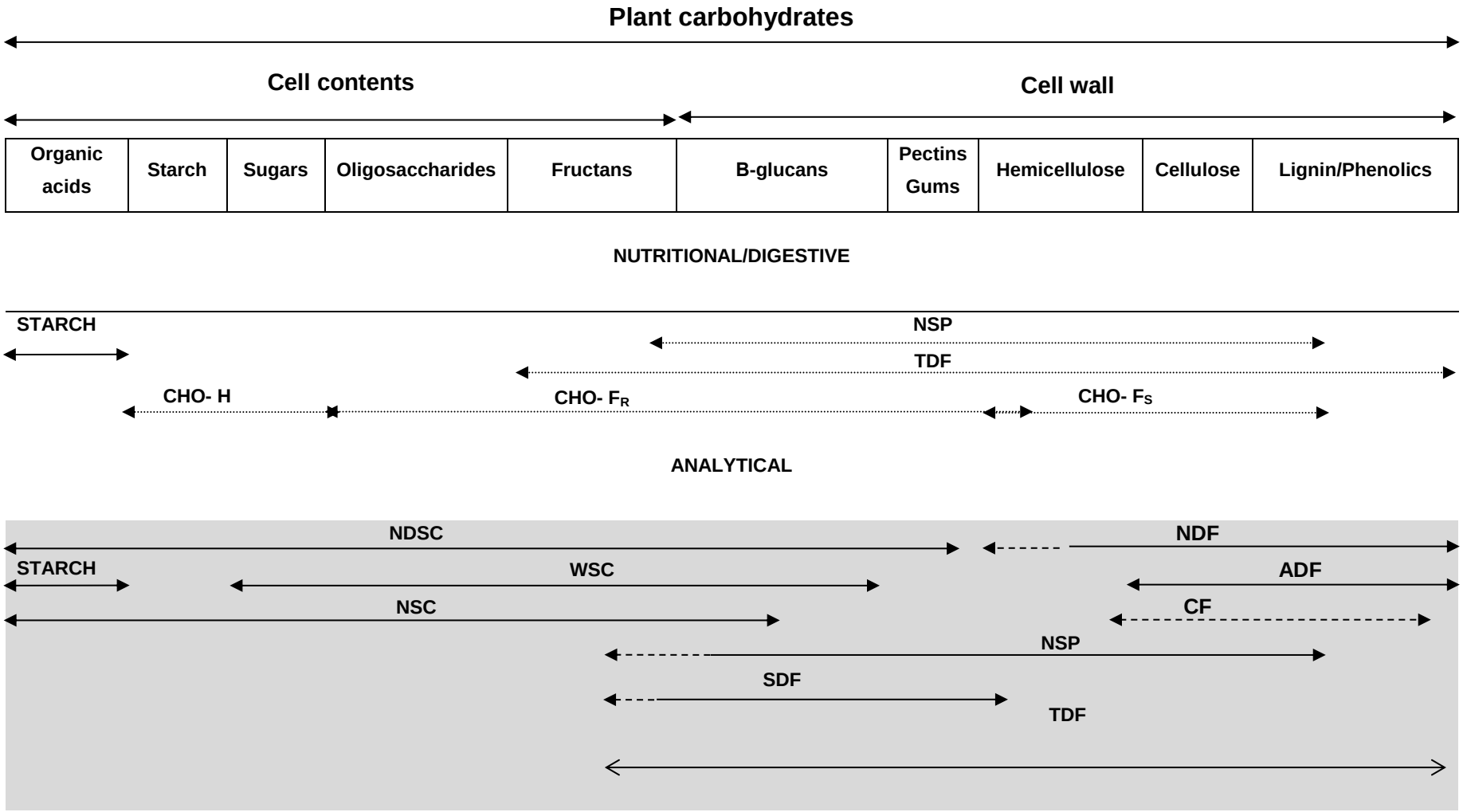


Figure 2.4: Fractionation of carbohydrates in monogastric animals (Adapted from Geor, 2007), where;

- Nutritional or physiological definitions, upper, un-shaded area, with dotted lines
- Analytical methods (grey shaded area) of fibre analyses- dashed lines show incomplete recovery
- CHO-OH – hydrolysable CHO
- CHO-S –slowly fermentable carbohydrate
- CHO-R –rapidly fermentable carbohydrate
- NDSC- neutral detergent soluble fibre
- WSC- water-soluble carbohydrates
- NSC-non-structural carbohydrates
- NDF- neutral detergent fibre
- ADF- acid detergent fibre
- SDF- Soluble dietary fibre

Current classification systems attempt to categorise NSP based on hydrolytic or fermentative degradability, and on solubility. For example, **Figure 2.4** is a generalised scheme showing the fractionation of plant carbohydrates and related compounds. The difficulty in meeting the criteria for validity is apparent in the incomplete recovery of some compounds, overlap of different extractions, and in the lack of overlap between the analytical and the nutritional or digestive categories of DF. Geor (2007) identified the following examples of complications in fibre analyses:

- Some non-carbohydrate components can be extracted in fibre fractions.
- A variable fraction of resistant starch may be included in the fibre fraction
- Some hemicellulose may be soluble in neutral detergent and are not extracted as NDF fraction.
- Solubility of NSP may depend on methodology used.
- The amount of cell wall constituents included in CF analysis varies by feed.
- The analytical method for NSP may recover a variable amount of fructan polysaccharide.
- The analytical method for TDF (and SDF) does not recover oligosaccharides and may recover a variable amount of fructan polysaccharides.

2.5.1 Non-enzymatic gravimetric methods

2.5.1.1 Crude fibre

Two methods are described for crude fibre (CF): AOAC method 962.09 (ceramic fibre filter method) and AOAC method 978.10 (fritted glass crucible method). Crude fibre recovers cellulose, non-cellulosic polysaccharides and lignin. Despite its reproducibility, a major weakness of CF is that, depending on the type of feed, variable quantities (40-50% of the cellulose, 15-20% of the pentosans, and 5-90% of the lignin) of fibre components are recovered (Mertens, 2003). Conceptually therefore, CF is not premised on an acceptable nutritional definition of DF. Consequently, CF has been largely abandoned and is restricted to application in quality control and specification of minimum fibre in feeds by regulatory agencies.

2.5.1.2 The detergent (Van Soest) fibre system

The Van Soest methods (Van Soest and Wine, 1967; Van Soest *et al.,* 1991) use detergents to progressively extract neutral detergent fibre (NDF) (cellulose, hemicelluloses, and lignin), acid detergent fibre (ADF) (hemicelluloses and lignin), and acid detergent lignin (ADL). A main limitation of this approach is that it does not account for soluble dietary fibre (SDF).

Neutral detergent fibre recovers cellulose, hemicellulose and lignin (Van Soest and Wine, 1967). Important nutritional attributes of NDF are that for many feedstuffs, it approximates the insoluble dietary fibre (IDF) and is highly reproducible (Mertens, 2003). The difference between TDF and NDF estimates the soluble NSP.

Acid detergent fibre is determined using AOAC Official Method 973.18 [Fibre (acid detergent) and lignin in animal feed] ADF was originally developed as a preparatory step in the determination of cellulose, lignin, acid detergent insoluble nitrogen (ADIN), acid-insoluble ash (AIA), and silica (Van Soest *et al.,* 1991). Acid detergent fibre recovers mainly (but not exclusively) cellulose and lignin. Therefore, NDF minus ADF estimates the hemicellulose fraction.

An alternative method is when ADF is measured sequentially (sADF) after neutral detergent extraction (Mertens, 2003). Ideally, it is advantageous to determine NDF, ADF and ADL sequentially because the sample required is reduced, and because the estimates of hemicellulose and cellulose by difference are more accurate (Van Soest *et al.,* 1991). However, sADF is usually lower than ADF because neutral detergent removes some components that are not removed by acid detergent, such as pectins and tannin or phenolic acid complexes (Mertens, 2003).

Acid detergent fibre has been modified to include treatment with amylase (Amylase treated neutral detergent fibre (aNDF); AOAC; Official method 2002.02/04) using heat-stable alpha amylases to remove contaminating starch. According to Mertens (2003), a major weakness of ADF is that whereas some acid-soluble hemicelloses are removed, some rapidly fermentable pectin is not. In addition, they also indicated that the precipitation of pectins in strong acid might give ADF results that are higher than NDF in feeds containing high proportions of pectin (e.g., immature lucerne, citrus pulp)

Acid detergent lignin recovers lignin (Van Soest and Wine, 1967). Acid detergent lignin using sulphuric acid (ADSL) is determined using AOAC Official Method 973.18 [Fibre (acid detergent) and lignin in animal feed]. Acid detergent fibre can be the preparatory step for determining lignin using permanganate (ADPL) (Mertens, 2003). In faeces, better recovery of lignin is obtained by Klason lignin, followed by treatment with permanganate (Van Soest and Robertson, 1985, cited by Van Soest *et al.,* 1991). Recovery of lignin allows for the calculation of cellulose from ADF by difference.

2.5.2 Enzymatic methods

Procedures that simulate the monogastric digestive system have become the basis of modern DF assays.

2.5.2.1 Enzymatic-gravimetric methods- Prosky Method

The Prosky method (Prosky *et al*., 1985) is an enzymatic-gravimetric procedure (AOAC Prosky method 985.29), which is based on the work of Asp (1978). In the procedure, the

non-fibre, low molecular weight non-structural sugars and the lipids are chemically extracted, followed by enzymatic degradation of protein and starch, after which the residue is weighed and corrected for ash and protein. The recovered compounds include polysaccharides, lignin, some resistant starch, waxes, phenolic compounds and Maillard reaction products. It excludes oligosaccharide and some types of resistant starch. Conceptually, TDF by method 985.29 is based on the "physiological-chemical" definition of DF (AACC, 2001; Mertens, 2003). In the methods superceding it (AOAC methods 991.41, 993.16, 991.42, 991.43, 992.16, 993.19 and 2001.25), DF is also partitioned into SDF and IDF. The separation is relevant to monogastric animals, in which DF solubility affects nutrient digestion and digesta transit in the GIT, and in satiety signalling.(Mertens, 2003). The Prosky-method based AOAC and equivalent American Association Of Cereal Chemists (AACC official methods of determining TDF are summarised in **Table 2.5**.

2.5.2.2 Enzymatic-chemical methods – Uppsala and Englyst Methods

The Uppsala method (AOAC method 994.13) (Theander *et al.,* 1995) for DF is enzymatic-chromatographic method which is based on the method originally described by Theander and Aman (1979). Starch and protein are hydrolysed enzymatically and the soluble NSP (sNSP) are either precipitated with aqueous ethanol or extracted by dialysis (Englyst *et al.,* 1994; Mañas *et al.,* 1994). Separation by dialysis is preferred to ethanol precipitation, to avoid soluble fibre loss (Mañas *et al.,* 1994). Individual sugars are quantified by gas chromatography (GC) or by high-performance liquid chromatography (HPLC). The neutral sugars are reduced to their alditol forms with alkaline sodium borohydride and acetylated with acetic anhydride, in the presence of methylimidazole as catalyst (Mongeau *et al.,* 2001). The uronic acids are measured separately in a procedure in which uronic acid-containing polysaccharides are treated with concentrated acid at high temperature and measured by colorimetry (Scott, 1979). The chromatographic analyses exclude lignin which is filtered and weighed as Klason lignin, and resistant starch, which is not digested.

The monomeric analyses are also not highly reproducible (Mertens, 2003). The monomeric composition can only be relevant if it can interpreted to reflect the metabolically and

physiologically active functional entities of NSP, and if the interpretation considers the cell wall matrix of the source (Mertens, 2003).

The Englyst method (Englyst and Cummings, 1988) is based on the method of Southgate (1969), and is similar of the Uppsala method. The method isolates lignin as Klason lignin, and removes resistant starch by solubilisation in dimethyl sulfoxide.

Table 2.5: Methods for DF analysis

AOAC		AACCI
Designation	**Description**	**Designation**
	Enzymatic-Gravimetric	
AOAC 985.29	Prosky method for TDF in Foods. First official DF method adopted. Does not allow for separation of SDF and IDF	AACC 32-05.01
AOAC 991.41	Insoluble DF in food and food products (Phosphate buffer). Extension of 985.29, quantitating IDF. SDF can be determined by subtraction.	-
AOAC 993.16	Soluble DF in food and food products, (Phosphate buffer).	
AOAC 991.42	Insoluble DF in foods and food (Phosphate buffer)	AACC 32-20.01
AOAC 991.43	Total, soluble and insoluble DF in foods. (MES-Tris buffer). Super ceded methods 991.41 and 993.16.	AACC 32-07.01
AOAC 992.16	Total DF. IDF and SDF ratios differ substantially from other AOAC methods, therefore this method is only for TDF	AACC 32-06.01
AOAC 993.19	Soluble DF in food and food products. (Phosphate buffer). Measures SDF directly	-
	Non-enzymatic-Gravimetric	
AOAC 993.21	Total DF in foods and food products with <2% Starch method. Applies primarily to fruits and vegetables where the analyst is sure the sample contains no starch	-
	Enzymatic - Chromatographic (Uppsala method)	
AOAC 994.13	Total DF (Determined as neutral sugar residues, uronic acid residues and Klason Lignin)-Gas Chromatographic- breaks DF down to its neutral sugars, uronic acids, and lignin which are summed as TDF.	AACC 32-25.01
AOAC 2009.01	Total dietary fibre, high molar and low molar weight. Modified Prosky method, divided DF into total high molar weight dietary fibre, including the resistant starches and low molar weight dietary fibre content .	AACC 32-45.01
AOAC 2011.25	Total dietary fibre, soluble and insoluble high molar weight and low molar weight. Extends method 2009.01 by sub-dividing the high molar weight dietary fibre into soluble and insoluble fraction, including the resistant starches, and the soluble high molar weight dietary fibre and the low molar weight dietary fibres or prebiotics.	AACC 32-505.01

Equivalent AOAC and AACC methods are listed horizontally.

2.6 Physiologically important physico-chemical properties of DF

Much of the older research on the nutrition of fibre in the growing pig focussed on the dietary level, and to a lesser extent on the type of fibre. However, fibre should be described more specifically by characterising the physico-chemical properties that are now known to influence its metabolic and physiological activity. These include particle size, ion exchange capacity, bulking, hydration and rheological properties, adsorption and fermentability (Guillon and Champ, 2000). These properties underpin the complex three-way interaction of the host animal, the fibre source and the microbial population in the lower gut (Eastwood and Morris, 1992; Zijlstra *et al.,* 2010). A complicating factor is the large variability among fibres (Mertens, 2003). The consequence is that despite the large volume of literature on the nutrition of DF, we still do not yet understand the mechanisms responsible for the digestive metabolic and physiological activities sufficiently to purposefully select the fibrous ingredients, and to match these to appropriate enzyme cocktails, in formulating diets for optimum nutritional, physiological and environmental outcomes.

In the aqueous contents of the gut, differences in secondary and tertiary structure cause the NSP to exist in different physical forms (Eastwood and Morris, 1992). The latter described three physical forms. On one extreme are the soluble NSP that exist as fluctuating, disordered coils, interacting with one another only by entanglement, which is readily accessible to enzymes. On the other extreme are the packed ordered assemblies, such as cellulose fibrils, which are extremely resistant to hydration, swelling and degradation. Between these extremes lie the hydrated, swelling networks.

2.6.1 Hydration properties

The hydration of DF is characterised by its solubilisation, swelling, water absorption, holding, binding or retention capacity where:

- *Swelling* described the volume occupied by a known weight of fibre under specific conditions
- *Water absorption* describes the kinetics of water movement under defined conditions.

- *Water retention capacity* (WRC) describes the amount of water retained without addition of mechanical forces.
- *Water holding or binding capacity* (WHC or WBC) describes the amount of water retained after centrifugation.

Swelling is the first step during solubilisation, whereby NSP are extended and disperse, which causes bulking and favours access by microbial enzymes (Thibault *et al.,* 1992). Rigid structures such as cellulose do not solubilise or swell significantly because the linear structure gives strength to the intermolecular non-covalent bonds (Eastwood and Morris, 1992).

Water binding depends on the structure of the NSP (Guillon and Champ, 2000). For instance, among the arabinoxylans, water-binding capacity is influenced by the degree of arabinose substitutions (Sternemalm *et al.,* 2008). Soluble arabinoxylans can absorb about ten times their weight of water (Choct, 1997). Galactomannans have generally high water-holding capacity (Reid, 1985). Generally, parenchymatous cell wall tissues, especially pectin, show greater hydration properties compared with secondary cell wall tissues, because of the more hydrophilic nature, in the case of pectin, resulting from a high electric charge (Thibault and Ralet, 2003). Environmental conditions that influence hydration include temperature, pH and ionic strength, particularly on fibres containing charged groups such as pectins, with carboxyl groups (Fleury and Lahaye, 1991). The hydration properties therefore change during transit through the different compartments of the GIT (Bach Knudsen, 2001). Examples of the swelling and WBC of different fibres are indicated in **Table 2.6.**

Table 2.6: Hydration capacities of different types of fibres

Source	DF (%)	Mean particle Size(μm)	Swelling (ml g^{-1})	WBC (g/g)
Sunflower heads	19.5	90		4.2
Psyllium seeds	7.9	240		10.1
Linseed hulls	13	190		8.1
Mustard hulls	24.4	340		5.8
Wheat bran	9.8	320		3.2
Sunflower hulls	57.4	302		3.7
Pea hulls	34.6	410		2.6
Cellulose	63.5	<63		2.0
Wheat bran		500-250		6.4
		900	11.9	6.8
		320	5.9	3.0
		1000-500	7.0	7.0
		Coarse	7.4	5.6
		Ground	6.4	6.6
Maize bran			5.7	2.4
Oat bran			5.5	3.5

Data from Guillon and Champ (2000), Kirstensen, and Jensen (2011)

2.6.2 Solubility and viscosity

In the monogastric GIT, solubility and viscosity of fibre have profound effects on nutrient digestion, absorption and digesta transit (Guillon and Champ, 2000). In addition to structure, the solubility of NSP is affected by temperature and ionic strength (Fleury and Lahaye, 1991). The pectins, glucomannans and galactomannans are largely water-soluble (Sinha *et al.,* 2011). Soluble DF also includes gums, mucilage, and variable components of the hemicelluloses, while insoluble DF (IDF) includes cellulose, some hemicelluloses, and lignin (Roehrig, 1988; Davidson and McDonald, 1998). Generally, among the NSP, solubility decreases with the chain length (Guillon and Champ, 2000) and with association with other complex compounds in the cell matrix (Sevendran *et al.,* 1987). In pectins, the presence of charged substitution groups such as the carboxyl group increases solubility (Kristensen and

Jensen, 2011). Differences in galacturonic acid content, neutral sugars, degree of methyl esterification and acetylation, amide content and molecular weight also influence the solubility (Eastwood and Morris, 1992).

The arabinoxylans tend to be largely insoluble in water due to entrapment in cell walls, and due to strong ester-like cross-links, (Mares and Stone, 1973). "Junction zones" formed by intermolecular hydrogen bonding between un-substituted regions of the xylan backbone also reduce solubility (Fincher and Stone, 1986). For example, in wheat, of the 4-9% (DM) content of arabinoxylan content, only 0.3-0.9 % is constituted by the soluble fraction (Stone and Morelle, 2007). Corresponding values for rye and barley are 7-12% and 0.6-3%, respectively (Bengtsson and Aman, 1990).

The βGs have greater conformational flexibility and are therefore more soluble compared to rigid structures such as cellulose and the arabinoxylans (Guillon and Champ, 2000). Oat βGs are less soluble than the glucans in barley, due to the higher proportion of β-(1-4) linkage in the latter (Högberg and Lindberg, 2004). Wheat and rye also contain relatively insoluble βGs (Evers *et al.,* 1999). The solubility of both βGs and arabinoxylans may be reduced by linkage through hydrogen bonds (Izydorczyk and Dexter, 2008).

Soluble NSP increase the viscosity of digesta (Vahjen *et al.,* 2007). Viscosity depends on both intrinsic (structural) and extrinsic factors (concentration of the polysaccharide, solvent properties and temperature) (Guillon and Champ, 2000). In mannans, the mannose-to-galactose ratio influences their solubility and consequently, the viscosity (Reid, 1985). In pig diets, the βGs and the soluble arabinoxylans of grains such as wheat, oat and barley dominate the viscous forming properties of DF (Cummings and Stephen, 2007). Their effects on the viscosities of some feedstuffs in different conditions are apparent in **Table 2.7.**

Doubling of the molecular weight or the concentration of NSP increases the viscosity by a factor of 10 (Eastwood and Morrison, 1992). The viscosity of soluble arabinoxylans is influenced by the degree of arabinose substitutions (Sinha *et al.,* 2011). A high ratio of arabinose to xylose confers a rigid rod-like conformation to the molecule that confers high viscosity (Collins *et al.,* 2010).

The effect of pH on viscosity depends on the ratio of neutral to acidic residues, being sensitive to pH in NSP with acidic residues, and not in those with neutral NSP (Kristensenn and Jensen, 2011). Soluble NSP have higher intrinsic viscosity compared to the insoluble NSP and therefore, in feedstuffs that contain high levels of the viscous NSP, they have the dominant influence on overall viscosity (Guillon and Champ, 2000). However, because solid particles occupy space, they do modify the flow properties of digesta. The physical effect of viscosity on digestion and absorption of nutrients appears to be independent of the sources of NSP (Sinha *et al.,* 2011).

Table 2.7: Viscosity of different feedstuffs.

DF source	Dietary fibre (%)	Viscosity of 2% solution (Pa s x rpm)	Gastric viscosity AUC 4 h (Pa s x rpm)	Intestine viscosity AUC 12 h (Pa s x rpm)
Cellulose	99.1	1112	313	113
Oat bran	19.5	1358	739	693
Wheat bran	49.7	508	642	202
Soy hulls	79.5	979	-	-
Maize bran	73.2	529	-	-

Data from Dikeman *et al.* (2006)

2.6.3 Adsorption

The adsorption of organic compounds or mineral ions is affected by the regiochemistry of the surface layer of fibre particles (Guillon and Chap, 2000). Non-starch polysaccharides with large hydrophobic surfaces bind hydrophobic organic molecules such as the lipids (Guillon and Champ, 2000). Charged polysaccharides, such as pectins through their carboxyl groups have been shown to bind metal ions *in vitro* (Kristensen and Jensen, 2011). **Table 2.8** shows the variation in fat adsorption capacities of different types of fibres.

Table 2.8: Fat adsorption capacities of different types of fibres

Source	DF (%)	Mean particle Size (µm)	Fat adsorption capacity (g/g)
Sunflower heads	19.5	90	4.4
Psyllium seeds	7.9	240	0.8
Linseed hulls	13	190	1.8
Mustard hulls	24.4	340	1.1
Wheat bran	9.8	320	2
Sunflower hulls	57.4	302	3.2
Pea hulls	34.6	410	0.8
Cellulose	63.5	<63	2

Data from Kristensen and Jensen (2011)

2.6.4 Particle size and porosity

The main factors that determine the range of particle sizes in feeds are the type of cell walls in relation to the degree of processing (De Vries *et al.,* 2012). Porosity similarly depends on the cell wall matrix and on processing (Guillon *et al.,* 1998). Pectins are thought to play a dominant role in controlling cell wall porosity (Guillon *et al.,* 1998). Overall, particle size interacts with the surface regiochemistry and porosity to influence the solubility, viscosity, water retention and fermentation properties of the fibre (Guillon and Champ, 2000). Small particle size and porosity increase the surface area available for contact with digestive enzymes, thereby influencing degradation (De Vries *et al.,* 2012).

2.7 Fermentation

Most of the beneficial metabolic and physiological activity of DF derives from its fermentation (Williams *et al.,* 2001). Both the animal's digestive response and plant genomic factors influence fermentation. The factors relevant to the metabolic and physiological impact of fermentation are the site, kinetics of fermentation, metabolites and the gut microbial ecosystem.

2.7.1 Effects of source on the degradability of fibre

In the absence of endogenous enzymes that can hydrolyse the NSPs, their degradability in the GIT of monogastric animals is exclusively due to fermentation by microbes. Currently, there is interest in characterising the kinetics of fermentation of the fibre of pig feeds. The objective is to manipulate the supply of fermentable fibre to the GIT through diet formulation. The fermentation kinetics of fibre are routinely described using an *in vitro* fermentation test (Bindelle *et al.,* 2007a; Bindelle *et al.,* 2007b; Bindelle *et al.,* 2011). Cumulative fermentation gas production is measured over periods that mimic the transit times of the lower gut, typically from 48 to 72 hours. The data is then fitted into mathematical models that generate quantitative kinetic parameters that predict the *in vivo* rate and extent of fermentation. France *et al.* (1993) and Groot *et al.* (1996) described among the most commonly used models in pig fermentation studies, with the latter increasingly preferred for pig studies. The kinetic parameters of biological relevance are quantified by modelling, and include a lag phase, representing the delay in fermentation during colonisation by bacteria, a rate constant, which measures the rate of substrate degradation, which is alternatively indicated by the time to half decay, and the gas produced at infinite time, which measures the extent of fermentation or degradability of the substrate.

A growing database of the fermentation kinetics of fibre in pig feeds reveals high variability among plant species and among their morphological components (Bindelle *et al.,* 2011; Jha *et al.,* 2010; Jha *et al.,* 2011; Jonathan *et al.,* 2012). The differences in fermentation kinetics relate to the chemistry of the NSP and of the cell wall matrix. The soluble NSP are practically totally fermented, whereas insoluble cellulose and xylans are almost unfermented (Noblet and Le Goff, 2001). Differences in chain length, sugar composition, and bond types

influence the fermentation kinetics of fructo-oligosaccharides (Smiricky-Tjardes *et al.,* 2003). Jonathan *et al.* (2012) reported differences in the fermentation kinetics among purified fibres, which were related to the primary structure of the NSP. Glitsø *et al.* (1998) reported differences in the fermentation kinetics of arabinoxylans in cereals, which related to the degree of branching. Fermentation kinetics differed among wheat bran, wood cellulose, peas, pea hulls, pea inner fibre, sugar beet pulp, flax seed meal and maize distillers dried grains with solubles (Jha *et al.,* 2011). Different fermentation kinetics were also reported among barley cultivars, oat and wheat products (Jha *et al.,* 2010) and among barley cultivars and wheat products (Bindelle *et al.,* 2011). Glucans from both parenchymatous as well as secondary cereal cell wall tissue are almost completely degraded (Bach Knudsen and Hansen, 1991; Bach Knudsen *et al.,* 1993a; Bach Knudsen and Canibe, 2000). However, the βG from the endosperm tended to be more degradable than those from the aleurone layer or the pericarp and testa (Bach Knudsen and Hansen, 1991; Bach Knudsen *et al.,* 1993b; Bach Knudsen and Canibe, 2000). Arabinosyl and galacturonic acid residues originating from pea cotyledon are readily digested, whereas xylosyl and glucosyl residues originating from glucuronoxylan and cellulose from the hull are more difficult to digest (Canibe and Bach Knudsen, 2001). *In vitro*, pectic arabinan and arabinogalactan present as side chains were more easily fermented than the (rhamno) galacturonan backbone (Van Laere *et al.,* 2000; Voragen *et al.,* 2009).

The variation in fermentation kinetics among and within feeds is illustrated in **Table 2.9.** However, the quantitative kinetic variables presented in the table are not directly comparable between experiments, due to different set-ups and due to different mathematical models. The parameters are critical in the least cost formulation of diets designed to induce optimal conditions for beneficial fermentation in the lower gut. However, this assumption needs to be tested on a wide range of practical dietary mixes. The high fermentability of the βGs (Jha *et al.,* 2010), the pectins (Jha *et al.,* 2010), natural oligosaccharides (Smiricky-Tjardes *et al.,* 2003) and the soluble arabinoxylans (Glitso *et al.,* 1998) makes those feedstuffs that contain high levels of these NSP the target feed ingredients to enhance fermentation in the GIT. The possibility to similarly control the fermentability of DF by selective inclusion of fermentable-insoluble fibre rich feeds needs to be investigated.

2.7.2 Animal factors affecting the fermentability of fibre

The gut of pigs has higher capacity to ferment NSP compared to chickens (**Table 2.10**). The digestibility of fibre by pigs tends to increase with maturity (**Table 2.11**). Much of the increase in digestibility with pig maturity is linked to increased capacity to degrade cellulose (Noblet and Le Goff, 2001). The latter proposed two hypotheses to explain the effect of maturity or body size on the digestibility of fibre. The size of the hindgut relative to the live weight and to feed intake increases with increasing live weight, while the transit time of digesta increases, both of which favour the fermentation of fibre. At larger weights, the lower feed intake, relative to gut size, may augment the effect. The alternative hypothesis is that as the pig matures the composition of the microbial population of the gut shifts to more active species, which improves digestibility. They suggested that the apparent interaction between source of fibre and pig maturity on the digestibility of fibre could be explained by differences in the properties of fibre in different feedstuffs. For example, in sugar beet, the high level of pectic polysaccharides (with abundant attachment sites for bacteria) and high water holding capacity (with swelling, which increases surface area) facilitate easier colonisation and degradation of fibre, such that limited improvement in digestibility can be expected with maturity. For a feedstuff such as wheat bran, which is characterised by a high level of insoluble lignified fibre that is resistant to degradation, only limited improvement in digestibility with age can also be expected. On the other hand, the degradability of maize fibre, which is less lignified and therefore the fermentability of which depends on the properties of the constituent NSP, and on the retention time in the hindgut, is likely to depend more on the maturity of size of the pig.

2.7.3 Site of fermentation

The soluble NSP (sNSP) are rapidly and totally fermented in the foregut, while the bulk of the insoluble NSFP (iNSP) are fermented in the lower gut (Williams *et al.,* 2001; Houdijk *et al.,* 2002; Konstantinov *et al.,* 2004; Bach Knudsen and Canibe, 2000). Substrates readily fermented in the small intestines include the βGs (Bach Knudsen and Hansen, 1991; Jha *et al.,* 2010), fructo-oligosaccharides (Smiricky-Tjardes *et al.,* 2003), pectic polysaccharides (Longland *et al.*, 1993) and soluble arabinoxylans (Jha *et al.,* 2010). The acidic xylans and

cellulose, which are less readily fermentable, are largely fermented in the distal part of the large intestine (Canibe *et al.,* 1997; Canibe and Bach Knudsen, 2001).

Table 2.9: *In vitro* fermentation kinetics of different types of [a]fibre when fermented by pig faecal bacteria

	Gas model parameters									
	[b]France *et al.* (1993)				[c]Groot *et al.* (1996)					
Substrate	L	μ	G_f	*T/2*	A	B	C	R_{max}	$t_{½}$	Source
72 hour fermentation										
Lupins	7.5	0.09	325.0	14.4	-	-	-	-	-	Bindelle *et al.* (2007a)
Maize	7.1	0.12	279.0	12.1	-	-	-	-	-	
Peas	7.0	0.13	341.0	11.8	-	-	-	-	-	
Sugar beet pulp	7.1	0.20	268.0	10.3	-	-	-	-	-	
Soybean meal	6.9	0.11	303.0	12.7	-	-	-	-	-	
Wheat bran	7.0	0.10	149.0	13.2	-	-	-	-	-	
48 hour fermentation										
Wheat bran	2.83	0.07	124.0	12.4	-	-	-	-	-	Jha *et al.* (2011)
Peas	1.33	0.10	253.0	10.23	-	-	-	-	-	
Pea hulls	3.59	0.10	276.0	12.85	-	-	-	-	-	
Pea inner fibre	1.46	0.11	264.0	8.38	-	-	-	-	-	
Sugar beet pulp	2.91	0.20	237.0	9.39	-	-	-	-	-	
Flax seed meal	1.75	0.10	130.0	10.66	-	-	-	-	-	
Maize DDGS	1.20	0.05	158.0	19.75	-	-	-	-	-	
72 hour fermentation, purified fibres										
Wheat	-	-	-	-	192	15.5	1.76	7.7	-	Bindelle *et al.*(2011)
Wheat bran	-	-	-	-	168	17.1	1.68	6.0	-	
Common barley Mc Leod	-	-	-	-	190	12.5	1.88	9.6	-	
Common barley AC Metcalfe	-	-	-	-	183	12.1	2.01	9.9	-	
Hulless barley CDC Fibar	-	-	-	-	220	9.9	2.26	15.3	-	
Hulless barley SB94893	-	-	-	-	227	10.4	2.34	15.5	-	
48 hours fermentation										
Guar gum	-	-	-	-	318	10.3	-	-	16.6	Jonathan *et al.* (2012)
Konjac glucomannan	-	-	-	-	344	10.6	-	-	18.4	
Retrograded tapioca starch	-	-	-	-	162	6.5	-	-	4.8	
Retrograded maize starch	-	-	-	-	36	8.4	-	-	3.7	

	Gas model parameters									
	[b]France *et al.* (1993)				[c]Groot *et al.* (1996)					
Substrate	L	μ	G_f	*T/2*	A	B	C	R_{max}	$t_{½}$	Source
Cellulose	-	-	-	-	311	2.2	-	-	12.0	
Oat β-glucan	-	-	-	-	421	3.9	-	-	48	
Fructans										
Inulin	-	-	-	-	374	15.5			11.3	
Oligofructose	-	-	-	-	430	7.5	-	-	7.7	
Polyuronides					-	-	-	-	-	
Citrus pectin	-	-	-	-	-	-	-	-	-	
Alginate	-	-	-	-	166	1.2	-	-	3.6	
Heteroglucans										
Soybean pectin	-	-	-	-	23	4.8	-	-	5.9	
Xantham gum	-	-	-	-	339	3.3	-	-	12.3	
72 hours fermentation										
Hulled barleys	-	0.076–0.093	167–197	10.2–10.8	-	-	-	-	-	Bindelle *et al.* (2010)
Hulless barleys	-	0.08–0.011	188–235	8.3–10.5	-	-	-	-	-	
Oats	-	0.035–0.056	66–186	11.6–30.0	-	-	-	-	-	
Oat groats	-	0.091–0.0108	154–199	8.0–9.1	-	-	-	-	-	

[a]Natural fibres extracted as residues after pepsin-pancreatin hydrolysis (Boisen and Fernandez, 1997)

[b]Gas production data modelled according to France *et al.* (1993), where;

L lag time (h).

μ fractional rate of degradation at t = T/2

T/2 half time to asymptote (h).

G_f maximum gas volume (mL g^{-1}DM)

[c]Gas production data modelled according to Groot *et al.* (1996), where;

A the maximum gas volume for $t = \infty$, mL g^{-1} DM

B The time to half asymptote when G = A/2 (h)

C constant determining the slope of the inflexion point of the profile.

R_{max} Calculated maximum rate of gas production volume (mL g^{-1} DM)

t_{max}, the time to R_{max} (h^{-1})

Table 2.10: Digestibility coefficients of NSP of peas by chickens and pigs

Carbohydrate	Chickens[1]	Pigs[2]
NSP	0.12	0.84
Non-cellulosic NSP	-	1.02
Cellulose	-	0.54
Arabinose	0.13	1.04
Xylose	0.14	1.03
Mannose	0	0.72
Galactose	0.15	1.02
Glucose	0.18	0.71
Uronic acids	0.02	0.94

Data from [1]Jørgensen *et al.* (1996). [2]Goodlad and Mathers (1991) – values are digestibility of NSP in mixed pig diets, predicted by multiple linear regressions.

Table 2.11: Effect of level, type of fibre and of pig size on the digestibility of DF

	Wheat bran		Maize fibre		Sugar beet pulp	
	20-110 Kg Pigs	Adult Sows	20-110 Kg Pigs	Adult Sows	20-110 Kg Pigs	Adult Sows
CF	0.35	0.38	0.45	0.79	0.78	0.82
NDF	0.48	0.51	0.39	0.81	0.84	0.89
NDF-ADF	0.58	0.62	0.38	0.82	0.88	0.93
ADF	0.26	0.33	0.38	0.82	0.89	0.92
Non-Cellulosic polysaccharides	0.54	0.61	0.38	0.82	0.89	0.92
Arabinose	0.37	0.45	0.48	0.86	0.89	0.97
Xylose	0.62	0.67	0.31	0.81	0.96	0.55
Uronic acids	0.32	0.35	0.43	0.80	0.48	0.97
Cellulose	0.25	0.32	0.38	0.82	0.95	0.91
Insoluble NSP	0.43	0.50	0.34	0.81	0.87	0.91
NSP	0.46	0.54	0.36	0.82	0.87	0.92
DF	0.38	0.46	0.32	0.74	0.89	0.86
Change in diet digestibility coefficient per 10g NSP	-0.0127	-0.0104	-0.0103	-0.0038	-0.0028	-0.0021

Data from Noblet and Le Goff (2001).

2.7.4 Fermentation metabolites

The pathways and metabolites produced during fermentation in the GIT of pigs are outlined in **Figure 2.5.**

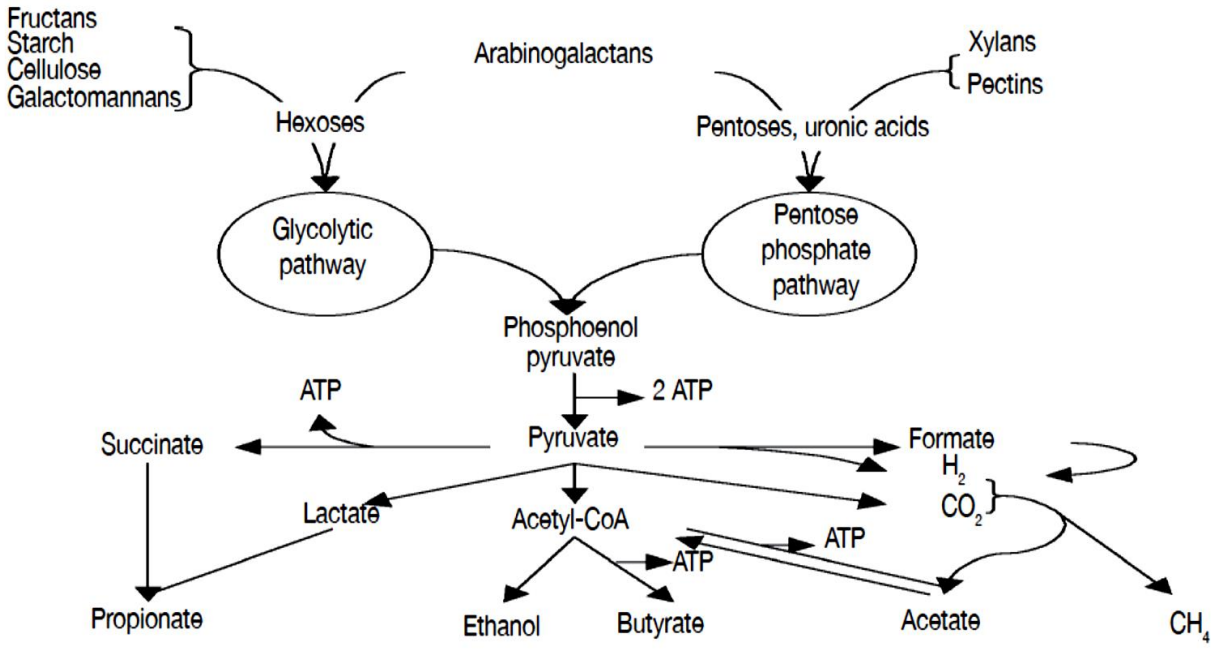


Figure 2.5: Fermentation pathways and metabolites (From Bindelle *et al.,* 2008)

The stoichiometry of the fermentation can be summarised in a general equation (Ewing and Cole, 1994):

$$\mathbf{57.5\ C_6H_{12}O_6 + 45\ H_2O \rightarrow 65\ Ace + 20\ Pro + 15\ n\text{-}But + 140\ H_2 + 95\ CO_2 + 288\ ATP}$$

However, the molar ratios of the SCFA are variable, depending on the substrate NSP (Williams *et al.,* 2001). The composition of SCFA varies by source of fibre (**Table 2.12).** Short chain fatty acid composition differed among barley cultivars and wheat products (Bindelle *et al.,* 2011), among barley cultivars, oat and wheat products (Jha *et al.,* 2010) and among purified fibres (guar gum, alginate, retrograded tapioca and maize starches, glucomannan, cellulose, oat βGs, oligofructose, high methyl esterified citrus pectin, soy pectin and xanthan gum) (Jonathan *et al.,* 2012). Short chain fatty acid composition also

differed in pigs fed complete diets containing wheat bran, DDGS, pea hulls and pea inner fibre to growing pigs (Jha and Leterme, 2012). Barley-based diets produced higher molar proportions of Pro and But in the caecum and colon, and a lower molar proportion of Ace compared to oat-based diets (Reilly *et al.,* 2010). Compared to wheat bran, maize cob produced higher Ace and lower But in the caecum (Carneiro *et al.,* 2008).

2.7.5 Microbial interactions

The distal ileum and the lower gut are host to a large and diverse range of microbes. Over 50 genera and over 500 species of bacteria are described (Jha *et al.,* 2010). Bacterial populations increase from 10^9-10^{10} in the distal small intestine to 10^{10}-10^{11} in the large intestine (Gaskins, 2001). Approximately 90% of the bacteria are Gram-positive, strict anaerobes, consisting mostly of Streptococcus, Lactobacillus, Eubacterium, Clostridium and Peptostreptococcus species. Ten percent are Gram-negative Bacteroides and Prevotella groups (Gaskins, 2001; Leser *et al.,* 2002). Application of metagenomic technology has allowed a more complete characterisation of the diverse bacterial species in the lower gut (Brownley *et al.,* 2011).

While each bacterial species occupies a particular niche, they interact antagonistically or synergistically (Williams *et al.,* 2001). Rapid transit in the stomach and small intestines affect adhesion and proliferation and limits the bacterial population size and diversity (Pluske *et al.,* 2002). By manipulating these interactions, it may be possible to manipulate bacterial population composition and size, and fermentation activity, through diet formulation. The effect of source of DF level on bacterial species composition is illustrated in **Table 2.13.**

Table 2.12: *In vitro* SCFA production by different types of fibre[a] when fermented by pig faecal bacteria

			% of total SCFA					Source
	pH	NH_3	Ace	Pro	But	BCFA	Total SCFA	
Ileal digesta of diets containing different protein and fibre source (mMol/g DM)								
Wheat bran	6.36	6.5	88.3	4.4	7.3	0.03	0.230	
Pea hulls	6.64	8.5	89.7	1.7	8.4	0.03	0.269	
Pea inner fibre	7.10	5.5	92.3	1.9	5.7	-	0.166	
Sugar beet pulp	6.57	6.5	90.1	2.0	7.7	-	0.243	
Maize distillers grains	7.08	5.6	93.9	1.3	4.7	-	0.191	Jha and Leterme (2012)
Colonic digesta of diets containing different protein and fibre source (mMol/g DM)								
Wheat bran	6.65	99.5	63.6	19.6	12.4	2.8	0.941	
Pea hulls	6.13	132.1	57.8	21.0	15.3	2.6	0.122	
Pea inner fibre	6.03	68.1	59.9	24.1	11.9	2.0	0.119	
Sugar beet pulp	5.96	73.3	61.1	21.8	13.5	2.0	0.113	
Maize distillers grains	6.37	106.3	60.5	22.1	11.8	2.8	1.000	
***In vitro* (48 hours) fermentation of pepsin-pancreatin digesta of feedstuffs (mMol/g DM)**								
Wheat bran	-	-	65	20	11	2	2.0	
Peas	-	-	61	22	13	1.7	4.0	
Pea hull fibre	-	-	70	19	8	0.5	4.5	
Pea inner fibre	-	-	60	26	11	1.1	4.4	Jha *et al.* (2011)
Sugar beet pulp	-	-	69	21	7	1	3.8	
Flax seed meal	-	-	62	27	7	1.7	2.4	
Maize DDGS	-	-	66	20	10	2.7	3.3	
***In vitro* (48 hours) fermentation of ileal digesta of diets (mMol/g DM)**								
Low fibre diet	-	-	48.0	29.0	16.0	6.0	4.2	Anguita *et al.* (2006)
Standard diet	-	-	45.0	33.0	13.0	9.0	3.6	

	pH	NH_3	% of total SCFA				Total SCFA	Source
			Ace	Pro	But	BCFA		
High fibre diet	-	-	54.0	31.0	10.0	5.0	3.9	
***In vitro* (48 hours) fermentation of commercial grade fibres (mMol/g OM)**								
Mannans								
Guar gum	-	-	60	31	9	-	7.17	
Konjac glucomannan	-	-	60	35	6	-	7.71	
Homoglucans								
Retrograded tapioca starch	-	-	65	22	14	-	4.47	
Retrograded maize starch	-	-	61	27	12	-	5.08	
Cellulose	-	-	50	44	6	-	8.11	
Oat β-glucan	-	-	52	22	19	-	5.81	
Fructans	-	-				-		Jonathan *et al.* (2012)
Inulin	-	-	53	34	13	-	4.52	
Oligofructose	-	-	53	35	12	-	6.56	
Polyuronides	-	-				-		
Citrus pectin	-	-	78	17	5	-	4.01	
Alginate	-	-	80	16	4	-	6.58	
Heteroglucans						-		
Soybean pectin	-	-	64	32	4	-	9.01	
Xantham gum			67	22	11	-	3.06	
***In vitro* fermentation of pepsin-pancreatin digesta**								
Hulled barleys	-	-	55.9–57.4	20.3–22.1	14.9–16.9	-	-	
Hulless barleys	-	-	54.4–52.5	22.0–24.5	15.1–17.2	-	-	
Oats	-	-	59.7–71.2	19.1–23.3	7.6–12.0	-	-	Bindelle *et al.* (2010)
Oat groats	-	-	53.8–56.7	24.6–26.6	12.3–13.3	-	-	

[a]Fibre extracted as residues after pepsin-pancreatin hydrolysis (Boisen and Fernandez, 1997)

Table 2.13: The effect of source of fibre on the microbial species composition in fermented pepsin pancreatin digesta*

Cereal type	Hulled barleys	Hulless barleys	Oats	Oat groats
CHO composition (minimum-maximum: g kg^{-1} DM)				
Starch	549–598	488–604	322–459	442–600
Amylose/amylopectin	0.08–0.85	0.39–0.47	0.30–0.37	0.27–0.32
β-glucan	41–59	46–127	29–51	48–92
tNSP	118–172	77–153	166–261	123–153
iNSP	71–123	40–89	142–204	44–92
sNSP	14–77	23–101	23–68	45–104
IVDMD (%)	59.2–73.3	49.7–77.5	62.4–71.6	87.5–90.6
Bacterial Terminal restriction fragment (%)				
90	4.30–5.57	2.48–10.37	4.49–6.35	2.32–7.13
144	3.75–17.57	7.67–16.84	0.82–2.90	4.49–7.37
204	0–4.70	0–4.32	1.67–3.89	1.72–2.80
281	5.45–11.02	0–7.42	11.22–23.67	4.09–6.48
496	1.64–2.92	1.63–3.17	1.82–5.95	2.88–4.73

Data from Bindelle *et al.* (2010)

**In vitro pepsin-pancreatin hydrolysis and gas test fermentation of residues for 72 hours according to Bindelle et al. (2007).*

***Terminal restriction fragment, most likely species identity: TRF 90 — Bacteriodes-like; TRF 144 — Anaerotruncus colihominis; TRF 204 — Roseburia faecalis, Clostridium sulfatireducens; TRF 281 — Ruminococcus flavofaciens, C. xylanolyticum; TRF 496 — C. subterminale, C. butyricum.*

The influence of fibre on microbial population composition is widely reported in literature. Oligofructose, galacto-oligosaccharides and lactulose increased *Bifidobacteria* and *Lactobacilli* in the large intestine of humans (Macfarlane and Macfarlane, 2003). Estrada *et al.* (2001) observed increased numbers of *Bifidobacteria and* decreased numbers of total anaerobes and *Clostridia* in faeces of pigs fed diets enriched with fructo-oligosaccharides. Pigs offered oat-based diets had higher populations of *Bifidobacteria* in the ileum and colon, and *Lactobacilli* in the colon compared with pigs fed barley-based diets (Smith *et al.,* 2010). Maize, wheat and barley based diets also differed in the bacterial populations (Drew *et al.,* 2002). In experiments by Pieper *et al.* (2008), hulless barley varieties with high sNSP content favoured xylan- and βG-degrading bacteria whereas βG-supplemented hulled

barleys favoured *Lactobacilli*. Sugar beet pulp, a highly fermentable fibre, reduced clostridia species which, however, tended to increase with dietary protein level (Jeaurond *et al.,* 2008). Owusu-Asiedu *et al.,* (2006) reported increased *Bifidobacteria and Enterobacteria* populations in the ileal digesta of growing pigs fed diets supplemented with guar gum or cellulose compared to a standard diet.

The variation in microbial activity in response to different sources of fibre was apparent in **Table 2.12 and Table 2.13**. Microbial activity can further be controlled by the quantities of fermentable NSP. *In vitro,* SCFA production increased with the level of fermentable fibre in the diet (Anguita *et al.,* 2006). In diets containing lupin hulls, maize cob, wheat bran and alfalfa stems, Stanogias and Pearce (1985a) reported a linear relationship between NDF intake and the concentration of SCFA in the proximal colon of pigs. Added fibre increased SCFA concentration both *in vitro* (Awati *et al.,* 2005) and *in vivo* (Awati *et al.,* 2006). *In vivo*, Jorgensen and Just (1988) reported a five-fold increase in microbial activity in the GIT of pigs fed diets containing high levels of wheat bran (102 g NSP/kg feed) or oat bran (93 g NSP/kg feed). Increased microbial activity was also observed in the intestines of pigs fed diets enriched with pea fibres and pectin (Jensen and Jorgensen, 1994).

2.8 Metabolic, physiological and environmental impacts of fibre

Fibre has profound influences on the metabolic and physiological functions of the GIT of pigs (**Table 2.14 and Table 2.15**) which depend on its dietary concentration, composition and physico-chemical properties as described in section 2.6 above.

2.8.1 Transit time of digesta in the GIT

The flow of digesta in the GIT is important given the influence on nutrient digestion and absorption (Wilfart *et al.,* 2007; Solà-Oriol *et al.,* 2010), and on satiety signalling (Kristensen and Jensen, 2011).

The impact of fibre on transit of digesta in the GIT was demonstrated in experiments by Wilfart *et al.* (2007) and Solà-Oriol *et al.* (2010). Solid and liquid markers were used to study digesta flow kinetics as affected by type and level of fibre, and level of feed intake. The data is presented in **Table 2.16** and **Table 2.17**. Overall, the findings indicate that insoluble fibre increases intestinal flow rate of the fibrous fraction, while soluble fibre tends to delay gastric emptying of fluid digesta. In addition, the data indicates that the cereal source influences the impact of fibre on the transit of digesta in the GIT.

The acceleration of gastric, intestinal and total transit times by IDF support earlier findings. Jorgensen *et al.* (1996) reported a 5- to 6-fold increase in the flow of digesta through the terminal ileum of pigs fed a high fibre diet. Le Goff *et al.* (2002) and Van Leeuwen *et al.* (2006) observed a positive association between the level of IDF and total tract transit time. However, Potkins *et al.* (1991) and Van Leeuwen *et al.* (2006) did not observe an effect of fibre on transit time in the small intestine. Dietary fibre actually increased mean retention time in the terminal ileum (Van Leeuwen *et al.,* 1997). The inconsistent findings may be related to dietary factors such as particle size (Potkins *et al.,* 1991), water holding capacity (Stanogias and Pearce, 1985b) and viscous gel forming properties of some fibres (Solà-Oriol *et. al.,* 2010).

Table 2.14: Main metabolic and physiological activities of NSP in the gut of growing pigs

Factor	Metabolic and physiologic effects
Changes in digesta viscosity	Reduced mixing of digestive enzymes and substrates
	Hindered effective interaction of digestive enzyme at the intestinal mucosal surface
	Increased residence time of the digesta
	Increased intestinal SCFA production
	Reduced absorption of minerals especially sodium ion
	Impaired nutrient digestion and absorption
	Reduced animal performance
Alteration in the gastric emptying and rate of passage	Reduced rate of gastric emptying
	Increased rate of passage of stomach content
	Delayed intestinal absorption of glucose
	Reduced plasma cholesterol and glucose levels
Alteration of gut physiology	Hinder endogenous secretion of water, proteins, electrolytes and lipids
	Enhanced bile acid secretion, and significant loss of these acids in the faeces
	Hampered absorption of lipids and cholesterol in intestine
	Limited intestinal enzyme activity
Alteration in the gut morphology	Increased size and length of digestive organs
	Changes in cell turnover in the intestinal mucosa
	Changes in *villi* height, crypt depth and *villi* height: crypt depth ratio
	Impaired water absorption, which can lead to dehydration
	Increased rate of turnover of intestinal mucosal cells
Alteration in the native gut microflora	Stimulated microbial fermentation in intestine.
	Enhanced SCFA production
	Lower pH of intestinal tract; in long term may disturb the normal microbiota prevailing in gut
	Influenced bioavailability of dietary minerals
	Decreased oxygen tension, favouring development of anaerobic microbiota
Alteration in gut mucus layer	Increased concentrations of luminal mucin in stomach and small intestine
Ammonia and odour compounds	Reduced NH_3 emission
	Sparing of protein fermentation in the lower gut, which reduces the production of odour causing and enterotoxigenic metabolites

Adapted from Sinha *et al.* (2011)

Table 2.15: Relationships between solubility of fibre and its physiological activities[a]

Effects in GIT	Soluble dietary fibre	Insoluble dietary fibre	Total dietary fibre
Stomach			
Viscosity	***	*	**
WBC	**	***	**
Emptying	*(*)	*	*
Small intestines			
Viscosity	**	-	*
WBC	**	***	***
Glucose absorption	(*)	-	-
Large intestine			
Fermentation	****	***	***
Bulking	*	****	****
Transit time	-	***	***
Energy supply			
Ileum	***	***	***
Faeces	-	****	****

[a] - No relation; * relative strengths of relation. Data from Bach Knudsen (2001).

Table 2.16: Mean retention time of solid and liquid phase markers

	DF (g/kg)		
DF level			
Total DF	143·8	182·1	234·8
Insoluble fibre	115·1	161·7	199·4
Soluble fibre‡	28·7	20·5	35·4
Solid phase marker (YbO_2)	**Transit times (hours)**		
Stomach	1·0	1·1	1·3
Small intestine	4·3	3·9	3·7
Large intestine	44·4	39·4	35·6
Total tract	49·7	44·3	40·5
Liquid phase marker (Cr-EDTA)			
Stomach	0·8	0·8	0·9
Small intestine	4·4	4·0	3·9
Large intestine	41·3	36·1	24·9
Total tract	46·6	41·0	29·7

Data from Wilfart *et al.* (2007)

Table 2.17: Effects of cereal source and feeding level on transit of digesta

	Cereal source		Feeding level (g feed/kg $W^{0.75}$/day)	
	Rice	Oats	Low	Normal
Fibre				
ADF	10.3	26.6		
NDF	3.3	11.4		
Solid phase Cr-mordanted fibre				
Digesta flow (%/h)	13.3	16.7	14.8	15.2
T_{lag} (h)	2.82	3.71	3.05	3.48
Mean retention time (h)	10.6	10.2	10.11	10.68
Liquid Titanium dioxide (TiO2)				
Digesta flow (%/h)	30.5	29.1	31.9	27.6
T_{lag} (h)	2.66	3.89	2.95	3.61
Mean retention time (h)	6.2	7.9	6.6	7.7

Data from Solà-Oriol *et al.* (2010)

The mechanism by which fibre influences transit times in the GIT are not clear. By increasing the viscosity of the GIT contents, the soluble DF fraction may reduce stomach emptying and increase transit time (Johansen *et al.,* 1996). Wenk (2001) hypothesised that fibre stimulates peristaltic action. Wilfart *et al.* (2007) argued that by increasing DF, DM digestibility is reduced (Le Goff *et al.,* 2002) thereby increasing the quantity of indigestible DM present in the digestive tract (Owusu-Asiedu *et al.,* 2006). The increase in bulk increases peristaltic action in the intestines, which stimulates propulsive colonic motility (Le Goff *et al.,* 2002). A second hypothesis is that SCFA are involved in regulating colonic motility and therefore, the transit of digesta. *In vivo*, infusion of SCFA into the colon reduced its motility and resulted in an increased transit rate in rats Cherbut *et al.* (1998).

2.8.2 Control of satiety

When fed low energy, high fibre diets, pigs respond to the low dietary energy density by increasing intake, until physical factors become limiting (Noblet and Le Goff, 2001). In the gut, satiety is induced via both gastric and intestinal signals. Gastric induced satiety is largely of mechanical origin, while intestinal satiety is also nutrient dependent (Kristensen and Jensen, 2011). The gastric satiating properties of DF are attributed to increased water binding by the SDF, which delays the gastric emptying rate of the liquid phase (Kristensen

and Jensen, 2011). By absorbing water, soluble, viscous fibre swells and causes the distension of the stomach, which activates mechanoreceptors, triggering vagal inhibition of feed (Bach Knudsen, 2001).

Viscous fibres are also indirectly involved in intestinal appetite regulation through their effects on nutrient diffusion and absorption. Nutrients flowing into the small intestine stimulate cells in the intestinal mucosa to release regulating peptides that regulate intake (Kristensen and Jensen, 2011). The main hormones that influence intake include cholecystokinin, glucose-dependent insulinotropic polypeptides, glucagon-like peptides, pancreatic polypeptide, oxyntomodulin, secretin, and ghrelin (Kristensen and Jensen, 2011). An increase in the viscosity of digesta prolongs transit time of the liquid phase of digesta and slows diffusion, thereby inhibiting nutrient absorption in the small intestines; the accumulation of nutrients causes the release of appetite suppressing hormones (Kristensen and Jensen, 2011).

Short chain fatty acids are also implicated in the control of satiety (Sleeth *et al.,* 2010)

2.8.3 Nutrient digestion and absorption

Poor digestion of nutrients will reduce animal performance and increase the C, N and mineral footprint (Aarnink and Verstegen, 2007). Reviews by Noblet and Le Goff (2001), Wenk (2001), Aarnink and Verstegen (2007) and Bindelle *et al.* (2008) indicate variable effects of fibre on nutrient digestion. The evidence shows that where there is any effect, it is generally negative, particularly with respect to precaecal digestibility. The effects relate to the type and level of DF. Different mechanisms are proposed to explain the variable effects of DF on nutrient digestion. High viscosity of digesta characteristic of oat, wheat, rye and barley based diets slows diffusion of both substrates and enzymes, which slows nutrient digestion and absorption (Bach Knudsen, 2001; Kristensen and Jensen, 2011). Binding of NSPs to the intestinal brush border increases the thickness of the unstirred water layer adjacent to the mucosa, leading to impaired nutrient digestion and absorption (de Lange *et al*., 2000). Plant cell walls may hinder the access of hydrolytic enzymes to nutrients inside the cells (Bach Knudsen *et al.,* 1993b).

No evidence has been presented on the direct inhibition of intestinal enzyme synthesis by NSPs (Sinha *et al.,* 2011). The activities of most enzymes may be reduced through coupling to NSPs or physical restriction of enzyme access to substrates (Schneeman, 1978; Pettersson and Åman, 1989). Increased endogenous intestinal secretions may reduce nutrient digestibility (Choct, 1997). Faster throughput in the small intestines limits the time feed particles are exposed to digestive processes (Wilfart *et al.,* 2007; Serena *et al.,* 2008). This shifts the digestion of some of the nutrients to the colon (Anguita *et al.,* 2006), where there is no absorption of nutrients other than the short chain fatty acid products of fermentation.

The effects of the cereal type on nutrient digestibility are apparent in **Table 2.18**. The data reflects the negative impact of the high concentration of the viscous forming NSP in wheat barley and rye on precaecal nutrient digestibility. The variability in the partial ileal and caecal digestibility of NSP is apparent.

Table 2.18: Effect of basal cereal on the ileal and caecal digestibility of nutrients

	Ileal digestibility (%)		Caecal digestibility (%)	
Nutrient	Maize	Wheat-barley-rye	Maize	Wheat-barley-rye
DM	60.1	46.8	70.2	61.4
Starch	90.1	89.9	93.4	94.8
Energy	60.4	46.9	68.9	64.0
CP	67.5	63.2	-	-
NSP	38.1	13.5	21.8	22.7
Arabinose	23.2	−9.4	22.6	11.7
Xylose	12.8	−7.5	29.9	43.0
Mannose	7.3	−9.7	39.5	61.6
Glucose	77.5	56.9	61.6	47.4
Galactose	15.3	−20.9	2.0	1.4

Data from Willamil *et al.* (2012)

2.8.4 Energy metabolism

Energy systems are based on the concept of a gross energy (GE) value of a feed ingredient, which is measured as the heat generated from complete oxidation of a unit of the feed under standard conditions (NRC, 1998). The GE value depends on the degree of oxidation of the organic components of feed, which is defined by the ratio of C and H to O in the ingredient (McDonald *et al*., 2002). Thus, the GE largely depends on the composition of the major dietary energy substrates such as glucose (15.5 kj g^{-1}), starch (17.6 kj g^{-1}) protein (23.4 kj g^{-1}), and lipids (39.3 kj g^{-1}), and when fermented, on the composition of the major fermentation products that include Ace (14.6 kj g^{-1}), Pro (20.9 kj g^{-1}), But (24.7 kj g^{-1}) and methane (54 kj g^{-1}) (McDonald *et al*., 2002).

Digestible energy (DE), metabolisable (ME) and net energy (NE) systems have been described for pigs (NRC, 1998). Respectively, these energy systems progressively partition the GE in the feed ingested by an animal into fractions that are either lost to the environment without benefit to the animal during digestion and metabolism or are retained by the animal for its maintenance and productive requirements. The apparently DE fraction of the GE is the energy absorbed in the GIT, measured without consideration for endogenous substances. In standard growing pig diets, the apparent DE varies between 0.70 and 0.90 (Sauvant *et al*., 2004), with an average of 0.82 for typical maize-soybean diets (Kil *et al*., 2013). Most of the variation in the apparent DE content of standard growing pig diets is likely due to DF (Noblet and van Milgen, 2004). The increase in the capacity to ferment fibre with pig maturity also means that feeds have higher DE values for mature compared to growing pigs (Noblet and van Milgen, 2004).

Digestible energy is further partitioned into metabolisable energy (ME), which is calculated as the DE less the energy lost in urine and in fermentation gases. Standard growing pig diets have an average DE:ME ratio of 0.96 (Noblet *et al*., 1994; NRC, 1998). Approximately 1-3% of DE is lost in fermentation gases, and depends on the composition, and dietary level of DF (Shi and Noblet, 1993). The use of high fibre diets will therefore increase the gaseous energy loss through increased hindgut fermentation (Kil *et al*., 2013).

The energy lost in urine is equivalent to the GE of the N containing urinary metabolites and depends on the dietary amino acid balance and the energy:protein ratio (Kil *et al*., 2013). The excretion of urea in urine can cost the animal up to 7% of total energy expenditure (Eisemann and Nienaber, 1990, cited by Williams *et al.,* 2001). In the ME system, the energy that is consumed during the synthesis of urea is not accounted for, However, a correction is made to account for the eventual energy lost in urine as N containing metabolites by using an ME value relevant to N equilibrium (ME_n), which is calculated by subtracting 38.4 kj ME g^{-1} N retained (Morgan *et al*., 1975). Recently,(Cozannet *et al*., 2010), it has been proposed that ME_n values be standardized to 50% N retention, based on the assumption that on average, 50% of the absorbed N is used for protein synthesis(Kil *et al*., 2013).

The fraction of ME that is used for maintenance plus body tissue synthesis is defined as net energy (NE), calculated as the difference between the ME and the energy lost as heat increment from nutrient metabolism. The efficiency with which ME is converted to NE is defined by the ratio NE/ME. The average NE:ME ratio of feeds is 0.74 (Noblet *et al*., 1994).

When fibrous ingredients are included at high levels in growing pig diets, there can be economic benefit in that, highly degradable, cheap fibrous substrates substitute for expensive energy sources. The actual energy supply from the dietary NSP depends on the level and composition of the NSP in relation to its degradability, the cost of its propulsion through the digestive system, and the impact on the digestibility of other nutrients (Noblet and Le Goff, 2001).

In pigs, the contribution of DF to the energy requirement is low. Average degradability of DF is low (0.40-0.50) and highly variable (from zero in high lignin and iNSP rich feedstuffs to 0.80-0.90 in high pectin, sNSP rich feedstuffs) (Noblet and Le Goff, 2001). For instance, in cereal grains, highly soluble arabinoxylan from parenchymatous tissues has high and variable coefficients of apparent total tract digestibility ranging from 0.82 for oats (Bach Knudsen *et al.,* 1993), 0.73–0.83 for rye (Glitsø *et al.,* 1998) and 0.68–0.94 for wheat (Bach Knudsen and Hansen, 1991). On the other hand, insoluble, branched chain arabinoxylan from lignified, secondary cell wall tissues in wheat is practically un-degradable (Bach

Knudsen and Hansen, 1991; Glitsø *et al.,* 1998; Bach Knudsen and Canibe, 2000). The effects of source of fibre on the degradation of its chemical fractions in the GIT of growing pigs are indicated in **Table 2.19.**

Table 2.19: Effect of source of fibre on the digestibility of fibre fractions in growing pigs

	Sugar beet pulp	Soybean hulls	Wheat bran	Wheat straw
NDF-ADF	0.601	0.679	0.404	0.150
ADF	0.540	0.622	0.190	0.112
NSP	0.695	0.791	0.458	0.163
Change in diet digestibility coefficient per 10g increase in NSP	-0.0080	-0.0083	-0.0125	-0.0177

Data from Noblet and Le Goff (2001)

The fermentation of fibre produces SCFA, gases ($C0_2$, H_2 and CH_4), urea, heat and bacterial mass (Noblet and le Goff, 2001). Energy is supplied to the host in the form of the SCFA that are absorbed into the bloodstream (representing energy intake), some of which are assimilated into bacterial mass (Bach Knudsen, 2001). The bacterial mass is excreted, which represents a loss to the energy value of the fibre (Noblet and Le Goff, 2001). The bulk of the SCFA are absorbed in the large intestine (Macfarlane and Macfarlane, 1993; Jorgensen *et al.,* 1997). The mechanisms of absorption depend on the pH and on the fluxes of water, protons and inorganic ions (Bugaut, 1987). Short chain fatty acids, particularly But are metabolised by ceco-colonic epithelial cells, with the remainder available as a source of energy by other tissues (Wong *et al.,* 2006). Liver cells use the residual But and Pro for gluconeogenesis. They also use 50 to 70% of the Ace, while skeletal and cardiac muscles use the rest (Roberfroid, 2007).

The composition of gas produced during fermentation varied with the pig live weight and the type of diet (Noblet and Le Goff, 2001). Dietary fibre increased methane and carbon dioxide emission in pigs by 5-9% (Varel and Yen, 1997). From an energy supply point of view, only the combustible gases, methane and hydrogen are important. Carbon dioxide is important from an environmental point of view. The production of hydrogen by pigs is low (Jensen and

Jorgensen, 1994), less than that of methane (Christensen and Thorbek, 1984; Zhu *et al.,* 1993). The energy lost in gases represents a tenth of the energy of fermented DF (Noblet and Le Goff, 2001). This estimate is lower (Zhu *et al.,* 1993), but higher (Noblet and Shi, 1993; Jorgensen *et al.,* 1996) compared to values estimated by stoichiometric calculation. Noblet and Le Goff (2001) suggested that the differences could be attributed to energy lost as hydrogen, which is usually not measured.

The efficiency of utilisation of fermented DF energy is low, equivalent to about 0.70 of the energy from hydrolysable carbohydrates (Noblet and Le Goff (2001). In ruminants, Hungate (1966) suggested the heat of fermentation represents about 0.06 of fermented energy. The higher energy cost of ingestion and propulsion, increased fermentation heat production and the relatively inefficient intermediary metabolism of short chain fatty acids increase the thermic effect of feeding fibrous diets (Noblet and van Milgen, 2004). Where fibre induces amino acid imbalance, leading to inefficient intermediary N metabolism, there is an added cost of energy used to excrete urea (Williams *et al.,* 2001). The net energy contribution of fibre is further reduced if discounted for the depression on digestion and absorption of other energy substrates (Noblet and Le Goff, 2001).

An analysis of findings on the energy value of fibre in growing pigs (Noblet and Le Goff, 2001) suggested that in general, although DF may seem to contribute significantly to apparent DE, due to its negative effect on other nutrient digestion, the energy cost of physical processing and the inefficient utilisation of metabolites, fibre may contribute negligible quantities of net energy in growing pig diets. However, it appears highly fermentable fibre may contribute significant amounts of energy to the animal requirement. SCFA provided up to 30% of the maintenance energy requirement (Varel and Yen, 1997), in excess of 10% of the total DE (Anguita *et al.,* 2006), and contributed as much as 17.6% of total available energy of growing pigs (Anguita *et al.,* 2006).

2.8.5 Nitrogen and amino acid metabolism

A clear understanding of the effects of DF on the digestive metabolism of dietary N and amino acids is complicated by the endogenous secretion of protein, and by the microbial modification of amino acids in the GIT (Sauer *et al.,* 1991). Depending on the diet, up to

30% of N in distal ileal digesta is of microbial origin (Dierick *et al.,* 1983, cited by Bindelle *et al.,* 2008). Depending on the availability of fermentable energy, the bulk (up to 90%) of total N in faeces can be of bacterial origin (Sauer *et al.,* 1991; Rubio, 2003). The greater microbial activity in the colon, relative to the ileum, makes the ileal, as opposed to the faecal digestibility of amino acids, the method of choice in estimating the availability of dietary amino acids in the gut.

2.8.5.1 Endogenous protein

The endogenous loss of amino acids may affect the animal requirement (Libao-Mercado *et al.,* 2006). The mucins secreted in the GIT are particularly rich in threonine and serine (Faure *et al.,* 2002). Endogenous loss may also account for the fibre induced increased requirement for threonine (Zhu *et al.,* 2005) and for methionine + cysteine (Zhu *et al.,* 2007). Blank (2009), cited by Susenbeth *et al.* (2011), reported increased requirement of pigs for ileal digestible threonine with increase in ingested NDF from wheat bran. Purified pectin, a soluble, highly fermentable non-starch polysaccharide (NSP), and wheat shorts, rich in insoluble, poorly fermentable fibre, both increased specific endogenous protein losses (Libao-Mercado *et al.,* 2006). Apparently, the SDF increased the endogenous secretion through the increased production of mucin proteins in the colon, and not in the ileum (Libao-Mercado *et al.,* 2007). Intestinal mucins were secreted in proportion to the bulk forming properties of both SDF and IDF, and was linked to the proliferation of goblet cells (Lerteme *et al.,* 1996; Leterme *et al.,* 1998). Libao-Mercado *et al.* (2007) suggested that mucosal protein synthesis could be mediated via hormones, controlled by the viscosity of digesta, or indirectly through the release of components such as galacturonic acids and or other products of bacterial fermentation, such as short chain fatty acids or ammonia.

2.8.5.2 Microbial metabolism of amino acids in the small intestines

The fermentative catabolism of dietary and endogenous amino acids by bacteria in the upper gut of pigs can reduce both the availability of dietary amino acids and the recovery of endogenous amino acids, and therefore increase their requirement (Sauer *et al.,* 1991; Colombus *et al.,* 2010). On the other hand, N of both exogenous and endogenous protein

can also be used for *de novo* synthesis of amino acids in the small intestines, which may subsequently be absorbed by the host (Libao-Mercado *et al.,* 2009). Holmes *et al.* (1974) reported concentration of threonine, proline and glycine in the distal ileal digesta of pigs fed high fibre, protein free diets. Torrallardona *et al.* (2003) suggested that such microbial amino acids may contribute to the amino acid requirement, which might account for apparent reduced tryptophan requirement (Zhu *et al.,* 2007) on fibrous diets. Sauer *et al.* (1991) observed net synthesis in the large intestine of Met, with disappearance of threonine, cysteine, glycine, proline and serine. They suggested that the disappearance of other amino acids may be due to preferential degradation of endogenous foregut glycoproteins which are rich in these amino acids.

2.8.5.3 Microbial metabolism of N in the lower gut

In some diets, the supply of fermentable energy may limit microbial fermentation in the lower gut of the pig, where NSP are the main energy substrate (Houdijk *et al.,* 2002). Protein can be fermented when the supply of fermentable NSP becomes limiting, or when there is substantial flow of undigested dietary and endogenous N into the lower gut (Piva *et al.,* 1996; Houdijk *et al.,* 1998; Macfarlane and Macfarlane, 2003).

In vitro, the fermentation of protein produced less gas compared to NSP, and occurred in the initial hours of incubation, apparently because the major part of protein was soluble (Cone *et al.,* 2005). The fermentation of proteins increases the yields of Iso-But, Val-a and Iso-Val-a, which are derivatives of the branched-chain amino acids such as valine, leucine and isoleucine. About 30% of the protein mass is converted to SCFA, of which BCFA constitute between 16 and 23%, depending on the substrate type (Macfarlane *et al.,* 1992; Cone *et al.,* 2005). Metabolites from the fermentation of protein include ammonia and amines (Cone *et al.,* 2005) as well as odorous compounds such as indoles and sulphides (Jensen *et al.,* 1995).

Ureolytic bacteria maintain a concentration gradient that favours a net transfer of urea into the caecal lumen (Younes *et al.,* 1995; Mosenthin *et al.,* 1992). The assimilation of BUN increases the amount of N present in the faeces and decreases N excretion as urea in urine (Younes *et al.,* 1995). Canh *et al.* (1997) reported a ratio of faecal to urinary N of 3.8 to 1.2

on high, compared to low DF diets, respectively. Sugar beet pulp, containing highly fermentable soluble fibre, also increased the faecal to urinary N ratio to 2.171, compared to 1.177 for less fermentable oat hulls (Bindelle *et al.,* 2009). Ammonia generated in the colon readily passes across the gut wall, thereby gaining access to other tissues of the body (Rowland, 1992) which can be detrimental to health (Nollet *et al.,* 1999; Cone *et al.,* 2005). It has also been found to be a predisposing factor for post-weaning diarrhoea (PWD) in pig (Gaskins, 2001).

2.8.5.4 Strategies to reduce protein fermentation in the lower gut

Reducing dietary protein is one strategy to reduce its wasteful fermentation by gut microbes (Bikker *et al.,* 2006; Nyachoti *et al.,* 2006; Htoo *et al.,* 2007). Protein is spared from fermentation in the presence of readily fermentable NSP (Awati *et al.,* 2006). For instance, fermentable fibre reduced ammonia and branched-chain fatty acids (BCFA) both *in vitro* (Awati *et al.,* 2005) and *in vivo* (Awati *et al.,* 2006). Jha *et al.* (2011) investigated the fermentation characteristics of diets enriched in fibre using wheat bran, pea hulls, pea inner fibres, sugar beet pulp or maize distillers dried grains with solubles, which also contained different levels of CP and of indigestible CP. The highly fermentable fibre (pea fibres, sugar beet pulp) decreased both NH_3 concentration in the colon digesta and faecal N excretion. These effects confirm the greater incorporation of N into bacterial proteins in the presence of fermentable DF.

2.8.6 Gut health

Low nutrient intake, immature immune functions, and the stress caused by weaning predispose piglets to enteric diseases (Kim *et al.,* 2012). During the immediate post-weaning period, conditions in the GIT are critical for the maintenance of good health. The gastric pH of between 3-4 is bactericidal for many pathogenic bacteria (Verstegen and Williams, 2002). However, weaned pigs have higher gastric pH compared to their sow reared peers due to low acid secretion, and low lactic acid due to the absence of lactose (Kim *et al.,* 2012).

Globally, to prevent drug resistance, the prophylactic use of antibiotics is increasingly restricted by legislation, to prevent drug resistance (Verstegen and Williams 2002). A nutritional approach is preferred.

2.8.6.1 The association between fibre and enteric diseases

The association between fibre and the disease resistance mechanisms of the GIT presents promising opportunity to control gut health by dietary manipulation (Kim *et al.,* 2012). Pathogenic bacterial diseases that are linked to the activities of DF include post-weaning colibacillosis (PWC) caused by serotypes of enterotoxigenic *Escherichia coli*, the proliferative enteropathies, caused by *Lawsonia intracellularis*, salmonellosis caused by *Salmonella Spp*., porcine intestinal spirochaetosis caused by *Brachyspira piloscicoli*, and swine dysentery caused by *Brachyspira hyodysenteriae* (Kim *et al.,* 2012).

Research suggests that while some types of fibre have a positive influence on gut health, others may be detrimental (Bach Knudsen *et al.,* 2012, Kim *et al.,* 2012). Inclusion of fermentable carbohydrates in weaner pig diets decreased post-weaning collibacilosis by promoting the proliferation of commensal microbiota (Awati *et al.,* 2006). The incidence of swine dysentery was also reduced when pigs were fed diets containing fructan-rich chicory roots and sweet lupins (Thomsen *et al.,* 2007). Wheat bran decreased the number of pathogenic E. coli in the faeces and reduced the incidence of post-weaning diarrhoea (Molist *et al.,* 2010). Smith and Halls (1968) reported that incorporation of barley hull, which is mostly insoluble fibre, prevented expression of PWC. Supplementation of oat hulls in cooked rice based diets (Mateos *et al.,* 2006), extruded rice based diets (Kim *et al.,* 2008) based diets decreased the incidence PWC. The health promoting actions of fibre include the reduction of enterotoxic fermentation metabolites such as indoles, volatile phenols, ammonia and amines (Brownlee *et al.,* 2011).

On the other hand, McDonald *et al.* (2001), Hopwood *et al.* (2002), and Montagne *et al.* (2004) reported positive relationships between soluble NSP and the expression of post weaning colibacillosis. Pluske *et al.* (2003) reported increased incidence of clinical swine dysentery in growing pigs and diarrhoea in weanling pigs fed with diets containing soluble

NSP and RS. Supplementation of βG to nursery pigs also tended to increase their susceptibility to *Streptococcus Suis* infection (Dritz *et al.,* 1995). Smith and Halls (1968) reported that incorporation of barley meal that contains soluble βG increased expression of PWC in enterotoxigenic *Escherichia coli* challenged pigs.

Overall, the evidence suggests that generally, whereas viscous sNSP may predispose piglets to enteric infections, they protect the piglets from enteric infections (Kim *et al.,* 2012). Choct (1997) suggested that the ability IDF to hold large amounts of water may reduce the solubilisation of the sNSP. This presents the possibility that at an appropriate ratio, the anti-nutritive effect of the SDF may be minimised. The hypothesis has not been tested, but would be consistent with a diet formulation strategy whereby the viscosity-increasing soluble NSP content is restricted in diets that contain iNSP at some minimum level (Kim *et al.,* 2012).

2.8.6.2 Mechanisms by which fibre maintains or may perturb gut health

The mechanisms by which the DF may influence gut health are complex. A dynamic equilibrium involving the gut-associated lymphoid tissue (GALT), mucin proteins secreted by goblet cells, commensal bacteria and the intestinal mucosa defines "*gut health*" (Brownlee *et al.,* 2011). A popularised illustration of this equilibrium is presented in **Figure 2.6**.

a) Gut associated lymphoid tissue

The GALT detects antigenic factors in the intestinal lumen (Montagne *et al,* 2003). The effects of fibre on GALT are thought to be mediated through changes in gut micro flora (Sharma and Schumacher, 2001).

b) Mucus secretion

A second immune function of the gut epithelium is the secretion of protective mucins. Mucins are synthesized and secreted by goblet cells (Bach Knudsen *et al.,* 2012). The mucins primarily protect the gut from the damaging agents and shear forces (Montagne *et al.,* 2004). The link between fibre and mucus secretion was discussed in section 2.8.5.1. The mechanims that control mucus secretion are illustrated in **Figure 2.7.**

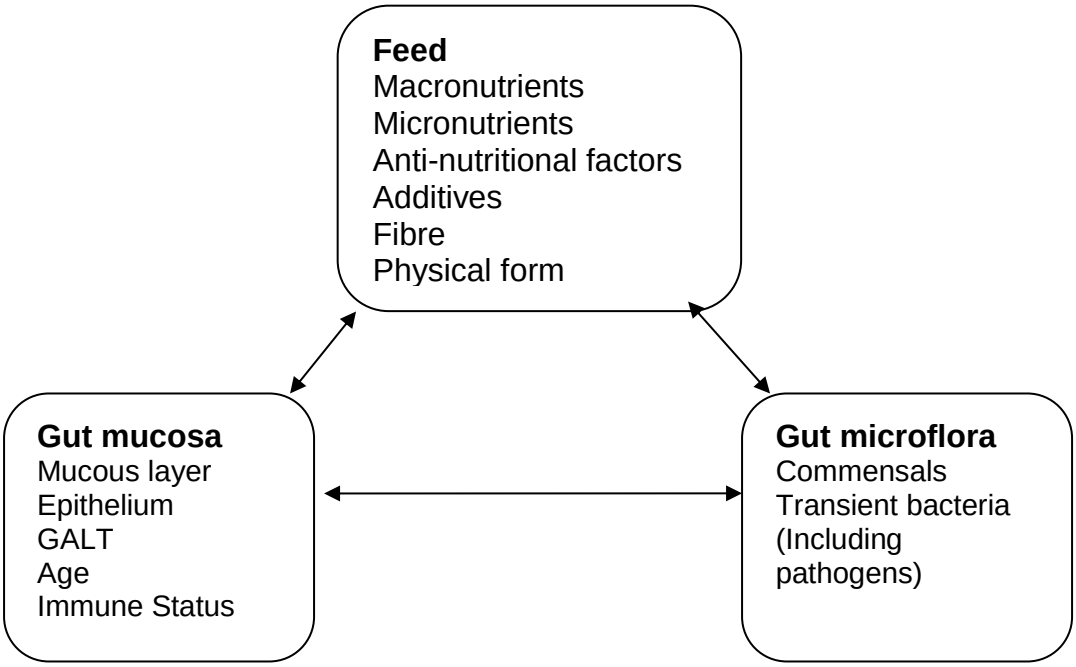


Figure 2.6: Dynamic equilibrium which defines "gut health" (From Montagne *et al.,* 2003)

c) The gut mucosa

The epithelium of the intestines is a semipermeable membrane that controls fluid and solute exchange between the internal and external environments (Bach Knudsen *et al.,* 2012). The glycoprotein (mucus) and other secretions of the brush border membrane influence the adherence and the metabolic activity of bacteria (Kelly *et al.,* 1994).

The epithelium contains *villi* and crypts. In the mature animal, the *villi* reduce in size from the proximal (320–350 µm) to the distal small intestine (220–260 µm) (Jin *et al.,* 1994). In the colon, only the crypts remain, the *villi* are barely present (Brunsgaard, 1997). For optimal digestive and absorptive function, long *villi* (Heo *et al.,* 2012) and deep crypts (Bach Knudsen *et al.,* 2012) are desirable. The *villus* height/crypt depth ratio is a useful criterion for estimating the likely digestive capacity of the small intestine (Bach Knudsen *et al.,* 2012). Post weaning, there is a period of *villi* atrophy and crypt hyperplasia in the ileal epithelium (Heo *et al.,* 2012). The causes are not clear.

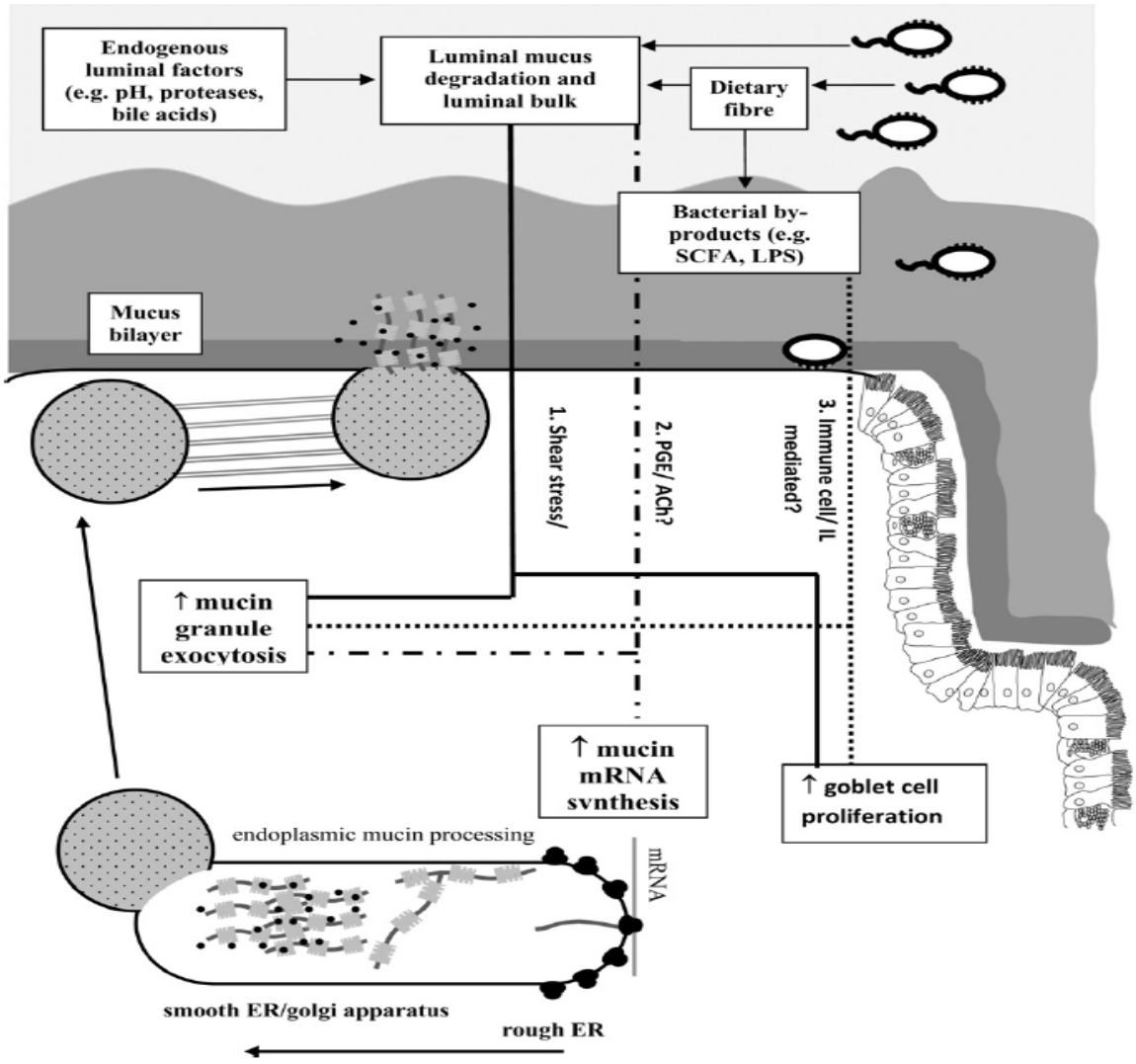


Figure 2.7: Modulation of mucus secretion in the GIT (From Brownlee *et al.,* 2011)

The effect of viscosity on ileal epithelial morphology is inconsistent. Highly viscous SDF increased the rate of *villus* cell losses leading to *villus* atrophy (Hedemann *et al.,* 2006). However, low viscous SDF increased *villus* height (McDonald *et al.,* 2001). Fermentable NSP interferes with the cell cycle of the crypt cells (Święch *et al.,* 2010). Fermentable (IDF) fibre increased the depth of intestinal crypts and the width of intestinal *villi* in jejunum and ileum, and increased the rate of cell proliferation, and depth of crypts in the colon (Jin *et al.,* 1994). These changes were accompanied by significant reduction in the concentrations of DNA in the crypt cells in the mucosa of jejunum and ileum, indicating programmed cell death, while the concentrations of RNA in the colon were increased. An increase in the rate of crypt-cell proliferation, when accompanied by reduction in concentrations of DNA,

indicates an increase in the rate of turnover of intestinal mucosal cells (Sinha *et al.,* 2011). The effect of fibre on colonic morphology is also conflicting. It depends on the botanic origin and fermentation properties of the fibre and the composition of the microbiota in the colon of pigs (Williams *et al.,* 2001).

The changes in the intestinal epithelial morphology in response to feeding fermentable fibre is attributed to the trophic effect of SCFA, especially butyrate, which is an important energy source for the colonic epithelium, and also regulates cell growth and differentiation (Sakata, 1987). Wang *et al.* (2005) reported that feeding weanling piglets with But increased the height of the intestinal *villi*. Kotunia *et al.* (2004) reported a reduction of the *villi* height in the duodenum and an increase in the *villi* height of the jejunum and ileum when But was supplemented to neonatal piglets. Brewers grain increased jejunum *villus* height and width, and jejunum and ileum crypt depth of piglets, and increased jejunum *villus* width, jejunum, and ileum crypt depth when compared to wheat bran (Martins *et al.,* 2010).

On the other hand, Biagi *et al.* (2007) and Tonel *et al.* (2010), did not find any effects on intestinal morphology when But was added to piglet diets. Supplementation of low viscosity fibre to a cooked rice-based diet increased the small intestinal *villus* and crypt depth in newly weaned pigs, but did not affect the shape of *villi* (McDonald *et al*., 2001).

The inconsistent findings are attributed to differences in gastrointestinal tract, breed, diet, experimental conditions and the difficulty in standardising the measurement of *villi* height and crypt depth. Therefore it difficult to interpret data on changes in intestinal morphology across different experiments (Tonel *et al.,* 2010).

d) Colonisation resistance

The concept of "*colonisation resistance"* describes the tendency to exclude newly ingested pathogenic microorganisms (Van der Waaij *et al.,* 1971) from the gut by the host and its mucus bound commensal bacteria. Factors involved include bacteriocins, extra-cellular enzymes, SCFA production, competition for nutrients and attachment sites, and stimulation of the immune system (Zoetendal *et al.,* 2004). Animal factors include pH control, changes

in gastric motility (peristalsis) and digesta flow, mucus secretion, epithelia cell desquamation and the production of IgA and non-specific adherence blocking factors by the GALT system (Williams *et al*, 2001).

e) Prebioses

"Prebioses" is an effect where "a selectively fermented ingredient allows specific changes, both in composition and or activity of microbiota that confer benefits upon the host's wellbeing and health' (Gibson *et al.,* 2004). The mechanism of action of prebiotics is thought to be through selective stimulation of proliferation and activities of favourable bacteria. In the GIT, there is a delicate balance between commensal and pathogenic bacteria (Williams *et al.,* 2001). Pathogenic bacteria include *E. coli*, *Salmonella spp*, *Camphylobacter spp, Chlostridium spp*. (Montagne *et al.,* 2003). Bacteria associated with a healthy gut include *Bifidobacteria, Lactobacilli* and *Eubacteria* (Heo *et al.,* 2012*).* Non starch polysaccharides for which prebiotic effects have been reported include the oligosaccharides (Heo *et al.,* 2012), resistant starch (Verstegen and Williams, 2002), arabino-xylans and βGs (Reilly *et al.,* 2010; Jha *et al.,* 2010) and fructans and inulin (Kim *et al.,* 2012).

2.9 NSPases in pig nutrition

Fibrolytic enzyme technology is intended to increase the nutritive value of fibrous feedstuffs by degrading the complex carbohydrates to remove the deleterious effect on the digestive physiology. The two main classes of fibre-degrading enzymes currently used in pig feed are xylanases and β-glucanases (Paloheimo *et al.,* 2011). It is noteworthy that historically, the development of enzyme technology targeted poultry on standard, European type diets in which the specific target are the gel forming βGs soluble arabinoxylans of wheat, oats, barley or triticale (Zijlstra *et al.,* 2010). Unlike in poultry, due to longer transit times, the digestive system of the pig is not as prone to the negative effects of soluble, viscous gel forming fibre (Barletta, 2011). Instead, given the greater fermentation capacity of the GIT of pigs, depending on the age of the pig, it can be hypothesised on such diets, the benefits associated with the fermentability of NSP may be more important than the deleterious impact of their viscosity. Therefore, whenever reported, the efficacy of carbohydrase in pigs might in large part reflect the positive effects of fermentation on gut health (Nofrarías *et al.,* 2006).

Currently, there is renewed interest to address the uncertainty on the efficacy of exogenous enzyme technology in growing pigs, particularly on maize-based diets (Barletta, 2011). Given the global dominance of maize in the feed industry, and the trend toward greater use of co-product feeds, the enzymes should be tailor-made for co-product feed enriched maize-soybean diets.

2.9.1 Mode of action

Current enzymes were largely developed for poultry, and were not designed to degrade NSP to monomeric sugars within the GIT transit times (Choct, 2006). The extent of degradation in pigs is not clear, but could be substantial due to the longer GIT transit times. The effects of enzymes are therefore likely largely limited to alteration of the physico-chemical and fermentation properties resulting from the partial depolymerisation.

Three hypotheses on the mode of action of enzymes are proposed (**Figure 2.8**). Enzymes degrade the cell walls, thereby releasing entrapped nutrients (Barletta, 2011). Shortening of

the polymer chains and or removal of side chains may alter the viscosity and water binding capacity (Choct, 1997; Bedford, 2000). The third proposed mechanism is through depolymerisation of the complex NSP to release highly fermentable oligosaccharides (Choct and Cadogan, 2001: Van der Meulen *et al.,* 2010;). However, theoretically, enzymes may also reduce fermentation in the lower gut if efficient nutrient digestion in the upper tract diverts nutrients to the host, away from the microbes in the lower gut (Bindelle *et al.,* 2011; Jonathan *et al.,* 2012).

Combining different enzymes tends to increase the efficacy (Barletta, 2011). It is not clear whether such effects are additive, or synergistic.

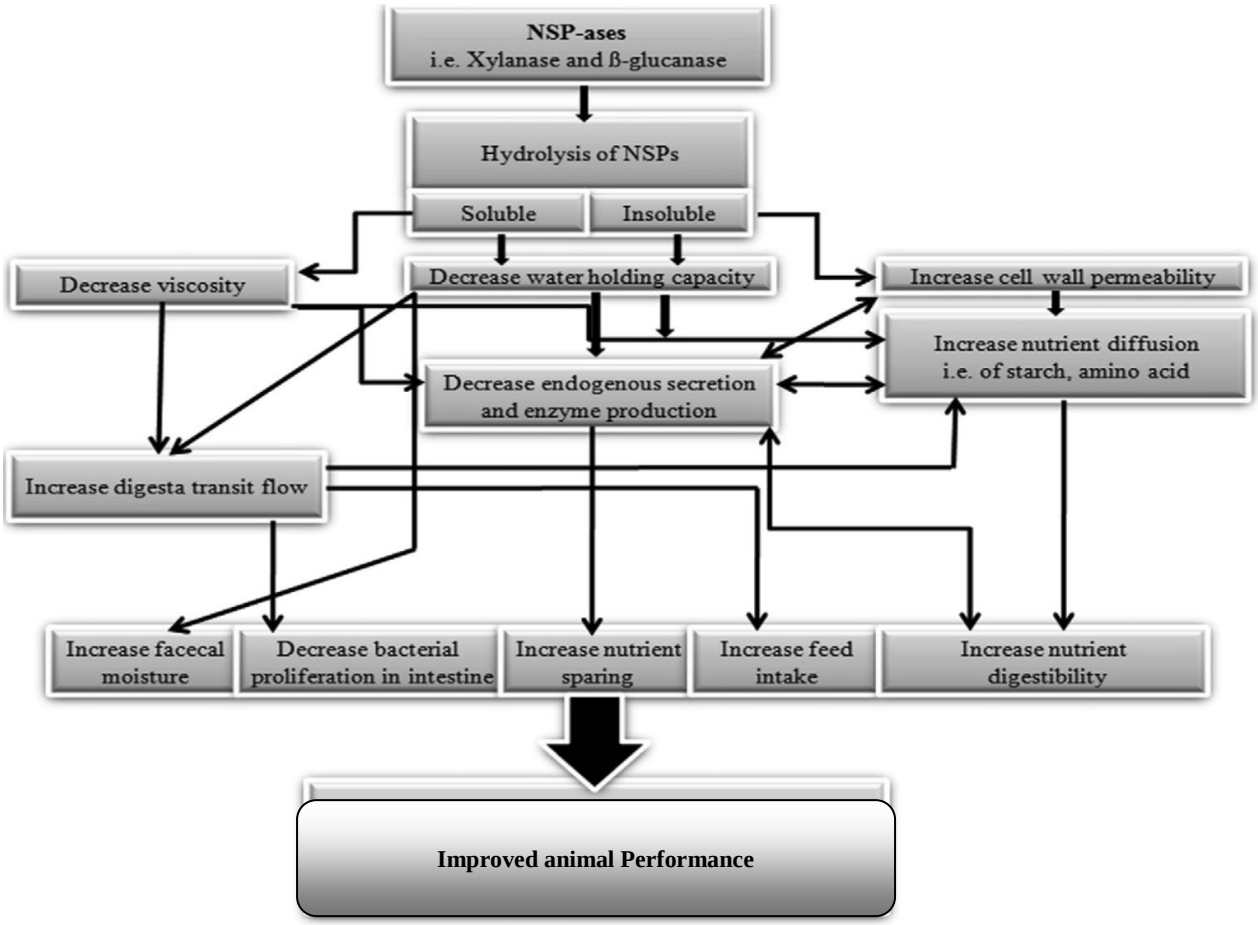


Figure 2.8: Modes of action of NSP degrading enzymes (Modified from Sinha *et al.,* 2011)

2.9.2 Sites of action

Review of literature shows high variability in optimum conditions for different enzymes (Svihus, 2011). The latter concluded that depending on the enzyme source, pH optimum ranged between 4.0 and 5.0, and temperature optimum between 45 and 65°C. They estimated the average pH of stomach contents of pigs at around 4.0 with a higher pH following feeding and a lower pH after a long time of feed withdrawal. A pH between 6.0 and 7.5 has been reported (Mathew *et al.,* 1996; Cuche and Malbert, 1998; Nyachoti *et al.,* 2006).

Transit times reported in literature are similarly highly variable and depend on the diet, meal size and feeding regime. Gastric mean retention time ranged from 7.2 and 11.2 h (Partanen *et al.,* 2007), 3 and 6 h (Van Leeuwen and Jansman, 2007) to as short as approximately 1 h (Wilfart *et al.,* 2007). Retention time in the small intestine ranged between 4 and 10 h (Potkins *et al.,* 1991; Partanen *et al.,* 2007; van Leeuwen and Jansman, 2007; Wilfart *et al.,* 2007). Overall, it appears that conditions in the upper tract should not limit enzyme activity. More research is needed to investigate the extent to which diet and feeding regime can affect the ability of the enzymes to exert meaningful biological responses. For example, Svihuis (2011) suggested that retention time in the stomach could be limiting under conditions of *ad libitum* feeding. .

The future challenge for the feed industry is, therefore, to produce purer, tailor-made, multi-potent, highly efficacious enzymes with capacity for extensive degradation of the NSP within the conditions and transit times of the pig's GIT.

2.9.3 Effects on nutrient digestibility and growth performance

In growing pigs, there is uncertainty on the efficacy of exogenous enzyme technology, particularly on maize-based diets enriched with chemically complex insoluble fibres (Barletta, 2011). Overall, there is greater effect on nutrient digestion and performance of young, compared to older pigs (Noblet and Le Goff, 2001), and greater effect in wheat, barley, rye and oat diets as opposed to maize based diets (Ji *et al.,* 2008).

Inconsistent findings on the efficacy of enzymes are partly attributed to failure to target specific anti-nutritive properties of the dietary NSP and or the failure to match the enzymes to the dietary NSP (Zijlstra *et al.,* 2010). Matching the enzymes to the NSP is complicated by the diversity among the feed grains and their processed components in the molecular composition of NSP (Bach Knudsen *et al.,* 2001). The inconsistent findings are also attributed to factors such as diet composition (type and level of fibre) and age of the animal (Souffrant, 2001), biased experimental results or poor optimisation of the dietary carbohydrase levels (Owusu-Asiedu *et al.,* 2010). It is also difficult to analyse and compare some of the findings because NSPase activities of diets are often not indicated (Zijlstra *et al.,* 2010).

Supplementing enzymes to barley and wheat based diets improved the growth performance and nutrient digestibility of 6 kg pigs (Omogbenigun *et al.,* 2004). Adding xylanase to diets containing wheat co-products improved precaecal apparent digestibility of dry matter, crude protein, and energy in 15 kg pigs fed diets containing high levels of DF (Yin *et al.,* 2000).

In growing pigs fed wheat based standard diets, xylanase increased ileal and total tract digestibility of energy and also improved digestible Ca and P (Nortey *et al.,* 2007). Yin *et al.* (2000) observed a minor effect of enzyme supplementation on precaecal the digestibility of DM, CP and energy in pigs weighing greater than 30kg body weight. In pigs of initial weight 14.7 kg and fed rye based diets, Roxazyme G2 increased the precaecal digestibility of total amino acids from 61.7% to 70.8%, and improved the digestibility coefficients of NSP constituent sugars arabinose + xylose (685%) and pentosans, mannose, galactose and glucose (110%) (Nitrayova *et al.,* 2007).

Xylanase reduced feed intake with improved feed efficiency in growing pigs on standard wheat based diets (Nortey *et al.,* 2007). A combination of phytase and a cocktail containing xylanase, amylase and protease improved growth performance in weanling pigs fed standard diets marginally deficient in energy (Olukosi *et al.,* 2007). Enzymes improved feed conversion (Owusu-Asiedu *et al.,* 2010). Carbohydrase supplementation improved ADG in pigs fed barley, hull-less barley or wheat-based diets (Bedford *et al.,* 1992; Van Lunen and

Schulze, 1996; Baidoo *et al.,* 1998). Zijlstra *et al.* (2004) observed increased energy and DM digestibility, ADFI and ADG, but not feed efficiency.

On the other hand, other reports do not show enzyme efficacy across a range of diets and animal types. Carbohydrase supplementation did not improve ADG and nutrient digestibility in pigs fed rye-based or barley-based diets (Zijlstra *et al.,* 2004; Thacker *et al.,* 1992; Baas and Thacker, 1996). In pigs weighing above 30kg, β-glucanase and xylanase enzyme supplementation to standard wheat, barley, rye plus soy based diets did not affect the precaecal digestibility of DM, and in fact reduced the digestibility of CP (Susenbeth *et al.,* 2011). Enzymes did not affect faecal the digestibility of energy and of protein and did not improve weight gain and feed intake (Owusu-Asiedu *et al.,* 2010).

The research on the efficacy of enzymes is predominantly on wheat, rye or barley based diets. There is limited information on the effects in maize based diets. Supplementation of growing/finishing pigs fed maize-soy diets increased growth performance in pigs, but with minimal effects on nutrient digestibility (Pettey *et al.,* 2002). Supplementing growing pigs with a β-glucanase and protease cocktail in maize-soya based standard diets improved faecal digestibility of dry matter, Organic matter, energy, protein and minerals (Ji *et al.,* 2008). The enzyme blend also shifted NDF and hemicellulose digestion from the colon to the ileum. Ji *et al.* (2008) reported increased precaecal digestibility of NDF and hemicelluloses when 38.5kg pigs were fed a maize soybean diet with a glucanase-protease enzyme supplement. However, enzymes had no effect in growing pigs fed on maize-soybean based diets, with significant effects on similarly formulated, wheat-rye-oat diets (Willamil *et al.,* 2012).

The impact of enzymes on amino acid nutrition is of particular concern to the productivity and cost of feeding pigs. Feeding maize-soya based diets containing an enzyme blend with glucanase and protease activity to growing pigs improved the apparent and standardised ileal digestibility of Met, Ala, Ser, Arg, Gly, Thr and Val (Ji *et al.,* 2008). Xalanase added to wheat-based diets improved precaecal digestibility of most essential amino acids in pigs weighing less than 30kg (Barrera *et al.,* 2004). β-glucanase and xylanase supplementation improved apparent ileal digestibility of Lys, Gly, Thr, His and Val in weaner diets (Yin *et al.,*

2001). Xylanase also increased ileal and total tract digestibility of Iso-Leu, Phy, Lys, Thr and Val in growing pigs fed wheat based diets (Nortey *et al.,* 2008).

On rye-based diets, RX increased apparent ileal digestibility of amino acids, particularly of arginine and glutamic acid (Nitrayova *et al.,* 2007). In pigs weighing above 30kg, β-glucanase and xylanase enzyme supplementation to standard wheat, barley, rye plus soy based diets did not affect threonine absorption, endogenous protein and amino acid losses (Susenbeth *et al.,* 2011). B-glucanase supplementation to barley and wheat-soybean diets increased the apparent ileal digestibility of amino acids in the barley-based diet and not in the wheat based diets (Li *et al.,* 1996). Xylanase supplements to wheat diets with high levels of wheat co-product feeds did not influence apparent ileal digestibility of protein (Yin *et al.,* 2000). There is dearth of information on the effect of enzymes in pigs fed maize based diets.

2.9.4 Influence of NSPases on fermentation in the hindgut

The influence of enzymes on the fermentation of NSP in the hindgut is not clear. Enzymes may alter the rate, type, extent or site of fermentation in the gut. This may in turn alter the overall fermentation induced physiological activity of fibre in the GIT. *In vitro* studies by Bindelle *et al.* (2011) suggested NSP-degrading enzymes can affect fermentation in the lower gut. Their findings on the influences of enzymes on SCFA production and microbial species are summarised in **Tables 2.20-2.22**, whereby enzymes reduced gas and SCFA production, shifted fermentation from Pro to Ace and increased the cellulolytic *Ruminococcus* and xylanolytic *Clostridium*-like bacteria. Barley-based diets had high total VFA but lower Enterobacteriaceae population compared to oat-based diets. However the oat-based diets enhanced beneficial microbial populations, which suggested greater prebiotic potential of oat derived βG. NSPases altered the concentration of SCFA in the small intestine of growing pigs (Diebold *et al.,* 2004). Hirsch *et al.* (2006), cited by Bindelle *et. al.* (2011) reported increased total bacteria, *Lactobacilli* 16S rRNA gene abundances, and the enhancement of specific *Lactobacillus spp* in the jejunum of weaned pigs. On barley-based diets, NSPases altered the composition of short chain fatty acids in the colon in favour of improved gut health (Reilly *et al.,* 2010). However, there was interaction between cereal

type and enzyme supplementation on total SCFA production and on the proportions of the individual acids.

The impact of altering fermentation patterns on gut health and odour emissions have not been investigated extensively. Kiarie *et al.* (2007) reported that products of enzyme hydrolysis of NSP might help maintain the intestinal barrier function during enterotoxigenic *E. Coli* infection. The hydrolysis products may interfere with the attachment of pathogens to the intestinal mucosa, which is an important first step in infection (Lange *et al.,* 2011). The hydrolysis products may also act as prebiotics, favouring the proliferation of lactic acid-producing bacteria (Högberg and Lindberg, 2004; Kiarie *et al.,* 2007), which inhibit the growth of pathogenic species (Choi *et al.,* 1994).

Table 2.20: The effect of enzymes on *in vitro* gas production kinetics*

Enzyme	Feed	Fermentation parameters**				
		A ($mL.g^{-1}$ DM)	B (h)	C	R_{max} ($mL.g^{-1}$ DM x h)	t_{max} (h)
	Diet	180	17.7	1.45	6.2	5.5
+	Diet	171	18.6	1.51	5.6	6.4
-	Wheat	220	11.5	1.80	11.9	5.7
+	Wheat	192	15.5	1.76	7.7	7.5
-	Wheat bran	183	15.1	1.69	7.5	6.7
+	Wheat bran	168	17.1	1.68	6.0	7.6
-	Wheat bran	183	15.1	1.69	7.5	6.7
+	Wheat bran	168	17.1	1.68	6.0	7.6
-	Common barley Mc Leod	207	11.7	1.89	11.3	6.3
+	Common barley Mc Leod	190	12.5	1.88	9.6	6.7
-	Common barley AC Metcalfe	201	10.8	1.98	12.0	6.1
+	Common barley AC Metcalfe	183	12.1	2.01	9.9	7.0
-	Hulless barley CDC Fibar	233	8.5	2.15	18.4	5.3
+	Hulless barley CDC Fibar	220	9.9	2.26	15.3	6.5
-	Hulless barley SB94893	240	10.6	2.13	15.2	6.5
+	Hulless barley SB94893	227	10.4	2.34	15.5	7.0

Data from Bindelle *et al.* (2011)

*Microbial communities after 72-h fermentation using a pig faecal inoculum of hydrolyzed ingredients when the NSP-degrading enzymes were added during the pepsin and pancreatin hydrolysis of the (Boisen and Fernandez (1997) procedure.

**Modelled according to Groot *et al.* (1996) where;

G (mL g^{-1} DM) denotes the gas accumulation to time

A (mL g^{-1} DM) the maximum gas volume for t =1,

B (h) the time to half asymptote when G = A/2

C is a constant determining the slope of the inflexion point of the profile.

R_{max}, the calculated maximum rate of gas production (mL g^{-1} DM × h),

t_{max}, the time at which R_{max}

Table 2.21: Effects of enzymes on the bacterial species composition

Enzyme	ingredient	Bacteria species TRF (bp)										
		32	139	190	218	281	292	465	490	516	551	Shannon index
-	Wheat	0.000	0.074	0.011	0.049	0.175	0.168	0.079	0.079	0.248	0.116	0.862
+	Wheat	0.000	0.082	0.025	0.045	0.207	0.144	0.063	0.079	0.268	0.087	0.853
-	Wheat bran	0.000	0.046	0.034	0.034	0.415	0.150	0.063	0.037	0.203	0.0	0.730
+	Wheat bran	0.000	0.047	0.053	0.067	0.495	0.124	0.068	0.021	0.109	0.016	0.712
-	Common barley Mc Leod	0.000	0.057	0.042	0.094	0.466	0.080	0.119	0.009	0.124	0.010	0.680
+	Common barley Mc Leod	0.041	0.046	0.070	0.093	0.443	0.100	0.066	0.025	0.086	0.030	0.786
-	Common barley AC Metcalfe	0.000	0.041	0.009	0.006	0.389	0.098	0.053	0.032	0.315	0.058	0.679
+	Common barley AC Metcalfe	0.009	0.037	0.019	0.018	0.421	0.097	0.051	0.039	0.273	0.036	0.713
-	Common barley AC Metcalfe	0.000	0.043	0.024	0.021	0.341	0.101	0.052	0.050	0.296	0.072	0.761
+	Hulless barley CDC Fibar	0.030	0.044	0.030	0.025	0.354	0.105	0.056	0.034	0.283	0.040	0.767
-	Hulless barley CDC Fibar	0.030	0.044	0.030	0.025	0.354	0.105	0.056	0.034	0.283	0.040	0.767
+	Hulless barley SB94893	0.000	0.057	0.015	0.030	0.220	0.139	0.062	0.103	0.203	0.169	0.855
-	Hulless barley +SB94893	0.008	0.114	0.008	0.015	0.342	0.138	0.044	0.025	0.214	0.092	0.688

Data from Bindelle *et al.* (2011)

*Microbial communities after 72-h fermentation using a pig faecal inoculum of hydrolyzed ingredients when the NSP-degrading enzymes were added during the pepsin and pancreatin hydrolysis of the (Boisen and Fernandez (1997) procedure.

Species identities: TRF 32, *Butyrivibrio fibrisolvens*; TRF 139, *Clostridium innocuum*; TRF 190, *Clostridium aminovorans*, *Clostridium bogorii*; TRF 218, *Eubacterium hallii*, *Eubacterium limosum*; TRF 281, *Ruminococcus flavefaciens*, *Clostridium xylanolyticum*; TRF 292, *Clostridium ramosum*; TRF 465, uncultured; TRF 490, uncultured; TRF 516, *Clostridium butyricum*, *Clostridium botulinum*, *Clostridium cellulovorans*, *Clostridium tyrobutyricum*, *Clostridium acetobutylicum*; TRF 551, *Streptococcus* spp, Leuconostoc spp.

Table 2.22: The effect of cereal type and enzyme inclusion on microbial populations (log_{10} cfu/g) digesta

Cereal	Barley		Oats	
Enzyme	−	+	−	+
Ileum				
Bifidobacteria spp.	4.9	3.8	6.7	5.3
Enterobacteria spp.	1.1	1.0	1.5	0.6
Lactobacilli spp.	6.1	2.5	7.0	4.5
Caecum				
Bifidobacteria spp.	4.7	5.7	7.2	6.8
Enterobacteriaceae spp.	0.9	1.0	2.3	1.8
Lactobacilli spp.	4.7	6.1	7.3	6.9
Colon				
Bifidobacteria spp.	6.3	6.5	7.5	7.0
Enterobacteria spp.	1.3	0.9	2.6	4.0
Lactobacilli spp.	5.8	6.5	6.9	7.1

Data from Smith *et al.* (2010)

The potential application of enzymes in controlling ammonia and odour emission is not clear. NSPases reduced the emission of ammonia, depending the dietary protein level and source of fibre (O'Connell *et al.,* 2005). On diets high in protein and in DF, glucanase and xylanase NSPases reduced the tendency of NSP to favour the excretion of N through faeces, and to lower faecal pH, thereby increasing the emission of ammonia (O'Connell *et al.,* 2005). On barley-based diets, NSPases had no effect on the skatole concentration in the colon (Reilly *et al.,* 2010). On similar diets, NSPases reduced the proportions of Iso-Val-a and iso-But in the caecum and in the colon (O'Connell *et al.,* 2005), suggesting the sparing of protein from fermentation, due to the increased fermentability of the NSP. However, there were no significant effects on the low protein diets.

2.9.5 Synergistic effects of enzyme and processing technologies

Combining processing and enzyme technologies is ideal because of the synergistic effects on the nutritive value of feeds (De Vries *et al.,* 2012) Cell wall degrading NSPases may degrade the viscous polymers that are solubilised during processing, while the breakdown of the cell wall matrix improves the accessibility of NSP to NSPases (de Vries *et al.,* 2012). This synergy hypothesis is supported in *in vivo* experiments. Enzyme reduced viscosity 3–4 times more in diets containing heat-processed ingredients than in the corresponding control diets (Gracía *et al.,* 2003, Gracía *et al.,* 2008, Gracía *et al.,* 2009).

The effects of milling on the activity of NSPases are not clear. Fine milling of flax seed increased the effect of enzyme compared with coarse milled flax seed (De Vries *et al.,* 2012). In contrast, the effect of enzyme was positive in crushed peas, but negative when peas were more finely milled (Daveby *et al.,* 1998). De Vries *et al.,* (2012) hypothesised that the NSPases could be effective in solubilizing and degrading NSP in the crushed peas, whereas there could be no such additive effect of the enzyme on ground peas where cell wall structures were already broken down.

2.10 Evaluating pig feeds

The success in manipulating the digestive function using the variable fermentative properties of DF among feedstuffs, and by using NSPases, is underpinned by robust hydrolytic and fermentation assays.

Given the wide range of potentially usable fibrous ingredients that are characterised by extremely high physico-chemical heterogeneity of the fibre, there is need for rapid, inexpensive techniques for screening these for inclusion in pig diets. Both *in vivo* and *in vitro* methods can be used to evaluate the hydrolytic and fermentation characteristics of fibrous feedstuffs and diets for pigs. *In vitro* methods are preferred as they are rapid and less expensive and have less ethical concerns (Williams *et al.,* 2005).

2.10.1 Simulation of upper tract digestion

The *in vitro* hydrolytic procedure developed by Boisen and Fernandez (1997) is commonly used to predict the energy values of pig feeds. The procedure involves hydrolysis by porcine pepsin, then by pancreatin, followed by hydrolysis by viscozyme. The procedure can be used to evaluate degradation kinetics of feedstuffs in the proximal gastrointestinal tract of the pig by estimating digestibility at different time points. Using this approach, Pérez-Vendrell and Torrallardona (2010) observed differences in digestion kinetics of diets containing 60% of rice, rice supplemented with wheat bran, barley, maize, oats, or naked oats. Bindelle *et al.* (2009) used pepsin-pancreatin hydrolysis prior to subjecting feedstuffs to the fermentation gas production test and concluded that it is important to hydrolyse substrates before fermentative evaluation.

2.10.2 Simulating fermentation in the lower gut

There is increasing interest in research on methods of estimating the fermentability of DF in pigs. Bindelle *et al.* (2007a) adapted the *in vitro* fermentation gas production technique originally described for ruminants (Menke and Steingass, 1988), to simulate fermentation of

DF in the pig's hindgut, with the convenient use of the *in vitro*, PP digesta (Boisen and Fernandez, 1997), and of pig faeces as inoculum.

The gas production technique measures cumulative gas production, which provides for the description of the kinetics of the microbial activity by fitting the profiles on appropriate mathematical models. However, questions remain on the validity of the gas production technique. Weaknesses of the procedure are that PP extraction of DF does not account for endogenous secretions and variable nutrient digestion and absorption in the small intestines, which influence fermentation (Wilfart *et al.,* 2008). In addition, the use of washed PP digesta excludes the soluble NSP. However, the fact that soluble NSP is excluded simulates the depletion of soluble NSP through fermentation in the distal ileum (Bindelle *et al.,* 2011).

The development of modern high-technology feed evaluation equipment presents further opportunity to make these simulations more rapid, with greater accuracy and precision. The *in vitro* hydrolysis procedure of Boisen and Fernandez (1997) has recently been adapted (Akinsola *et al., personal communication*) for use in an Ankom Daisy^{II} Incubator. A high correlation coefficient between *in vitro* and *in vivo* DM of 99.3% was reported. Although it is designed for small samples, the Ankom Daisy^{II} Incubator has the capacity to handle up to ninety-six samples at a time, such that residues of the PP stages of the *in vitro* assay for subsequent fermentation tests can be accumulated rapidly.

A modern ANKOM^{RF} Gas Production System is available on the market, with similarly rapid and sensitive gas production tests, and retains functionalities such as the evaluation of gas and SCFA composition, and microbial species growth and their interactions. However, given that fermentation *in vivo* is also influenced by endogenous secretions and continuous absorption of metabolites by the animal, which cannot be simulated *in vitro*, there is need for validation tests across different substrates (Williams *et al.,* 2005).

2.11 Metabonomics

The metabolic effects of feeding different types of diets have traditionally been evaluated by chemical analyses of plasma metabolites. ^{1}H-NMRS metabonomics is increasingly applied to complement traditional chemical analytical methods in studying biochemical changes in body fluids that are caused by feeding grain processing co-products (Yde *et al.,* 2011).

An important application in co-product nutrition is the detection of both nutritionally beneficial compounds and anti-nutrients. Examples of important metabolic modifiers detectable by ^{1}H-NMRS are dimethylsulfone and betaine. Dimethylsulfone or methylsulfonylmethane [$(CH_3)_2SO_2$] is found in a variety of fruits, vegetables and grains in low amounts (Pearson *et al.,* 1981) and is widely a applied dietary supplement for various ailments in humans (Brien *et al.,* 2008). Betaine or trimethylglycine is known to influence protein and fat deposition and to improve meat quality in finishing pigs (Matthews *et al.,* 2001; Lawrence *et al.,* 2002; Huang *et al.,* 2009). Betane also increased protein deposition in growing pigs on restricted feeding regime (Fernandez-Figares *et al.,* 2002). High plasma concentrations of both compounds were observed in sugar beet pulp and pectin enriched diets (Yde *et al.,* 2011).

2.12 Summary

Overall, the available evidence suggests that there is scope to expand the role of fibrous ingredients, provided there is application of modern processing and enzyme technologies, and provided high precision energy and amino acid evaluation systems are used.

The beneficial effects of increasing the supply of fermentable fibre on gut health and odour are increasingly recognised. Much progress has also been made in developing methods to characterise the fermentative properties of fibre for pigs. However, there is dearth of information on whether or to what extent the inclusion of the *in vitro* determined fermentation parameters in least cost formulation of diets can produce predictable, desirable fermentation patterns that achieve the intended objectives.

Given the potential of NSPases to expand the range of usable feedstuffs, and given the pressure to increase the dietary levels of fibrous ingredients, there is need for further

research to address the uncertainty on the efficacy of exogenous NSPases in pigs, particularly on maize-soybean based diets. Much of the available data on the efficacy of NSPases is on standard diets differing in the type of fibre, as influenced by the basal cereal ingredient. Research on the effects of DF has also targeted standard, wheat, barley and oats diets. There is limited information on maize based diets, and on high fibre diets. The influence of NSPases on lower gut fermentation in relation to its beneficial effects needs further investigation.

Overall, the challenge remains to clarify the mechanisms that control the activity of fibre in the GIT of growing pigs, sufficiently to select the supplementary fibrous ingredients, and to match these to appropriate enzyme cocktails, in formulating high fibre diets for optimum nutritional, physiological and environmental outcomes. A concerted, modelling approach is recommended. In such an approach, the comparability of findings is of paramount importance. There is therefore need for researchers to adopt a consistent, nutritionally relevant definition of fibre, with characterisation of the test diets for the properties that are known to control its metabolic and physiological activities in the GIT of pigs.

CHAPTER 3

HYDROLYSIS BY GLUCANASE AND XYLANASE ENZYMES AND FERMENTABILITY OF DIETARY FIBRE BY PIG FAECAL BACTERIA

Abstract

In vitro procedures that mimicked pig digestion were used to evaluate Roxazyme® G2 (RX) and Viscozyme L ® V2010 (VZ) activities, and the fermentation characteristics of DF in feed ingredients and in fibrous, complete maize-soybean growing pig diets. Maize (*Zea mays*) grain, its hominy chop and dehulled soybean (*Glycine max*) were used as the basal ingredients in a standard (141 g TDF g^{-1} DM), and in five high fibre (246 g TDF g^{-1} DM) growing pig diets, each enriched in DF using maize cobs, soy hulls, brewer's (barley; *Hordeum vulgare L*) grains, lucerne (*Medicago sativa*) hay or wheat (*Triticum aestivum*) bran. Among the feed ingredients, dissimilar, source-dependent ($p \leq 0.001$) activities on DF were observed between RX (0.02 to 0.12) and VZ (0.04-0.33). The lowest ($p \leq 0.05$) RX activity was observed on the maize and soybean fibres. The source of fibre influenced ($p \leq 0.0001$) fermentation gas (51.8-299.4 mL g^{-1} DM) and SCFA (2.3-6.0 mMol g^{-1} DM) production. Dehulled soy bean, maize meal and hominy chop fibre extracts were the most fermentable, while maize cob and brewers' grain fibre were poorly fermented ($p \leq 0.05$). Among the fibrous complete diets, the source of DF influenced ($p \leq 0.001$) the enzyme activities, with the activity of RX (0.03-0.06) substantially lower ($p \leq 0.05$) than that of VZ (0.16-0.22). The DF source influenced ($p \leq 0.001$) fermentation gas (126.6-187.6 mL g^{-1} DM) similarly ($p \leq 0.05$) affected the SCFA (4.1-5.4 mMol g^{-1} DM) production. On the STD DF, the activity of RX was less ($p \leq 0.05$) (0.03) than that of VZ (0.25), with higher ($p \leq 0.05$) (205.3 mL g^{-1} DM) gas, but similar ($p > 0.05$) (5.0-mMol g^{-1} DM) SCFA production compared to the fibrous diets. The leguminous fibres produced higher percent Ace and with less But ($p \leq 0.05$), and, except for the dehulled soybean meal fibre, less ($p \leq 0.05$) Pro. Except for MC, the cereal fibres produced higher ($p \leq 0.05$) percent Pro and less ($p \leq 0.05$) Ace. Maize meal and hominy chop fibres produced relatively high percent But ($p \leq 0.05$). Compared to the

standard diet, fibre extracted from the high fibre diets produced higher (p≤0.05) percent Ace, and less (p≤0.05) Pro and But. Among the diets, correlation was strong (r = 0.99, p≤ 0.001) between the partial degradability of VZ and the SCFA production, justifying its status as the standard enzyme for *in vitro,* pig digestibility studies. The source-depended variation in the fermentability of DF among the high fibre diets suggested substantial scope to increase DF fibre fermentability by screening the ingredients on fibre fermentability. The results suggested RX may not be effective in pigs fed high DF maize-soybean diets in which the DF is dominated by maize co-products.

Keywords*; growing pigs, fibre, non-starch polysaccharides, enzymes, fermentability*

3.1 Introduction

In growing pigs, the objective to control gut fermentation is motivated by its profound influence on the pig's metabolism and digestive physiology. Examples include prebiotic (Williams *et al.,* 2001), bactericidal (Verstegen and Williams, 2002) and enterotrophic (Tonel *et al.,* 2010) effects of the SCFA products. Short chain fatty acids are also known to control satiety (Sleeth *et al.,* 2010) and are involved in glucose (Theil *et al.,* 2011) and lipid (Fushimi *et al.,* 2006) homeostasis. Short chain fatty acids provided in excess of 10% of the total DE, equivalent to 17.6% of the total energy supply of the growing pig (Anguita *et al.,* 2006).

Fibre fermentability is a concept increasingly considered critical in a broader strategy toward the productive use of low cost, typically fibrous grain processing co-product feed ingredients (Chen *et al*, 2013). To control fermentation, the fermentation kinetics of DF in the feed ingredients need to be quantified, followed by the application of the parameters in feed formulation. Non-starch polysaccharide degrading enzymes may provide additional controls to influence fermentation (Bindelle *et al.,* 2011). A range of commercial NSPase cocktails are available on the market. There is uncertainty on their efficacy in pigs, particularly on maize-soybean based diets (Bedford and Cowieson, 2012).

Effective characterisation of the fermentation kinetics of DF and the matching of enzymes to the NSP composition are still constrained by methodology. *In vitro* assays are preferred if

they can predict the hydrolytic and fermentative degradation of NSP in the gut with less difficulty, low cost and in less time compared to *in situ* and *in vivo* studies. Bindelle *et al.* (2007) adapted the *in vitro* fermentation gas production technique originally described for ruminants (Menke *et al.,* 1979), to application in pig nutrition. The method conveniently uses faeces as inoculum to ferment fibre residues remaining after simulated gastric-ileal digestion by porcine pepsin + pancreatin. The procedure is a variation of the three-step *in vitro* method of Boisen and Fernandez (1997), which uses Viscozyme L ® V2010 (VZ) to predict the lower gut degradation of fibre. Viscozyme is a non-specific, multi-enzyme complex that contains a wide range of carbohydrases including arabinase, cellulase, β-glucanase, hemicellulose, pectinase, and xylanase (Cone *et al.,* 2005).

The aim of the study was to examine the influence of high levels of different unconventional feed ingredients on the fermentation characteristics, and the activity of Roxazyme® G2 (RX) on DF in complete, maize-soybean growing pig diets.

3.2 Materials and methods

3.2.1 Experimental feeds

Maize *(Zea Mays)* grain (MM) was obtained from the Animal Production Institute of the Agricultural Research Council (ARC). Maize hominy chop (HC) and cobs (MC), dehulled soybean (*Glycine max)* (dSBM) and soybean hulls (SH), brewer's (barley; *Hordeum vulgare L)* grains (BG), lucerne (*Medicago sativa)* hay (LH), wheat (*Triticum aestivum*) bran (WB) from OPTI FEEDS PVT LTD, Lichtenburg. The nutrient and fibre composition of the feed ingredients are described in **Table 3.1.** From these ingredients, six growing pig diets (**Table 3.2** and **Table 3.3**) were formulated; a standard, low fibre (141 g total dietary fibre (TDF) kg^{-1} dry matter (DM)) growing pig diet and five similarly nutritionally balanced (NRC, 1998) but high fibre (246 g TDF kg^{-1} DM) diets. The high level of DF was achieved by substituting variable proportions of maize and dehulled soybean meals in the STD diet with WB, MC, BG, SH or LH.

Samples of feedstuffs and diets were milled through a 1 mm screen using an Ika® analytical mill and were subsequently oven-dried for 18 hours in a forced draught oven at 100 ºC to

determine DM content. The samples were cooled for 30 minutes in a desiccator. Ankom® F57 filter bags (ANKOM Technology, Macedon, New York, USA) were rinsed in 99% acetone, air-dried and labelled using a solvent resistant marker. Feed samples (0.5 g ± 0.001) were weighed into the bags, and heat-sealed using a 200 mm AIE 60 Hz impulse sealer.

3.2.2 Chemical analyses

Feeds were analysed using AOAC (2000) Official method 934.01 for moisture, AOAC (2006) Official method 965.17 for phosphorus and AOAC (1999) methods 990.03 for crude protein (CP), 968.08, 942.05 and 920.39, for calcium, ash and fat (EE), respectively. Dietary fibre was analysed as soluble plus insoluble fibre, using the AOAC (2007) Official method 991.43. Crude fibre (CF), Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) were analysed according to van Soest *et al.* (1991). Gross energy (GE) was determined using a DDS isothermal CP500 bomb calorimeter.

Table 3.1: Chemical composition (g kg^{-1} DM) and gross energy (MJ kg^{-1} DM) content of pig feed ingredients

Chemical Component	[1]Feed Ingredients							
	BG	HC	LH	MC	MM	dSBM	SH	WB
Dry matter	875	896	946	944	901	904	913	902
Organic matter	956	979	910	944	989	929	951	951
Gross energy	22	18	16	17	16	18	16	17
Ash	44	21	90	56	11	71	49	49
Crude protein	241	97	161	42	62	515	119	167
Fat (ether extraction)	73	71	17	11	36	13	11	32
Crude fibre	212	59	381	352	13	43	421	90
Neutral detergent fibre	752	331	518	811	121	148	703	443
Acid detergent fiber	280	103	454	669	29	66	542	131
Acid detergent lignin	57	18	94	47	20	13	36	41
Soluble dietary fibre	18	9	26	5	4	7	14	17
Insoluble dietary fibre	627	249	600	871	113	152	773	363
Total dietary fibre	646	258	627	876	118	158	787	380
Calcium	2.1	<0.01	8.0	2.7	<0.01	3.1	5..2	0.6
Phosphorous	4.8	3.7	2.2	0.7	2.3	6.5	1.2	8.2

[1] BG (brewer's grains), HC (maize hominy chop), LH (lucerne hay), MM (maize meal), MC (maize cobs), dSBM (dehulled soybean meal), SH (soy hulls), WB (wheat bran).

Table 3.2: Ingredient composition of the diets used in the growth trial (% fed basis)

Ingredients	[1]Diets					
	STD	SH	MC	WB	LH	BG
Dehulled soybean meal	30.0	32.9	35.0	26.2	30.3	26.7
Maize meal	56.0	39.0	39.4	20.9	38.3	40.1
Soybean hulls	-	16.9	-	-	-	-
Maize cob	-		14.2	-	-	-
Wheat bran	2.5		-	42.3	-	-
Lucerne hay	-		-	-	20.4	-
Brewer's grains	-		-	-	-	21.9
Maize hominy chop	6.5	6.5	6.5	6.5	6.5	6.5
L-lysine HCl	0.1	0.0	0.0	0.0	-	0.0
DL methionine	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin & mineral premix	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	2.6	2.3	2.4	2.4	2.1	2.5
Mono-calcium phosphate	1.1	1.1	1.1	0.3	1.1	1.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0

[1]STD - standard, maize-soybean diet and diets in which the basal ingredients were partially substituted by the fibrous feeds: BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).
(-) Feed ingredient not added.

3.2.3. *In vitro* digestion of feeds

Feed ingredients were digested using a modification of the three step procedure described by Boisen and Fernandez (1997). Pepsin (porcine, 200 FIP-U/g, Merck No, 7190), pancreatin (porcine, grade IV, Sigma No P-1750) and Viscozyme L ® V2010 (VZ) (Viscozyme 120 L, 120 fungal β-glucanase U/g; mixture of microbial carbohydrases, including β-glucanase, xylanase, arabinase, cellulase, Novo-Nordisk, Bagsvaerd, Denmark) were used in the same dosages used in the original procedure. In the third step, Viscozyme was compared to Roxazyme® G2 (Novo DSM, from T. Longibrachitum, with endo -1,4-β-glucanase (8000 U/g), endo-1,3(4)-β-glucanase (18 000 U/g) and endo-1,3(4)-β- xylanase (26000 U/g). To determine the minimum effective dose of RX, calibration started from the feed dose (100 mg/kg), by calculating the dose in proportion to the mass of the sample used

in the setup and increasing the dose until the maximum degradability was achieved for all the substrates.

Table 3.3: Chemical composition (g kg^{-1} DM) and energy content of a standard and high fibre maize-soybean growing pig diets

Chemical Component	[1]Diet					
	STD	BG	LH	MC	SH	WB
Dry matter	897	906	901	904	900	902
Organic matter	922	919	905	912	907	921
Gross energy (MJ kg^{-1} DM)	15.3	14.6	16.2	16.5	16.4	16.6
Ash	78	81	95	88	93	79
Crude protein	219	232	233	229	233	233
Crude fat	30	38	26	25	25	29
Crude fibre	27	65	103	76	95	56
Neutral detergent fibre	146	269	224	242	239	274
Acid detergent fibre	47	96	135	141	133	86
Acid detergent lignin	17	25	33	21	20	26
Soluble dietary fibre	6	8	10	5	7	10
Insoluble dietary fibre	135	235	234	243	243	234
Total dietary fibre	141	243	244	249	250	244
Calcium	13.4	13.3	13.3	13.3	13.3	11.3
Phosphorus	6.7	6.6	6.7	6.6	6.7	6.7
Indispensable amino acids						
Lysine	8.9	8.8	9.1	8.9	8.9	8.9
Methionine + Cysteine	5.5	5.4	5.4	5.4	5.5	5.4
Threonine	5.9	6.5	6.3	6.3	6.3	6.3
Tryptophan	2.8	3.0	3.0	3.1	3.0	3.4
Arginine	11.0	11.4	11.4	11.7	11.8	11.7
Isoleucine	6.6	7.6	7.2	7.1	7.2	7.0
Leucine	9.1	9.9	9.6	9.3	9.4	9.0
Phenylalanine.+ Tyrosine	11.3	13.1	12.2	11.4	11.2	12.1

[1]STD - standard, maize-soybean diet and diets in which the basal ingredients were partially substituted by the fibrous feeds - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).

3.2.3.1. Digestion by pepsin

Each digestion jar of the ANKOM Daisy^{II} Incubator used in the digestion accommodated a maximum of 24 samples. Accordingly, in each run of the digestion, 24 sample bags containing the 8 feed ingredients with 3 replicates, or 6 diets with 4 replicates were placed in one of 4 digestion jars on the incubator. One empty filter bag was included in each jar as a blank. To each jar, 600 mL of phosphate buffer solution (0.1 M, pH 6.0) and 240 mL HCl (0.2 M) were added. The pH was adjusted to 2.0 using a 1 M HCl or a 1 M NaOH solution. The jars were placed on a rotating rack in the incubator. Temperature was calibrated to 39 ^{o}C. Pepsin (0.6 g) and 12 mL of chloramphenicol (5 g, Sigma No. C-0378, per 100 mL ethanol) solution were added to the digestion medium in each of the jars and mixed gently. The samples were digested for 2 hours at a temperature of 39 ± 0.5^{o} C.

3.2.3.2. Digestion by pancreatin

After digestion by pepsin, 240 mL of phosphate buffer solution (0.2 M, pH 6.8) and 120 mL of 0.6 M NaOH solution were added to the medium in each of the jars. The pH was adjusted to 6.8 using 1 M HCl or NaOH solutions. Pancreatin (2.4 g, porcine, grade IV, Sigma No P-1750) was added to each jar. The samples were digested for 5 h at a temperature of 39 ± 0.5^{o} C. After the digestion, the bags were rinsed in warm tap water with minimum agitation to prevent the escape of the particulates from the bags. The bags were further rinsed for 5 minutes in 95% ethanol, and for a further 5 minutes in 99% acetone. The samples were then oven-dried at 85^{o} C for 18 hours in a forced draught oven and were cooled in a desiccator for 30 minutes.

3.2.3.3. Digestion by Viscozyme and Roxazyme

At the termination of pancreatin digestion, the media in the jars were completely discarded. To each jar, 750 mL of a freshly prepared phosphate buffer (0.1 M, pH 4.8) was added. Temperature was calibrated to 39 ± 0.5 ^{o}C. To each jar, 12 mL of VZ or 0.017 g of RX was added. The samples were incubated for 24 hours at a temperature of 39 ± 0.5 ^{o}C. At the completion of the fermentation process, the bags were washed, rinsed and dried as described section 3.2.2.2 above.

3.2.4 *In vitro* fermentation

3.2.4.1 Preparation of substrates and the experimental scheme

Fermentation tests were conducted on the filtered, washed pooled residues after several runs of pepsin-pancreatin (PP) digestion of feedstuffs and diets. The gas production setup accommodated 10 samples at a time. Accordingly, each fermentation run included the 8 feed ingredients + 2 blanks or the 6 diets + 2 blanks. The blanks contained the inoculum only.

3.2.4.2 Preparation of buffer and inoculum

A phosphate-bicarbonate buffer was prepared according to Marten and Barnes (1980). The temperature of the buffer was calibrated and maintained at 39 °C in a water bath.

The inoculum was prepared following the procedures described by Bindelle *et al.* (2007). Fresh faeces were collected directly from the rectum of three mature dry sows from the herd at the Agricultural Research Council, Animal Production Institute, Irene (Pretoria, South Africa). The sows were on an antibiotic-free, dry sow ration. The faeces were immediately placed into a sealable plastic bag filled with CO_2 and placed in a pre-warmed thermos flask. The flask was flushed with CO_2, and was closed tightly. The inoculum was prepared in 2 batches in stomacher bags, each batch containing 60 g of the pooled faeces suspended in 400 mL of the buffer at 39 ^{o}C. The faeces and buffer in each stomacher bag were subjected to 60 seconds of mechanical pummelling in a stomacher blender. The blended mixtures were filtered through 4 layers of mutton cloth into a pre-heated (39 °C) 2 litre flat bottomed flask, which was immediately flushed with CO_2 and closed with a stopper. The inoculum was kept tightly closed and submerged in a water bath at 39 ^{o}C.

3.2.4.3 Gas production measurements

Gas production was measured using an ANKOM^{RF} gas production system (ANKOM® Technology). Residues of PP digestion were dried to constant weight at 50^{o} C and stored in

a desiccator prior to weighing 0.75 g ± 0.001 g samples into a 200 mL glass bottle. To each bottle of the gas measurement module, 100 mL of buffer at 39 ^{o}C and 50 mL of inoculum were added to make up 150 mL of fermentation medium containing 0.05 g fresh faeces per mL of buffer. The gas bottle headspace was flushed with CO_2 before closing tightly and incubation in a warm bath at 39 °C. Gas production measurements were taken over 64 hours, at a recording interval of 5 minutes.

3.2.4.4 Analyses for short chain fatty acids in the fermentation residues

Acetic (Ace), propionic (Pro), n-butyric (But), iso-butyric (Iso-But) and n-valeric acids were determined using a modification of the procedures described by Webb (1994). Residues of fermentation were filtered through Cameo 30 (0.45 μm) filters before analyses for SCFA in 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), with Helium as the carrier gas. Ace, Pro, But, Iso-But and n-Valeric were used as standards. The temperature program started at initial column temperature of 50 °C for 2 minutes, which was increased at 15 °C per minute to a final temperature of 190 °C, for 5 minutes.

3.2.5 Statistical analyses

The PROC NLIN procedures of Statistical Analysis Systems (SAS) Institute software, version 9.3 (SAS, 2010) were used to estimate gas production kinetics using the monophasic model described by Groot *et al.* (1996);

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

Where;

GV (mL g-1 DM)	Gas accumulation to time t (hours)
A (mL g-1 DM)	Asymptotic gas volume (t = ∞)
T½ (hours)	Time to half asymptote when G = A/2

Analysis of variance was conducted on the partial *in vitro* digestible dry matter (IVDDM) coefficients and on fermentation parameters using the PROC MIXED procedures of SAS

software, version 9.3 (SAS, 2010). The IVDMD dry matter by PP ($IVDDM_{PP}$) and the fermentation parameters of the residues of the digesta were analysed using model I;

$$\mathbf{Y_i = \alpha_i + e_i} \qquad \textbf{Model I}$$

Where: μ is the overall mean, α_i the effect of the i^{th} source of fibre and e_i the random error.

Partial IVDMD by RX ($IVDMD_{RX}$) and VZ ($IVDMD_{VZ}$) were analysed using model II;

$$\mathbf{Y_{ij} = \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}} \qquad \textbf{Model II}$$

Where: μ is the overall mean, α_i the effect of i^{th} source of fibre, β_j the effect of the j^{th} enzyme cocktail, $(\alpha\beta)_{ij}$ the interaction of fibre source and enzyme cocktail and e_{ij} the random error.

Comparison of means was performed using the Bonferroni t-test at the α-level of 0.05.

3.3 Results

3.3.4 Degradability by Roxazyme and Viscozyme

The $IVDMD_{PP}$, $IVDMD_{RX}$ and $IVDMD_{VZ}$ of the feed ingredients and of the diets are indicated in **Table 3.4** and **Table 3.5**, respectively. $IVDMD_{PP}$; $IVDMD_{RX}$ and $IVDMD_{VZ}$ differed among the feed ingredients and among the diets. Compared to other feedstuffs, the activity of RX was lower ($p \leq 0.05$) on fibre extracted from dehulled soybean meal and from maize and its co-products. Interaction was also significant ($p \leq 0.001$) among the diets, whereby the activity of VZ was highest on fibre extracted from the STD and SH diets, whereas RX had among the least activity on the same diets.

Table 3.4: Coefficients of partial enzyme degradability of residues of pepsin+pancreatin digestion of pig feed ingredients

Partial Degradability	[1]Feed Ingredients								SEM	P Values		
	BG	HC	LH	MM	MC	dSBM	SH	WB		Feed	Enzyme	Feed*Enzyme
[2]$IVDMD_{PP}$	0.31^{f}	0.71^{c}	0.35^{e}	0.75^{b}	0.16^{g}	0.80^{a}	0.29^{f}	0.58^{d}	0.014	<0.001		
[3]$IVDMD_{RX}$	0.12^{c}	0.02^{g}	0.08^{de}	0.04^{fg}	0.05^{ef}	0.02^{fg}	0.06^{e}	0.12^{c}	0.010	<0.001	<0.001	<0.001
[4]$IVDMD_{VZ}$	0.12^{c}	0.10^{cd}	0.18^{b}	0.16^{b}	0.04^{fg}	0.17^{b}	0.33^{a}	0.12^{c}				

[1] BG (brewer's grains), HC (maize hominy chop), LH (lucerne hay), MM (maize meal), MC (maize cobs), dSBM (dehulled soybean meal), SH (soy hulls), WB (wheat bran).

[2]$IVDMD_{PP}$ - Degradability of feed dry matter by pepsin, followed by pancreatin digestion, according to Bindelle *et al.* (1997).

[3]$IVDMD_{RX}$ - Degradability of the dry matter (after pepsin+pancreatin digestion) by Roxazyme® G2.

[4]$IVDMD_{VZ}$ - Degradability of the dry matter (after pepsin+pancreatin digestion) by Viscozyme 120 L.

[abcdefg] For pepsin + pancreatin degradability coefficients, means in a row which do not share a common superscript are different ($p \leq 0.05$).

[abcdefg] Across the Roxazyme and Viscozyme degradability coefficients, means which do not share a common superscript are different ($p \leq 0.05$).

Table 3.5: Coefficients of partial enzyme degradability of residues of pepsin+pancreatin digestion of a standard and high fibre maize-soybean growing pig diets

Partial degradability	[1]Diets						SEM	P Values		
	STD	BG	LH	MC	SH	WB		Diet	Enzyme	Diet*Enzyme
[2]$IVDMD_{PP}$	0.70^{a}	0.64^{c}	0.67^{bc}	0.68^{b}	0.65^{c}	0.68^{b}	0.003	<0.0001		
[3]$IVDMD_{RX}$	0.03^{d}	0.04^{d}	0.06^{c}	0.03^{d}	0.04^{cd}	0.06^{c}	0.009	<0.001	<0.001	<0.001
[4]$IVDMD_{VZ}$	0.21^{a}	0.17^{b}	0.22^{a}	0.16^{b}	0.21^{a}	0.17^{b}				

[1]STD- standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).

[2]$IVDMD_{PP}$ - Degradability of diet dry matter by pepsin, followed by pancreatin, according to Bindelle *et al.* (1997a).

[3]$IVDMD_{RX}$ – Partial degradability of the dry matter (after pepsin+pancreatin digestion) by Roxazyme® G2.

[4]$IVDMD_{VZ}$ – Partial degradability of the dry matter (after pepsin+pancreatin digestion) by Viscozyme 120 L.

[abc] For pepsin + pancreatin degradability coefficients, means in a row which do not share a common superscript are different ($p \leq 0.05$).

[abcd] Across the Roxazyme and Viscozyme degradability coefficients, means which do not share a common superscript are different ($p \leq 0.05$).

3.3.5 Fermentation properties

The fermentation gas production profiles of the fibre extracted from the ingredients and the diets are indicated in **Figure 3.1** and **Figure 3.2**, respectively. Based on a regression model forced through the origin, the algorithm used to predict gas production achieved stronger prediction of the final gas (Y, at t = ∞) from the gas produced at t = 64 hours for the feed ingredients (Y=2.37X; R^2=0.78), compared to the complete diets (Y = 1.89X; R^2 = 0.74).

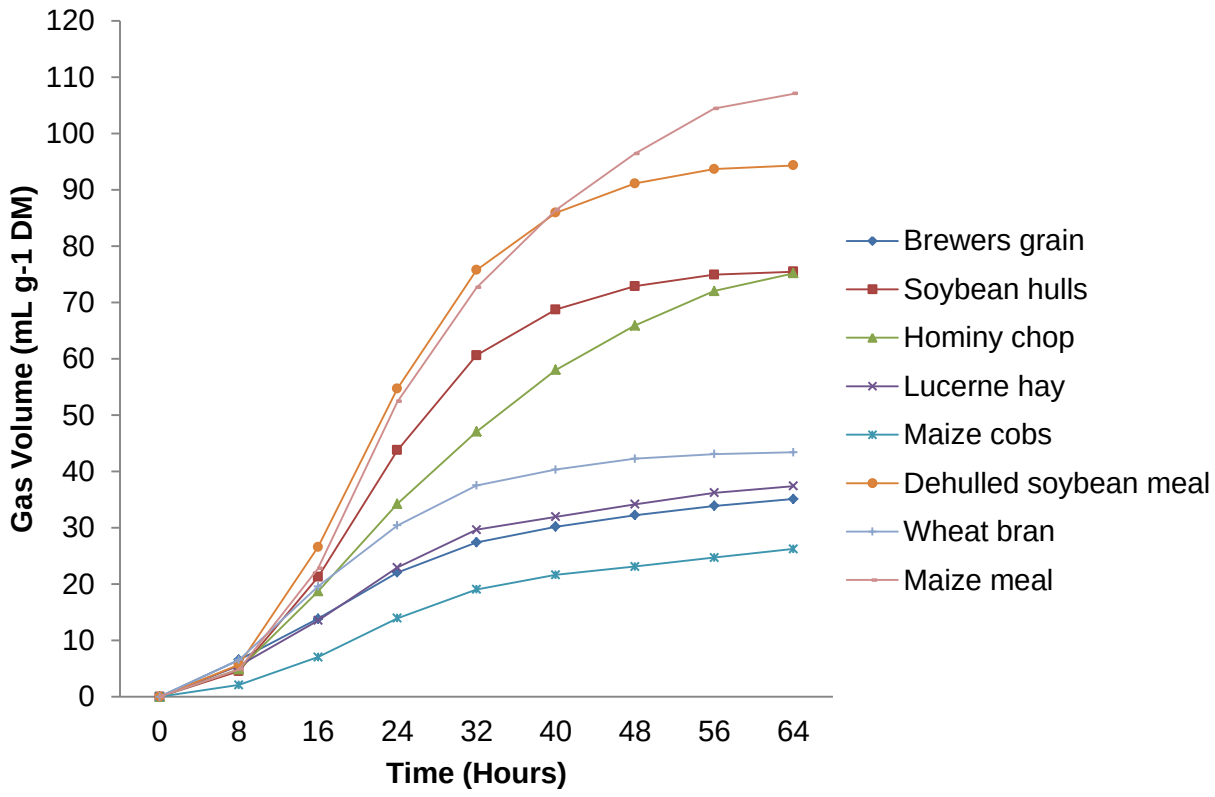


Figure 3.1: Gas production during fermentation by pig faecal bacteria of fibrous residues of pepsin, followed by pancreatin digestion of pig feed ingredients

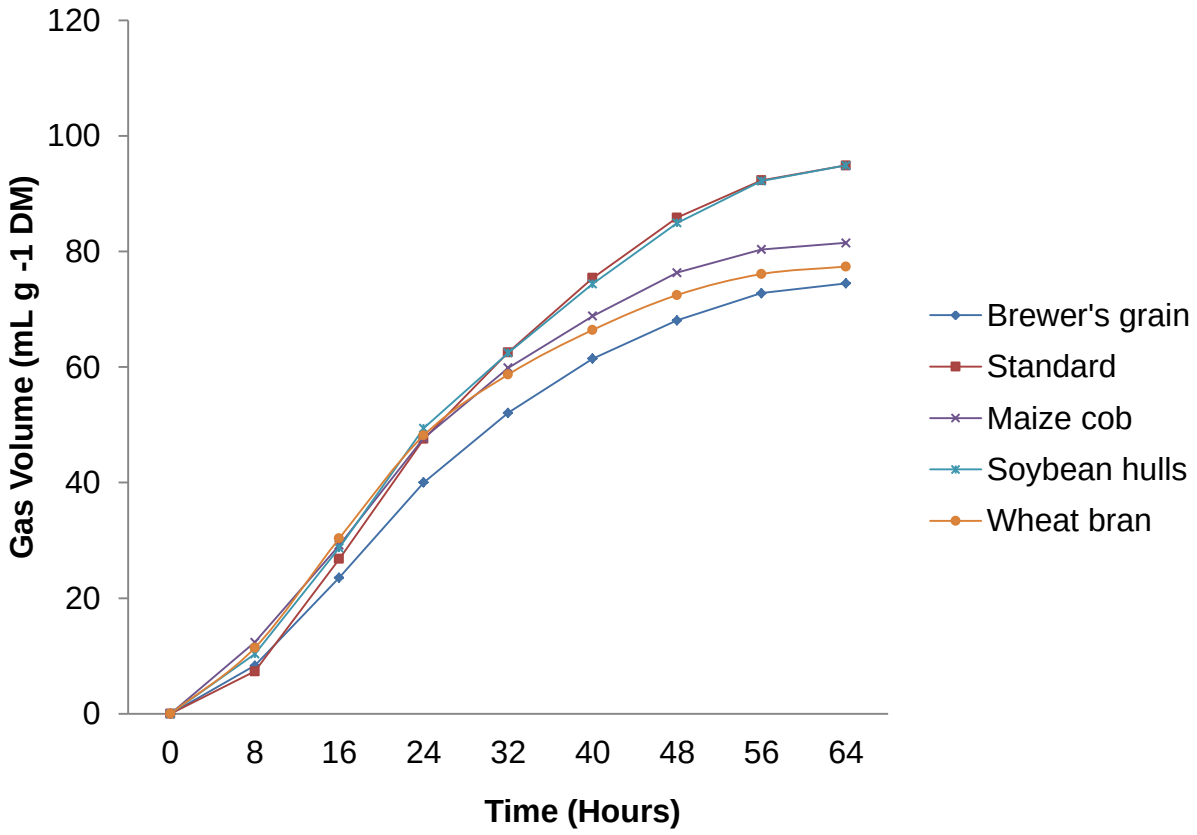


Figure 3.2: Gas production during fermentation by pig faecal bacteria of fibrous residues of pepsin, followed by pancreatin digestion of a standard, 141 g total dietary fibre kg^{-1} DM maize-soybean growing pig diets. In the fibrous diets, the basal ingredients were partially substituted with different feeds to contain 246 g total dietary fibre kg^{-1} DM

Fermentation parameters of the fibre extracted from the ingredients and the diets are indicated in **Table 3.6** and **Table 3.7**, respectively. Gas production differed by source of fibre among the feed ingredients and among the diets. There was greater variation in gas production among the feed ingredients, where maize and soybean fibres produced 3-4 times the gas produced by BG and maize cob fibres. The time taken to produce half the predicted gas volume was influenced by the source of fibre among the feed ingredients, but not among the diets. The source of fibre also influenced both the composition and the total (Ace, Pro, But, Iso-But, Val-a) SCFA concentration among the feedstuffs. Leguminous fibre tended to produce more percent Ace and less Pro and But. On the other hand, cereal fibre produced relatively high percent Pro and less Ace, and except for maize fibre, less But. Maize fibre had high Pro, low Ace and, except for maize cobs, high But.

Table 3.6 Fermentation characteristics of residues of pepsin+pancreatin digestion of common pig feed ingredients

Parameters		[1]Feed Ingredients								SEM	P Values
		BG	dSBM	HC	LH	MC	MM	SH	WB		
[2]Total SCFA (mMol g^{-1} DM)		2.3^{e}	6.0^{a}	4.3^{c}	2.4^{de}	2.3^{e}	5.3^{b}	4.5^{c}	2.9^{d}	0.29	<0.0001
SCFA (%)											
Acetate		51.4^{d}	53.9^{c}	51.3^{d}	61.0^{b}	59.6^{b}	48.8^{e}	66.7^{a}	51.7^{d}	1.21	<0.0001
Propionate		39.7^{a}	37.4^{ab}	35.8^{b}	32.0^{c}	32.0^{c}	35.4^{b}	26.1^{d}	38.8^{a}	0.88	<0.0001
Butyrate		7.3^{c}	7.0^{cd}	12.1^{b}	5.4^{e}	7.9^{c}	14.8^{a}	5.31^{e}	8.5^{c}	0.65	<0.0001
Iso-Butryrate		0.4^{c}	0.7^{ab}	0.3^{c}	0.8^{a}	0.2^{c}	0.3^{c}	0.5^{abc}	0.4^{c}	0.04	<0.0001
Valerate		1.2^{ab}	1.0^{bc}	0.7^{cd}	0.8^{cd}	0.3^{e}	0.7^{cd}	1.5^{a}	0.6^{de}	0.08	<0.0001
Ace:Pro:But ratio		52:40:08	55:38:07	52:36:12	62:33:05	60:32:08	45:39:16	72:23:05	52:39:09		
Gas production	*A*	61.3^{c}	299.4^{a}	173.5^{b}	65.2^{c}	51.8^{c}	245.2^{ab}	184.4^{ab}	67.6^{c}	18.11	<0.0001
	$T_{½}$	13.4^{b}	32.3^{a}	29.8^{ab}	18.5^{b}	30.1^{ab}	29.1^{ab}	20.6^{b}	12.7^{b}	2.95	<0.0001

[1]BG (brewer's grains), HC (maize hominy chop), LH (lucerne hay), MM (maize meal), MC (maize cobs), dSBM (dehulled soybean meal), SH (soy hulls), WB (wheat bran).

[2]Total short chain fatty acids (SCFA) - propionate+butyrate+Iso-butryrate+valerate.

[3]Ace - acetate; Pro - propionate; But - butyrate;

[4]Predicted using the monophasic model of Groot *et al.* (1996):

A - asymptotic gas volume (mL g-1 DM) at t = ∞.

T½ - time (Hours) to half asymptote when G = A/2.

[abc] Means within a row with different superscripts are significantly different (p≤0.05).

Table 3.7: Fermentation characteristics of pepsin+pancreatin digesta of high fibre, maize-soybean growing pig diets

Parameters		[1]Diet							
		STD	BG	LH	MC	SH	WB	SEM	P Values
[2]Total SCFA (mMol g^{-1} DM)		5.0	4.3	5.4	4.1	5.2	4.4	0.19	<0.05
SCFA (%)									
Acetate		45.4^{c}	48.6^{b}	51.0^{b}	47.5^{b}	53.0^{a}	50.0^{b}	0.63	<0.0001
Propionate		39.7^{a}	38.3^{ab}	36.1^{bc}	37.5^{ab}	34.8^{c}	37.8^{b}	0.41	<0.0001
Butyrate		13.5^{a}	11.6^{ab}	11.3^{b}	12.7^{ab}	10.7^{b}	10.9^{ab}	0.28	<0.01
Iso-Butyrate		0.4^{b}	0.4^{ab}	0.6^{a}	0.5^{ab}	0.4^{ab}	0.5^{ab}	0.03	0.22
Valerate		1.5	1.1	1.1	0.9	1.1	0.9	0.09	0.35
[3]Ace:Pro:But ratio		46:41:13	49:39:12	52:37:11	49:38:13	54:35:11	51:38:11		
[4]Gas production	*A*	205.3^{a}	126.6^{c}	155.2^{bc}	139.8^{c}	187.6^{ab}	141.0^{c}	6.36	<0.001
	$T_{½}$	25.2	14.2	19.3	17.8	23.4	21.8	1.03	<0.05

[1]STD - standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).

[2]Total short chain fatty acids (SCFA) = acetate + propionate + butyrate + iso-butyrate + valerate.

[3]Ace - acetate; Pro - propionate; But - butyrate.

[4]Predicted using the monophasic model of Groot *et al.* (1996):

A - asymptotic gas volume (mL g^{-1} DM) at t = ∞.

T½ - time (Hours) to half asymptote when G = A/2.

[abc] Means within a row with different superscripts are significantly different (p≤0.05).

Among the diets, SCFA production was not different, except for a tendency (p=0.069) towards higher SCFA in the LH diet, compared to the MC diet. Compared to the STD diet, the high fibre diets increased the proportions of Ace at the expense of Pro and But. Overall, due to the moderating effect of the highly fermentable maize and dehulled soybean fibres, there was less variation in fermentation parameters among the diets compared to the feed ingredients.

Short chain fatty acid production of the fibre extracted from the fibrous ingredients was correlated (r=0.89; p=0.045) to that of the diets in which they were used to increase DF. There was lower (r=0.550; p>0.337) correlation of the gas production. There was correlation (r=0.99; p <0.0001)) between SCFA and gas production among the feed ingredients, and not among the diets (r=0.67; p>0.05).

Different relationships were observed when the RX and VZ partial DM degradability were profiled in scatter plots against SCFA or gas production (**Figure 3**). Among the diets, correlation was significant (r = 0.99, p≤ 0.001) between the partial degradability of VZ and the SCFA production. The correlation was not significant for RX. Correlation of fibre degradability by enzymes to gas production was not significant for both the feed ingredients and the complete diets.

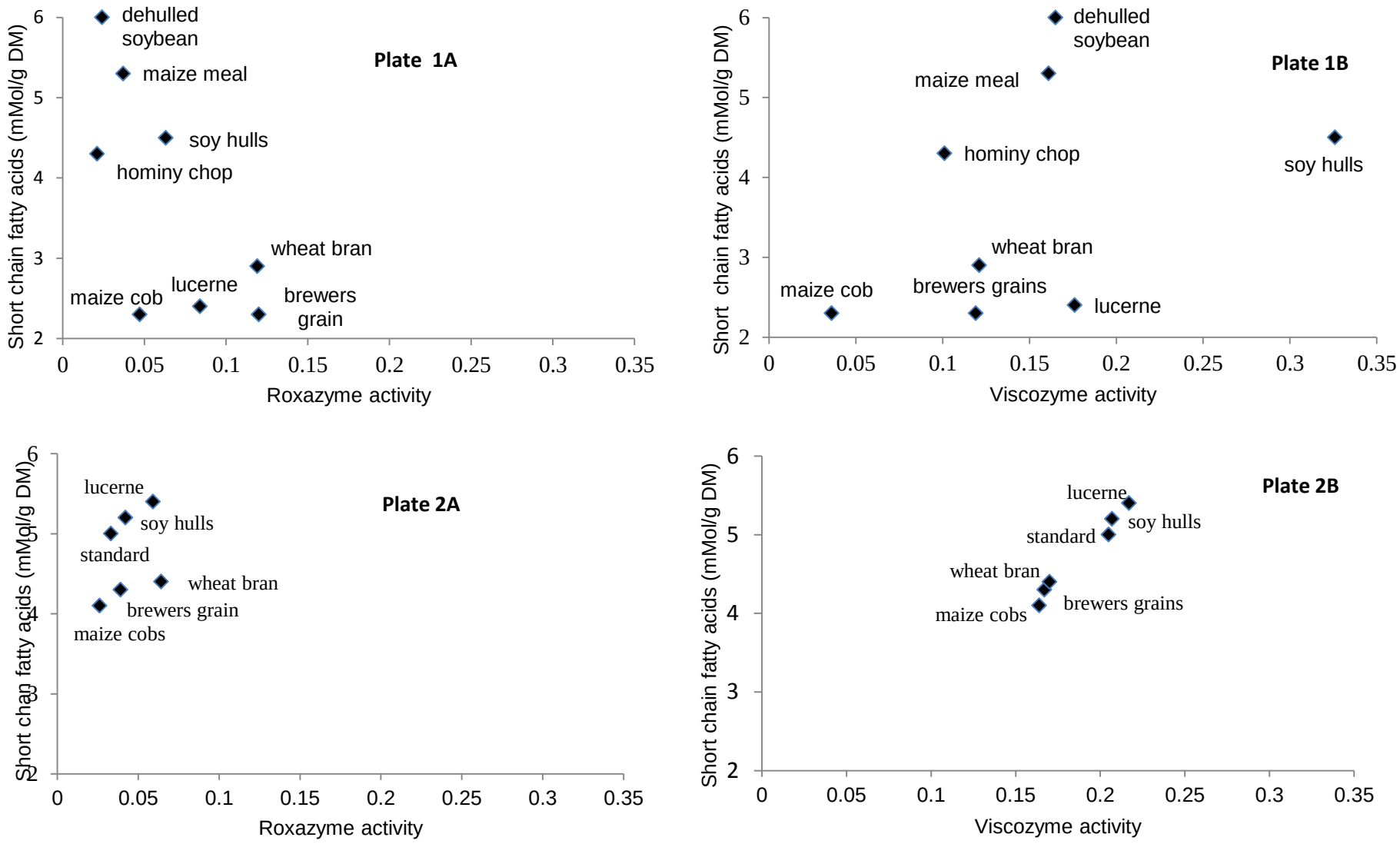


Figure 3.3: Relationships between Roxazyme and Viscozyme activity and short chain fatty acid production of fibre from feed ingredients (Plates 1A and 1B) and from high fibre, complete diets (Plates 2A and 2B).

3.4 Discussion

The dissimilar, source depended activities of VZ and RX on DF were attributed to differences in NSP specificities (Bedford and Cowieson, 2012). The low enzyme activities on maize fibre could be indicative of the presence of resistant starch (RS). Resistant starch is almost totally recovered in the filtered PP residues (Giuberti *et al.,* 2013).

Similar large variation in the fermentation properties of DF among and within feeds was previously reported (Bindelle *et al*, 2011; Jha *et al.,* 2011; Jha and Lerteme, 2012; Jonathan *et al*., 2012). The variation is attributed to differences in the NSP composition of DF (Bach Knudsen *et al.,* 2001). For example, cereal fibre consists mainly of arabinoxylans, β-glucans and cellulose, whereas legume fibre contains mainly pectins, cellulose and xyloglucan (McDougall *et al.,* 1996). Whereas NSP such as pectins and β-glucans are rapidly and practically totally fermented, insoluble cellulose and xylans are almost undigested (Noblet and Le Goff, 2001). In the present study, the low fermentability of brewer's grains and wheat bran was attributed to the concentration of the husk and pericarp, which typically contain the insoluble, lignified fibre. Chemical analysis of maize cobs and lucerne hay also showed that the fibre was largely insoluble, and for maize cobs, highly lignified. On the other hand, the high fermentability of the soybean fibre was attributed to the high content of pectin (McDougall *et al.,* 1996). Branching of the pectic polysaccharide chains provides numerous cleaving sites for enzymes (Jonathan *et al.,* 2012). The hulls contain relatively more insoluble, relatively slowly fermentable cellulose and xylans (de Vries, *et al.,* 2012) compared to the dehulled grain, hence were relatively less fermentable.

It is difficult to relate the observed profiles of fermentation SCFA to the NSP composition of DF, and to the likely gut microbial interactions. The general shift from Ace to Pro production in response to leguminous or high DF was previously associated with the presence of a diverse microbial population, in response to high substrate availability (Macfarlane and Macfarlane, 2003). Depending on the abundance of sNSP, a shift from Ace to But production by the maize meal and hominy chop fibres was previously associated with enhanced bacterial proliferation and a diverse microbial community (Jha *et al.,* 2011). In the

present study, the high But production by maize fibres could be attributed to RS, whose fermentation characteristically yields high But (Giuberti *et al.,* 2013; Jonathan *et al.,* 2013).

The fermentation test is premised on the assumption that *in vitro* gas and SCFA production predict *in vivo* degradation of of DF (Jonathan *et al.,* 2012; Chen *et al*., 2013). Interstingly, in the present study, there was strong correlation between the two parameters among the feed ingredients, but not among the diets. In feed ingredients, Jonathan *et al.* (2012) reported significant correlation in the early stages of fermentation, and not by the end of fermentation. They attributed the poor correlation to fermentation pathways which differently partition carbon into SCFA or gases. For example, lactate, which is metabolised early during fermentation to yield Pro, But and the gases, was detected after 72 hours of fermentation of oat β-glucans and inulin, and not in other substrates. CO_2 produced by bicarbonate buffering also depends on the quantitative and qualitative production of SCFA (Bindelle *et al.,* 2011). It is therefore important to determine the gas composition, and the intermediate metabolites during the course of the fermentation (Jonathan *et al.,* 2012).

Given strong prediction of the *in vivo* degradability of DF by the *in vitro* fermentation test (Chen *et al.*, 2013), the positive correlation of VZ activity to the SCFA production validated its prediction of the *in vivo* degradation of DF. The large variation in the fermentation characteristics of DF from different sources, and its expression in high fibre diets create scope to control fermentation through diet formulation. The *in vitro* methods used in this experiment are largely currently restricted to the characterisation of fibre in feed ingredients. There is need for further, *in vivo* evaluation to test whether the inclusion of high levels of insoluble fibre-rich ingredients, enzyme supplements and screening fibre sources for fibre fermentability can be effective tools to control fermentation, sufficiently to induce beneficial the digestive metabolic and physiological functions.

3.5 Conclusion

The influence of source of DF on enzyme activity and on its fermentation provides scope to formulate diets uniquely designed to induce desirable fermentation in the gut by screening

the fibrous feed ingredients based on the fermentation kinetics of the fibre, and by correctly matching the NSP compostion to NSPases. The use of VZ as the standard enzyme for *in vitro* pig feed digestibility studies was supported by the strong correlation of its activity with the fermentability of DF. The low activity of RX on maize and soybean fibres may result in low efficacy in pigs fed maize-soybean based diets. If validated in an *in vivo* model, the *in vitro* approach used in the study could be a practical method to match the fibre in complete diets to different enzyme cocktails, and to test combinations of different cocktails for a specific diet to exploit possible additive or synergistic enzyme action.

CHAPTER 4

EFFECTS OF FIBRE FERMENTABILITY AND ROXAZYME® G2 ON THE FERMENTATION CHARACTERISTICS OF ILEAL DIGESTA OF GROWING PIGS FED HIGH FIBRE, MAIZE-SOYBEAN BASED DIETS

Abstract

The study investigated the effects of formulating high fibre maize-soybean-coproduct growing pig diets for high DF fermentability, and the influence of RX, on the fermentation characteristics of the ileal digesta. The PP degradability and the fermentabilty of the washed PP digesta of the co-product feed ingredients as described in Chapter 3 were used to formulate two iso-nutritive, high fibre (319 g TDF) kg^{-1} DM) growing pig diets containing DF of high (HF) versus low (LF) fermentability. The *in vitro* digestion methods were similarly used to evaluate RX, VZ and combined VZ+RZ activities on DF, and its fermentation properties. The STD DF served as a control. The HF and LF diets were prepared in duplicate, and one mixture was fortified with 270 mg RX kg^{-1} of feed. Ileal digesta was collected during a feeding trial in which the four diets were fed *ad libitum* to eight intact male, ileal-cannulised, Large White X Landrace crossed growing pigs. The diets were allocated to the pigs in a duplicate 4 (period) x 4 (animal) Latin Square, with a 2 (enzyme) x 2 (fermentability) factorial arrangement of the treatments. The fermentation test was separately conducted on the ileal digesta. Viscozyme was more active ($p \leq 0.05$) than RX on the PP digesta of both the HF and LF diets. Combining VZ and RX increased ($p \leq 0.05$) the degradability of the PP digesta of the HF. The HF PP digesta produced similar ($P > 0.05$) gas and SCFA production to the STD PP digesta, and 65% more ($p \leq 0.05$) gas and 40% more ($p \leq 0.05$) SCFA than the LF PP digesta. The HF ileal digesta produced 40% more ($p \leq 0.05$) of both gas and SCFA. Compared to the STD, and for both the PP and ileal digesta, the HF and LF fibre shifted ($p \leq 0.05$) fermentation from Ace to Pro and But production, with greater ($p \leq 0.05$) effect by the HF DF. Roxazyme had no effect on the fermentation characteristics of the ileal digesta of

the HF and LF diets. Overall, the results supported the use of the *in vitro* methods to formulate fermentable insoluble fibre-rich, maize-soybean-mixed co-product diets, but did not justify the use of RX to control fermentation.

***Keywords**; growing pigs, fibre, enzymes, fermentability, maize-soybean*

4.1 Introduction

In growing pig nutrition, there is growing interest in targeting fermentable DF for its beneficial metabolic and physiological effects (Chen *et al.*, 2013). A fermentable fibre feeding strategy is yet to be fully developed for practical application in feeding fibrous, insoluble fibre-rich diets to growing pigs. To develop such a strategy, further research is critical to clarify and enable modelling (Zijilstra et al, 2010) of the complex interaction of animal, dietary, and microbial factors that control fermentation in the gut of pigs.

The key objective of a fermentable DF feeding strategy is to increase its flow to the lower gut (Reilly *et al.,* 2010; Jha *et al.,* 2011). Lower gut fermentation can be stimulated by adding purified fibres or sNSP-rich feeds to the diet (Zijlstra *et al.,* 2010; Martins *et al.,* 2010). Readily fermentable NSP known to stimulate and intensify fermentation in the gut of pigs include the soluble βGs and arabinoxylans typically present in relatively high concentrations in wheat, barley, rye or oat based diets (Reilly *et al.,* 2010; Jha *et al.,* 2010). However, the use of purified SDF is economically inefficient, and at high levels, viscous forming sNSP have deleterious effects on digestive function (Barletta, 2011). The alternative could be the cheap, insoluble, fermentable fibre rich co-product feeds.

Willamil *et al.* (2012) argued that improved performance by growing pigs in response to NSPases could be partly attributed to altered fermentation patterns that promote gut health. NSPases may stimulate fermentation through depolymerisation of the iNSP to highly fermentable oligosaccharides, or by disrupting the plant cell walls sufficiently to release bound or encapsulated nutrients (Choct and Cadogan, 2001; Smiricky-Tjardes *et al.,* 2003).

Screening co-product feeds to target fermentable DF, and matching the NSP composition to NSPases are complicated by the presence of chemically more diverse, complex iNSP which are variably and poorly degraded by exogenous and gut microbial enzymes (Jonathan *et al.*, 2012; Chen *et al.*, 2013). On the other hand, the applicability of current enzyme technology to control gut fermentation, and the overall impact of fermentation on pig performance remain unclear (Bedford and Cowieson, 2012).

The aim of the study was to evaluate the effectiveness of screening co-product feed ingredients based on *in vitro* quantified fermentation properties of insoluble DF, and the efficacy of RX, as tools to control the fermentation characteristics of digesta flowing into the lower gut of growing pigs fed high fibre maize-soybean diets.

4.2 Materials and methods

4.2.1 Experimental feeds

The feed ingredients described in Chapter 3 were used to formulate two iso-nutritive, high fibre (319 g total dietary fibre (TDF) kg^{-1} dry matter (DM)) growing pig diets (**Tables 4.1** and **Table 4.2)** that contrasted in the fermentability of the fibre component. To achieve the contrast in fermentability, the product of the coefficients of indigestibility by pepsin+pancreatin (PP) and the predicted asymptotic cumulative gas production from the fermentation of PP digesta of the feeds was used as an index of fermentability and was included in the least-cost diet formulation. The diets were balanced for essential nutrients according to the National Research Council (NRC) (1998).

4.2.2 *In vivo* digestion

The high fermentability (HF) and low fermentability (LF) diets were duplicated, and one mixture (HF+ and LF+, respectively) supplemented with 0.270 g RX kg^{-1} of feed. The diets were randomly allocated to eight Large White X Landrace pigs with T-cannula surgically inserted at the terminal ileum in a duplicate 4 by 4 (animal X period) Latin square design,

with a 2 (enzyme) x 2 (fermentability) factorial arrangement of the dietary treatments. The detailed experimental procedures for the feeding trial are described in Chapter 7.

4.2.3 *In vitro digestion*

The procedures used in simulating gastro-small intestinal digestion were as described in Chapter 3, section 3.2. The STD diet described in Chapter 3, section 3.2, Table 3.3 was similarly digested and used as the control in the fermentation tests. In addition to the separate RX and VZ digestion of PP digesta, a combined RX+VZ treatment was added. In each run of the PP digestion using the ANKOM Daisy^{II} Incubator, 24 sample bags containing the STD, HF an LF diets with 8 replicates per diet were placed in one of 4 digestion jars on the incubator.

4.2.4 Fermentation of dietary fibre

Fermentation characteristics of the PP digesta of the STD diet, the LF and HF diets, and of the ileal digesta of the HF, HF+, LF, LF+, including the gas production measurements and short chain fatty acid analyses were conducted as described in Chapter 3, section 3.3. In each fermentation run, the experimental scheme was as follows; two blanks + 2 duplicates of each diet or 2 blanks + 8 ileal digesta samples collected from the pigs in each period of *the vivo* trial), or ; two blanks + 2 duplicates of each of the diets.

4.2.5 Statistical analyses

Gas production kinetics were described using the monophasic model described in Chapter 3. ANOVA of the partial degradability of DM by PP ($IVDMD_{PP}$), RX ($IVDMD_{RX}$), VZ ($IVDMD_{VZ}$) and RX+VZ ($IVDMD_{RX+VX}$) were analysed using Mode ll, also as described in Chapter 3. Fermentation parameters of ileal digesta were analysed using model III;

$$\mathbf{Y_{ijkl}} = \mu + \boldsymbol{\alpha_i} + \boldsymbol{\beta_j} + \boldsymbol{\delta_k} + \boldsymbol{\gamma_l} + (\boldsymbol{\alpha\beta})_{\mathbf{ij}} + + \boldsymbol{\epsilon_{ijkl}} \qquad \textbf{Model III}$$

Where μ is the overall mean, α_i the effect of i^{th} fermentability, β_j the effect of the j^{th} RX level, δ_k the effect of the k^{th} animal, γl the effect of y^{th} run/period and $(\alpha\beta)_{ij}$ the interaction of the fermentability and RX, and e_{ijkl} the random error.

Comparison of means was performed using the LS MEANS and treatment means were separated using the Bonferroni t-test at the α-level of 0.05.

Table 4.1: Ingredient composition (%, as fed) of high fibre, maize-soybean and co-product growing pig diets contrasting in fermentability

	Diets	
Ingredients	High Fermentability	Low Fermentability
Dehulled soybean meal	32.2	28.0
Maize meal	28.2	27.3
Soy hulls	20.3	-
Maize cobs	5.7	10.8
Wheat bran	2.5	2.5
Brewer's grain	-	20.2
Hominy chop	6.5	6.5
DL methionine	0.2	0.2
Vitamin & mineral premix	0.2	0.2
Limestone flour	2.2	2.5
Mono-calcium phosphate	1.1	1.0
Salt	1.0	1.0

(-) Ingredient not added

Table 4.2: Chemical (g kg^{-1} DM) and energy composition of high fibre, maize-soybean growing pig diets

Component	High Fementability	Low Fermentability
Dry matter	921	926
Organic matter	918	921
Gross energy (MJ kg^{-1} DM)	17.1	17.7
Crude protein	226	227
Ash	82	79
Starch	286	278
Crude fibre	128	100
Neutral detergent fibre	301	337
Acid detergent fibre	187	163
Acid detergent lignin	22	27
Soluble dietary fibre	7	8
Insoluble dietary fibre	310	312
Total dietary fibre	318	320
Calcium	13.0	13.0
Phosphorus	7.0	6.0
Essential amino acids		
Arginine	13.0	13.7
Cysteine	0.4	10.9
Isoleucine	8.5	7.5
Leucine	12.7	12.4
Lysine	11.3	12.5
Methionine	6.6	4.5
Phenylalanine	9.9	8.8
Threonine	9.4	9.1
Tryptophan	3.0	3.5
Tyrosine	10.7	9.8

4.3 Results

The $IVDMD_{PP}$, $IVDMD_{RX}$, $IVDMD_{VZ}$ and $IVDMD_{RX+VX}$ are indicated in **Table 4.3**. VZ was more active (p≤0.05) in degrading the DF of both diets. Combining VZ and RX increased (p≤0.05) the degradability of DF of HF, and not of the LF diet.

Gas production profiles of the washed PP digesta and ileal digesta are indicated in **Figure 4.1** and **Figure 4.2,** respectively. The fermentation parameters are indicated in **Table 4.4** and **Table 4.5**, respectively. The T½ of the PP digesta was not affected by diet (p>0.05). T½ of the ileal digesta was also not affected by the diet, and by the enzyme (p>0.05). PP digesta of the HF and STD diets produced 65% and 75% more (p≤0.05) gas, and 40% and 50% more p≤0.05) short chain acids than the LF diet, respectively. Similarly, ileal digesta of the HF diet produced more (40%) gas and the same percentage of chain fatty acids than the LF diet. High level of DF and high fermentability resulted in a shift p≤0.05) from Ace to Pro and But production. NSPases had no effect on any of the measured fermentation parameters across all the substrates.

Table 4.3: Degradability coefficients of high fibre growing pig diets contrasting in fermentability

Partial degradability	[1]Diet		SEM	P Values		
	High Fermentability	Low Fermentability		Diet	Enzyme	Diet*Enzyme
[2]$IVDMDM_{PP}$	0.60[a]	0.55[b]	0.005	<0.001		
[3]$IVDMD_{RX}$	0.04[c]	0.07[c]				
[4]$IVDMD_{VZ}$	0.20[b]	0.17[b]	0.013	<0.01	0.001	0.001
[5]$IVDMD_{RX+VZ}$	0.25[a]	0.18[b]				

[1]Growing pig diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.
[2]$IVDMD_{PP}$ - Degradability of diet dry matter by pepsin, followed by pancreatin digestion, according to Bindelle *et al.* (1997).
[3]$IVDMD_{RX}$ - Partial degradability of dry matter (after pepsin+pancreatin digestion) by Roxazyme® G2.
[4]$IVDMD_{VZ}$ - Partial degradability of dry matter (after pepsin+pancreatin digestion) by Viscozyme 120 L.

[5]$IVDMD_{RX+VZ}$ - Partial degradability of dry matter (after pepsin+pancreatin digestion) by Roxazyme G2 and Viscozyme 120 L

[abc]For the pepsin+pancreatin digestion, means within the row with different superscripts are different ($p \leq 0.05$).

[abc]Across the Roxazyme, Viscozyme and Roxazyme+Viscozyme treatments, means which do not share a common superscript are different ($p \leq 0.05$).

Table 4.4: Fermentation characteristics of pepsin+pancreatin digestion residues of a standard and high fibre growing pig diets contrasting in fermentability

Parameters	[1]Diet				P Value
	Standard	High Fermentability	Low Fermentability	SEM	Diet
[2]Total SCFA (mMol g^{-1} DM)	5.4[a]	5.0[a]	3.6[b]	0.26	<0.02
[2]SCFA (%)					
Acetate	47.5[c]	55.2[a]	50.5[b]	0.96	<0.0001
Propionate	38.2[a]	33.8[b]	37.2[ab]	0.67	<0.01
Butyrate	12.6[a]	9.5[b]	10.9[ab]	0.42	<0.01
Iso-Butyrate	0.5	0.5	0.5	0.03	0.82
Valerate	1.3	1.1	1.0	0.1	0.44
[3]Ace:Pro:But ratio	56:34:10	51:38:11	48:39:13		
[4]Gas production					
A	173.8[a]	159.5[a]	96.6[b]	9.57	<0.0001
$T_{½}$	23.2	27.4	29.8	1.99	0.36

[1]Standard, 141 g total dietary fibre kg^{-1} DM diet, and 319 g total dietary fibre kg^{-1} DM diets formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.
[2]Total short chain fatty acids (SCFA) = acetate + propionate + butyrate + iso-butyrate + valerate.
[3]Ace - acetate; Pro - propionate; But – butyrate.
[4]Predicted using the monophasic model of Groot *et al.* (1996):
A - asymptotic gas volume (mL g-1 DM) at t = ∞.
T½ - time (Hours) to half asymptote when G = A/2.
[abc]Means within rows with different superscripts are significantly different (p≤0.05).

Table 4.5: Fermentation characteristics (N=4) of ileal digesta of growing pigs fed high fibre pig diets contrasting in fermentability

Parameters	[1]Diet				SEM	P Value				
	HF (+)	HF	LF (+)	LF		Period	Animal	Diet	Enzyme	Diet*Enzyme
Total [2]SCFA (mMol g^{-1} DM)	6.4[a]	5.7[a]	4.4[b]	4.4[b]	0.27	<0.0001	0.42	<0.0001	0.29	0.48
[2]SCFA (%)										
Acetate	58.6[ab]	60.1[a]	56.9[b]	57.3[ab]	0.85	<0.001	0.39	<0.01	0.13	0.41
Propionate	30.2[ab]	29.1[b]	31.8[a]	31.4[ab]	0.61	<0.001	0.17	<0.01	0.15	0.48
Butyrate	8.2	7.9	8.3	8.3	0.19	<0.01	0.94	0.32	0.56	0.65
Iso-Butyrate	0.9	1.0	0.9	0.9	0.04	<0.05	0.11	0.31	0.59	0.84
Valerate	2.2	2.0	2.2	2.1	0.02	<0.0001	0.39	0.48	0.32	0.24
[3]Ace:Pro:But ratio	61:31:08	62:30:08	59:33:08	59:32:09						
[4]Gas production										
A	67.1[a]	63.7[a]	46.5[b]	46.8[b]	2.35	<0.0001	0.08	<0.0001	0.14	0.18
$T_{½}$	6.6	9.2	7.6	7.0	0.43	<0.05	0.10	0.39	0.16	<0.05

[1]Diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF), each with (+) or without (-) 0.270 g kg^{-1} (as fed) Roxazyme® G2.

[2]Total short chain fatty acids (SCFA) = acetate + propionate + butyrate + iso-butyrate + valerate.

[3]Ace - acetate; Pro - propionate; But – butyrate.

[4]Predicted using the monophasic model of Groot *et al.* (1996):

A - asymptotic gas volume (mL g-1 DM) at t = ∞.

T½ - time (Hours) to half asymptote when G = A/2.

[abc]Means within rows with different superscripts are significantly different (p≤0.05).

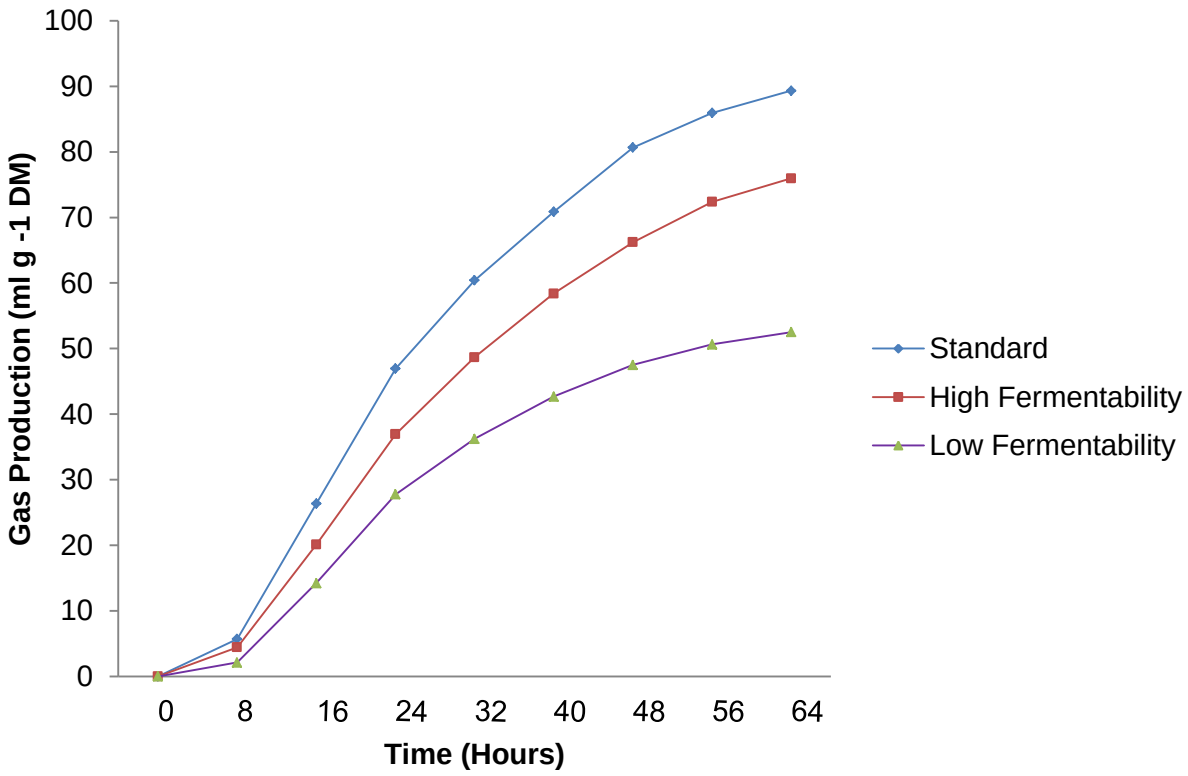


Figure 4.1: Gas production during fermentation by faecal bacteria of residues of pepsin, followed by pancreatin digestion of 319 g total dietary fibre kg^{-1} DM growing pig diets formulated to contrast in the fermentability of dietary fibre. The (+) indicates the diets with 0.270 g kg^{-1} (as fed) of Roxazyme® G2

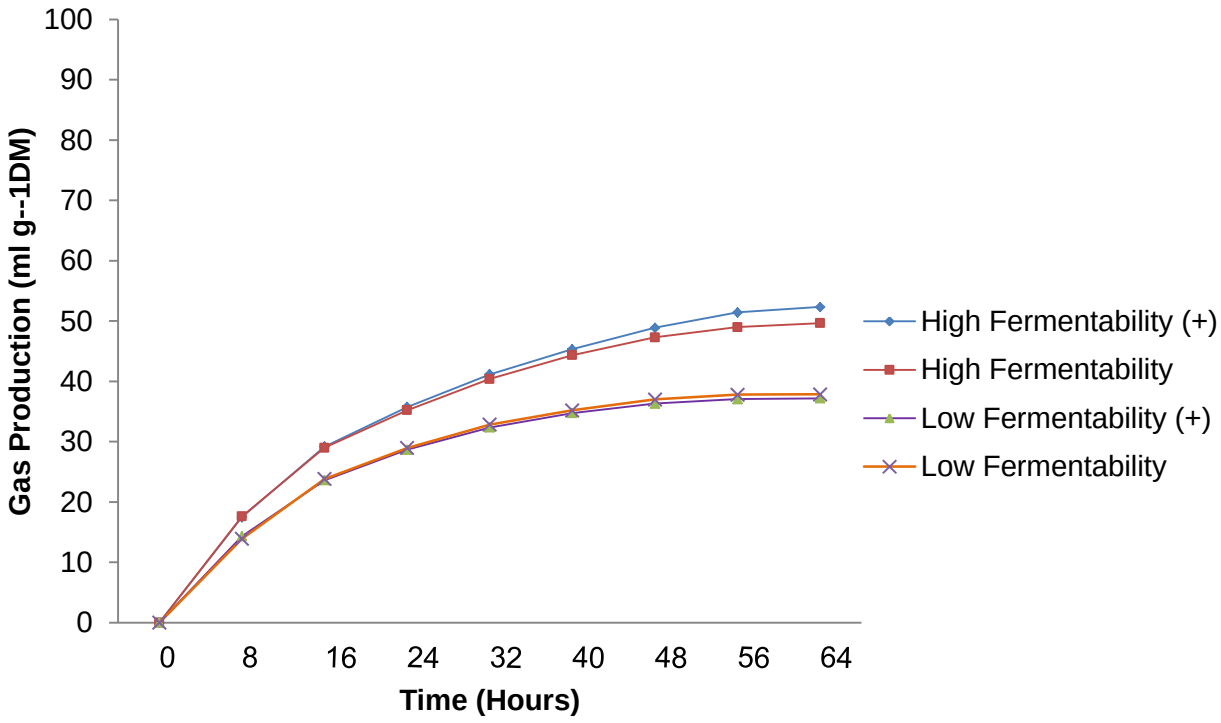


Figure 4.2: Gas production during fermentation by faecal bacteria of ileal digesta of 319 g total dietary fibre kg^{-1} DM growing pig diets formulated to contrast in the fermentability of dietary fibre. The (+) indicates the diets with 0.270 g kg^{-1} (as fed) of Roxazyme® G2

4.4 Discussion

Diets formulated to contrast in fermentation properties using the fermentability of washed PP digesta expressed the predicted relative fermentabilty of the ileal digesta, with similar SCFA composition between the *in vitro* and *in vivo* digesta of the diets. This was contrary to Chen *et al.* (2013), who argued that the *in vitro* fibre extraction method may yield material different to ileal chyme, due to endogenous secretion, and due to the loss of soluble DF. The higher fermentability of the PP, compared to the ileal digesta was explained by the buffering effects (Bindelle *et al.,* 2007) of endogenous mucins (Libao-Mercado *et al.,* 2007).

In the present study, compared to a STD diet, increasing DF to twice the STD DF using high fermentabilty fibre did not depress DF fermementability, but altered the molar ratios of the SCFA. This suggests that when feed intake is not limited by the DF, total fermentation in

the gut will be increased. Potential metabolic and physiological impacts of the increased fermentation and the altered SCFA composition need to be confirmed *in vivo*.

The lack of RX effect on the fermentation of ileal digesta was contrary to *in vitro* observations by Bindelle *et al.* (2011). The later included enzymes in the gastric-small intestinal phase of the *in vitro* assay, with subsequent tests on the effect on fermentation. They reported significant, source-dependent influences of the enzymes on fermentation patterns. Research in poultry suggests that the depolymerisation of NSP in the upper digestive tract of pigs is not complete. It is likely limited to chemically heterogeneous mixtures of oligosaccharides, whose composition and physical properties depend on the enzyme and on the fibre source (Choct, 2006). The implications of the alteration of physico-chemical properties of the DF need further investigation. For instance, *in vitro*, enzymes reduced the water holding capacity, but increased the viscosity of NSP (Courtin and Delcor, 2001). In the literature, the extent to which NSPases can exert significant biological effect in the upper tract of pigs is unclear. In this study, although the low *in vitro* activity of RX reported in chapter 3 may largely explain the lack of effect of the RX *in vivo*, the *ad libitum* feeding possibly accelerated digesta transit in the stomach and in the small intestines (Wilfart *et al.,* 2007; Solà-Oriol *et al.,* 2010). The digesta flow rate may limit the action of NSPases (Svihuis, 2011).

Indications of possible additive or synergistic effects when the different RX and VZ were combined to digest the HF diet suggest that, subject to cost, and depending on the diet, there could be benefit if Roxazyme ® G2 is cocktailed with complimentary gut NSPases. It is noteworthy that current enzymes were developed targeting the viscous forming NSP of barley, wheat, oat or rye and soybean based diets (Bedford, 2000). Multipotent enzymes are needed to target insoluble fibre, with the objective to enhance the fermentation of the fibre, while limiting possible deleterious changes in other physiologically important physico-chemical properties.

4.5 Conclusion

Similar fermentation patterns between the PP and ileal digesta of different diets justified the use of PP in place of ileal digesta in the fermentation test. The findings suggested the efficacy of RX in stimulating fermentation in the gut of pigs fed maize-soybean based diets could be limited. Overall, the results supported the use of the *in vitro* methods to enhance fermentation in the gut of growing pigs fed IDF rich, maize-soybean-mixed co-product diets.

CHAPTER 5

PERFORMANCE OF GROWING PIGS FED HIGH-FIBRE, MAIZE-SOYBEAN DIETS SUPPLEMENTED WITH ROXAZYME® G2

Abstract

Growth and slaughter performance were examined in growing pigs fed the diets described in Chapter 3. Each diet was duplicated and 200 mg RX kg^{-1} feed were added to one of the mixtures. Seventy-two intact Large White X Landrace male (32.0 ± 5.6 kg live weight (LW)) pigs were allocated to the 12 diets in two complete randomised weight blocks in a 2 (fibre source) X 2 (enzyme) factorial arrangement. The pigs were fed a*d libitum* for approximately 10 weeks. Cumulative live weight gain and feed intake were measured at 50, 70, 80 and 95 kg mean LW. Digestibility was estimated at 65-70 kg LW, using chromium oxide (200 g kg^{-1} DM) as an indigestible marker. Compared to the control-fed animals, high fibre reduced ($p \leq 0.05$) daily feed intake during the period up to 50 kg LW for the MC, and up to slaughter for the LH diet. Compared to the control, high fibre reduced ($p \leq 0.05$) the apparent digestibility of DM, OM and GE (all the high fibre diets), CP (all diets except MC), NDF (BG, LH) and ADF (MC, LH, WB). The apparent digestibility of ash (MC, SH), CP (MC), P (BG), Fat (L, BG) and ADF (SH) was higher ($p \leq 0.05$) in the fibrous diets. High fibre (LH) reduced live weight gain ($p \leq 0.05$) during growth to slaughter. MC increased ($p \leq 0.05$) the feed:gain ratio during growth up to slaughter. Pigs on the high fibre diets had similar slaughter performance to control-fed animals, except for lower ($p \leq 0.05$) dressing per cent of pigs on the LH and higher ($p \leq 0.05$) lean per cent of pigs on the LH and WB diets. No significant differences were observed on nutrient digestibility, growth and slaughter performance for RX. Across the dietary treatments, fermentability of DF was positively correlated with feed intake ($p \leq 0.05$), and to weight gain ($p \leq 0.01$) during growth to 50 kg live weight. Fibre solubility ($p \leq 0.05$), swelling ($p \leq 0.05$) and water binding ($p \leq 0.01$) capacity were negatively correlated with feed intake below 50 kg live weight. Solubility of DF was also negatively correlated weight gain and feed: gain ratio at all growth stages ($p \leq 0.01$). Fermentability was

positively correlated (p≤0.001) to the digestible DM, OM, energy, ADF and NDF, and to fat (p≤0.05). Swelling was negatively correlated with the digestible DM (p≤0.05) and to OM, energy and protein (p≤0.01), with a positive correlation (p≤0.05) to the digestible ash and P. Solubility was negatively correlated with the digestible DM, OM (P≤0.01), protein and to ADF and NDF (p≤0.001), with positive correlation to the digestibility of fat (p≤0.001). Water binding capacity was negatively correlated with the digestible DM and OM (p≤0.01), energy (p≤0.05) and NDF (p≤0.001). Water binding capacity was also positively correlated with the digestibility of fat (p≤0.001). Depending on the source of fibre, productivity was maintained in growing pigs fed maize-soybean-grain processing co-product diets containing up to twice the STD level of DF. Supplementing RX to growing pigs on such diets was not justified.

Key words: *enzymes, fermentability, fibre, grain-processing co-products, growing pigs, , maize-soybean diets, non-starch polysaccharides, Roxazyme G2*

5.1 Introduction

Currently, economic, anthropological and environmental factors favour the partial substitution of the cereal and legume grains in conventional growing pig diets with their cheaper, but typically fibrous co-products from agro-processing and biofuel production (Zijlstra and Beltranena, 2010). However, the generally low and highly variable nutritive value of these feedstuffs (Zijlstra and Beltranena, 2013) limit their usage in modern, high precision, high productivity pig production systems. Reviews by Noblet and Le Goff (2001), Aarnink and Verstegen (2007) and Bindelle *et al.* (2008) showed that generally, depending on the source and level to which fibre is included in the diet, reduced nutrient digestibility and restriction of feed intake limit nutrient, particularly energy intake, resulting in poor growth, slaughter performance, and may affect pork quality (Zijlstra and Beltranena, 2013).

The challenge is therefore to ensure cost-effective, predictable growth performance with minimal environmental footprint. However, the mechanisms that underpin the metabolic and physiological activity of fibre in the gut of pigs are not yet understood sufficiently to enable producers to formulate diets for optimum production, economic and environmental outcomes. The difficulty in predicting the effects of fibre on digestive function is a

consequence of the complex three-way interaction of the type of fibre, the responses of the animal and the microbial system resident in the lower gut (Williams *et al.,* 2001). The digestive metabolic and physiological responses of growing pigs to DF are largely controlled by the interactive effects of the shared properties of fermentability, viscosity, adsorption, and hydration (solubility, swelling and water binding capacity) (Bindelle *et al.,* 2008). These properties are extremely variable and primarily reflect the heterogeneous secondary and tertiary structures of the constituent non-starch polysaccharides (NSP) in plant matter, and secondarily, the variable plant cell wall architecture and molecular associations such as lignification (Bach Knudsen, 2001).

The use of NSPase technology is intended to improve the nutritive value of fibrous feedstuffs, thereby expanding the range of usable feeds, and enabling higher levels of inclusion in diets. However, in growing pigs, the efficacy of NSPases is questioned, particularly on maize-soybean diets (Barletta, 2011; Kerr and Shurson, 2013). The inconsistency in findings can be attributed to the difficulty in matching NSPases to the NSP (Zijlstra *et al.,* 2010) and to gut conditions and rheology (Barletta, 2011), which depend on factors such as type of fibre, level of fibre and animal age (Ji *et al.,* 2008), and processing (De Vries *et al.,* 2012). For example, due to the lack of adaptation by the microbial and the animal digestive system to fibre (Noblet and Le Goff, 2001), NSPases are more likely to have greater effect on nutrient digestion and growth performance in young pigs. In addition, due to high concentration of viscous non-polysaccharides (Bach Knudsen *et al.,* 2001), there is greater effect in wheat, barley, rye and oat diets, as opposed to maize based diets (Ji *et al.,* 2008; Willamil *et al.,* 2012).

The aim of the research was to examine nutrient digestibility, growth and slaughter performance of pigs fed maize-soybean based diets containing high levels of different fibrous feedstuffs and supplemented with Roxazyme® G2, in relation to the physicochemical properties of the fibre.

5.2 Methods and Materials

The feeding experiment was conducted at the Animal Production Institute of the Agricultural Research Council. The experimental procedures used for the care and treatment of the animals were approved by the ethics Committees of both the University of South Africa and the ARC.

5.2.1 Experimental diets

The diets and their nutritional characterises were described in Chapter 3, section 3.2., **Tables 3.1-3.3**. To prepare the bulk diets, the ingredients were hammer- milled through a 5 mm screen. Diets were then mixed in half-tonne lots in a vertical mixer. The mixing cycle was 15 minutes. Each diet was prepared in duplicate, one mixture with 200 mg RX kg^{-1} feed (as is) (RX+), and the other without (RX-).

Swelling and water binding capacity were determined following the procedures described by Canibe and Bach Knudsen, (2001), with modifications. For both measurements, triplicate 300 mg feedstuff or diet samples were weighed into centrifuge tubes into which were added 2 mL of an aqueous solution containing 9 g L^{-1} NaCl and 0.2 g L^{-1} NaN_3. The samples were incubated overnight in a shaking water bath at 39 °C. Swelling capacity was measured as volume occupied by the feedstuff expressed as mL g^{-1} DM feed. To determine water-binding capacity, the soaked samples were centrifuged at 4000 X g for 20 minutes at 25 ^{o}C. The supernatant was removed and the tubes were turned upside down to drain for 20 minutes and weighed. The samples were dried in an oven at for 48 hours at 103 ^{o}C and weighed. Water binding capacity was calculated as the water retained in g g^{-1} DM feed. The water binding and swelling capacities of the feeds are shown in **Table 5.1.**

Table 5.1: Water binding (g g^{-1} DM) and swelling capacity (mL g^{-1} DM) of the feed ingredients and growing pig diets

Feed	[1]Swelling capacity	[2]Water binding capacity
Feed ingredients		
Brewer's grains	4.7	3.2
Hominy chop	5.5	1.8
Lucerne	8.9	4.6
Maize cobs	7.9	4.6
Maize meal	4.0	1.7
Dehulled soybean meal	5.9	3.0
Soy hulls	6.7	3.8
Wheat bran	5.8	2.4
Diets		
Standard	3.7	1.7
Brewer's grains	5.2	2.1
Lucerne hay	4.7	2.4
Maize cobs	4.2	2.0
Soyhulls	4.7	2.2
Wheat bran	3.7	2.2

[1]Swelling capacity by the bed-volume technique, determined according to Canibe and Bach Knudsen (2001), with modifications.
[2]Water binding by centrifugation, according to Canibe and Bach Knudsen (2001), with modifications

5.2.2 Animal management and experimental design

Seventy-two weaned, intact male Large White X Landrace pig crosses were selected from the station herd. Pigs were weaned at 4 weeks and fed a commercial weaner diet for 2 weeks before being put onto a standard commercial grower diet. At 32.0 ± 5.6 kg live weight (LW), the pigs were allocated to the 12 experimental diets in two completely randomised weight blocks, in a 2 (fibre source) X 2 (enzyme) factorial arrangement. The animals were randomly allotted to individual steel crates measuring 75 cm high, 88 cm wide, 150 cm long and placed 25 cm above a concrete floor. Feed was offered *ad libitum* from self-feeders, and water was continuously available from nipple drinkers. The pigs were slaughtered at age 155 ± 3.5 days.

5.2.3 Pig performance measurements

Live weight gain and feed intake were measured at 3, 6 and 8 weeks into the experiment, and at slaughter (approximately 60, 70, 80 and 95 kg live weight), respectively. To estimate digestibility, chromium oxide was included in the diets at 200 mg kg^{-1} (as fed). The chromium diet was fed for seven days prior to and during sample collection. Faecal samples were collected over five days at 65-70 kg live weight. Faeces were collected from each pig on each day between 06:00 and 12:00 and placed in a deep freezer at -18 ^{o}C. At the end of the collection, the samples were pooled and freeze-dried for chemical analyses. Dressing per cent was calculated from the hot dressed carcass. Lean, fat (per cent) and meat colour were determined using a Hennessey® probe, after the carcasses were chilled at 4 ^{o}C for 24 hours. Measurements were taken on the chilled carcass at a point 60 mm from the mid line, between the 3rd and 4th ribs from the last rib, on the left side.

5.2.4 Statistical Analysis

ANOVA of the ATTD of nutrients, growth and slaughter performance indices was conducted using the PROC MIXED procedures of SAS software, version 9.3 (SAS, 2010) using model II as described in Chapter 3, with the inclusion of the initial pig live weight weight as a covariate.

Comparison of means was performed using the Bonferroni t-test at the α-level of 0.05. Values of $0.05<p<0.1$ were considered tendencies towards significance.

Pearson's product-moment correlation coefficients between the physicochemical properties of the diets and the digestibility coefficients and growth parameters and the associated probabilities were calculated using the PROC CORR statement of SAS software, version 9.3 (SAS, 2010).

5.3 Results

Nutrient digestibility, growth and slaughter performance indices are presented in **Table 5.2, Table 5.3 and Table 5.4**, respectively. Correlation coefficients between the physico-chemical properties of the fibre in the diets and the nutrient digestibility coefficients and growth performance measures are indicated in **Table 5.5** and **Table 5.6,** respectively.

5.3.1 Growth and slaughter performance

Compared to the control-fed animals, high fibre reduced (p≤0.05) daily feed intake during the period up to 50 kg LW for the MC diet, and up to slaughter for the LH diet. High fibre also reduced (p≤0.05) the apparent digestibility of DM, OM and GE (all high fibre diets), and of CP (all diets except MC), NDF (BG, L) and ADF (MC, L, WB). High fibre increased (p≤0.05) the apparent digestibility of ash (MC, SH), CP (MC), P (BG), Fat (L, BG) and ADF (SH). High fibre reduced daily gain (p≤0.05) in pigs on the L diet during growth to slaughter. MC increased the feed:gain ratio during growth up to slaughter. Pigs on the high fibre diets had similar slaughter performance to control-fed animals, except for lower dressing percentage of pigs on the L and higher lean percentage of pigs on the L and WB diets. RX did not affect (p>0.05) nutrient digestibility, growth and slaughter performance.

5.3.2 Correlations of performance measures to the physicochemical properties of dietary fibre

Fermentability of DF was positively correlated with feed intake (p≤0.05), and to weight gain (p≤0.01) to 50 kg live weight. Both swelling (p≤0.05) and water binding (p≤0.01) capacity were negatively correlated with feed intake below 50 kg live weight. Solubility of DF was negatively correlated with feed intake of pigs up to 50 kg (p≤0.05) and to all other parameters at all growth stages (p≤0.01).

Fermentability was positively correlated (p≤0.001) with digestible DM, OM, energy, ADF and NDF, and to fat (p≤0.05). Swelling was negatively correlated with digestible DM (p≤0.05) and with OM, energy and protein (p≤0.01). There was a positive correlation (p≤0.05) with

digestible ash and P. Solubility was negatively correlated with digestible DM, OM ($P \leq 0.01$), protein and with ADF and NDF ($p \leq 0.001$). There was a positive correlation with the digestibility of fat ($p \leq 0.001$). Water binding capacity was negatively correlated with digestible DM and OM ($p \leq 0.01$), energy ($p \leq 0.05$) and NDF ($p \leq 0.001$). Water binding capacity was also positively correlated with the digestibility of fat ($p \leq 0.001$).

Table 5.2: Apparent total tract digestibility coefficients of chemical components and energy by [1]pigs fed maize-soybean based diets containing high levels of different fibrous feedstuffs and supplemented with Roxazyme® G2

Component	[2]Diets						[3]Enzyme			P Value		
	STD	BG	LH	MC	SH	WB	-	+	SEM	Diet	Enzyme	Enzyme X Diet
Dry matter	0.85^{a}	0.80^{c}	0.82^{bc}	0.82^{bc}	0.83^{ab}	0.81^{bc}	0.82	0.82	0.005	<0.01	0.77	0.31
Organic matter	0.87^{a}	0.81^{b}	0.84^{b}	0.83^{b}	0.85^{b}	0.83^{b}	0.84	0.84	0.045	<0.01	0.88	0.28
Gross energy	0.85^{a}	0.79^{b}	0.81^{b}	0.81^{b}	0.82^{b}	0.81^{b}	0.81	0.81	0.005	<0.01	0.98	0.35
Crude protein	0.85^{ab}	0.81^{b}	0.83^{b}	0.87^{a}	0.81^{b}	0.84^{b}	0.83	0.83	0.005	<0.01	0.86	0.26
Crude fat	0.55^{b}	0.60^{b}	0.76^{a}	0.579^{b}	0.57^{b}	0.71^{a}	0.62	0.62	0.015	<0.01	0.94	0.70
Ash	0.61^{b}	0.63^{b}	0.66^{ab}	0.68^{a}	0.70^{a}	0.61^{b}	0.65	0.64	0.009	<0.01	0.32	0.40
P	0.54^{bc}	0.62^{a}	0.58^{abc}	0.64^{c}	0.60^{ab}	0.52^{c}	0.58	0.59	0.013	0.02	0.92	0.26
NDF	0.78^{a}	0.68^{b}	0.67^{b}	0.74^{a}	0.80^{a}	0.67^{b}	0.72	0.73	0.010	<0.01	0.76	0.49
ADF	0.59^{b}	0.49^{bc}	0.43^{c}	0.48^{c}	0.62^{a}	0.325^{d}	0.50	0.49	0.020	<0.01	0.90	0.19

[1]Male, intact, Large White X Landrace pigs. Digestibility was evaluated at 65-70 kg live weight.

[2]STD- standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).

[3]Roxazyme® G2.

[ab] Means within a row with different superscripts are significantly different (p≤0.05.

Table 5.3: Growth performance of [1]pigs fed maize-soybean diets containing high levels of different fibrous feedstuffs and supplemented with Roxazyme® G2

Mean live weight	[2]Diets						[3]Enzyme			P Value		
	STD	BG	LH	MC	SH	WB	-	+	SEM	Diet	Enzyme	Enzyme X Diet
	Feed intake (kg DM/day)											
50	1.912^{a}	1.686^{b}	1.611^{b}	1.800^{ab}	1.863^{ab}	1.738^{ab}	1.753	1.794	0.456	0.02	0.43	0.77
70	2.018^{ab}	1.966^{ab}	1.867^{b}	2.174^{a}	2.034^{ab}	1.952^{ab}	1.984	2.028	0.435	0.03	0.37	0.51
80	2.366^{ab}	2.337^{ab}	2.226^{b}	2.600^{a}	2.409^{ab}	2.287^{ab}	2.362	2.388	0.480	0.02	0.63	0.47
95	2.443^{ab}	2.464^{ab}	2.314^{b}	2.721^{a}	2.561^{ab}	2.356^{b}	2.462	2.500	0.511	<0.01	0.48	0.60
	Weight gain (kg per day)											
50	0.893^{ab}	0.745^{b}	0.742^{b}	0.740^{b}	0.896^{a}	0.855^{ab}	0.798	0.825	0.017	<0.01	0.42	0.29
70	0.902^{ab}	0.827^{ab}	0.797^{b}	0.833^{ab}	0.918^{a}	0.895^{ab}	0.842	0.881	0.012	<0.01	<0.05	0.71
80	0.859^{ab}	0.836^{ab}	0.785^{b}	0.866^{ab}	0.920^{a}	0.896^{a}	0.842	0.881	0.012	<0.01	0.04	0.94
95	0.834^{a}	0.813^{ab}	0.753^{b}	0.818^{ab}	0.860^{a}	0.854^{a}	0.809	0.835	0.009	<0.01	0.16	0.47
	Feed Conversion Ratio											
50	2.175^{ab}	2.269^{ab}	2.176^{ab}	2.430^{a}	2.135^{ab}	2.018^{b}	2.193	2.209	0.035	0.01	0.95	0.63
70	2.261^{b}	2.384^{ab}	2.332^{ab}	2.613^{a}	2.236^{b}	2.178^{b}	2.359	2.309	0.032	<0.01	0.54	0.62
80	2.770^{ab}	2.802^{ab}	2.83^{ab}	3.010^{a}	2.643^{b}	2.547^{b}	2.806	2.725	0.035	<0.01	0.09	0.14
95	2.861^{b}	2.964^{ab}	2.998^{ab}	3.282^{a}	2.887^{b}	2.727^{b}	2.973	2.931	0.040	<0.01	0.32	0.07

[1]Male, intact, Large White X Landrace pigs

[2]STD- standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet; diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran)

[3]Roxazyme® G2.

[abc]Means within a row with different superscripts are significantly different (p≤0.05).

Table 5.4: Slaughter performances of [1]pigs fed maize-soybean diets containing high levels of different fibrous feedstuffs and supplemented with Roxazyme® G2

Parameter	[2]Diets						[3]Enzyme		SEM	P Value		
	STD	BG	LH	MC	SH	WB	-	+		Diet	Enzyme	Enzyme X Diet
Slaughter weight (kg)	94.7	93.0	89.2	93.9	96.8	95.6	92.8	95.2	0.82	0.18	0.81	0.86
Dressing (%)	75.1^{a}	72.2^{a}	68.6^{b}	73.1^{a}	76.4^{a}	74.2^{a}	72.4	74.4	0.71	0.05	0.16	0.58
Carcass length (cm)	81.6	80.5	82.1	81.8	80.8	82.5	81.4	81.6	0.34	0.58	0.77	0.52
Meat colour	82.5	81.2	80.8	80.4	82.0	82.3	81.3	81.8	0.37	0.53	0.55	0.79
Lean (%)	68.4^{b}	69.6^{ab}	71.0^{a}	69.5^{ab}	70.0^{ab}	71.2^{a}	69.9	69.9	0.26	0.01	0.95	0.35
Fat (%)	15.0^{a}	12.9^{a}	10.1^{b}	13.3^{a}	12.6^{a}	11.9^{a}	12.9	12.5	0.37	0.01	0.19	0.52

[1]Male, intact, Large White X Landrace, initial live weight of 32 ± 5.6 kg.

[2]STD - standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).

[3]Roxazyme® G2.

[ab] Means within a row with different superscripts are significantly different (p≤0.05).

Table 5.5: Correlation coefficients of fermentability (mL gas g^{-1} DM), swelling capacity (mL g^{-1} DM), soluble fibre (g kg^{-1} DM) water binding capacity (g g^{-1} DM), and digestibility coefficients of nutrients in growing [1]pigs fed maize-soybean [2]diets

Component	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Swelling capacity		[5]Water binding capacity	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
Dry matter	0.466	***	-0.219	*	-0.239	*	-0.304	**
Organic matter	0.508	***	-0.213	*	-0.284	**	-0.344	**
Gross energy	0.446	***	-0.206	*	-0.268	**	-0.292	*
Crude protein	-0.051	ns	-0.230	*	-0.359	**	-0.204	ns
Crude fat	-0.242	*	0.515	***	-0.005	ns	0.423	***
Ash	0.033	ns	-0.148	ns	0.225	*	0.194	Ns
P	-0.150	ns	-0.184	ns	0.275	*	0.089	ns
NDF	0.534	***	-0.515	***	-0.151	ns	-0.403	***
ADF	0.403	***	-0.413	***	0.158	ns	-0.226	ns

ns not significant.
*Significant at p≤0.05.
**Significant at p≤0.01.
***Significant at p≤0.001.
[1]Male, intact, Large White X Landrace pigs, digestibility evaluated at 65-70 kg live weight.
[2]Standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted brewer's grains, lucerne hay, maize cobs, soy hulls, wheat bran to contain 246 g total dietary fibre kg^{-1} DM.
141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM with brewer's grain, lucerne hay, maize cobs, soy hulls and wheat bran.
[3]Fermentation by pig faecal inoculum of washed residues remaining after the diets were digested by pepsin (porcine, 200 FIP-U/g, Merck No, 7190), followed by pancreatin (porcine, grade IV, Sigma No P-1750), according to Bindelle *et al.* (2007)
[4]AOAC (2006) method 991.43.
[5]Water binding by centrifugation and swelling capacity by bed volume technique, of the whole feed matrix, determined according to Canibe and Bach Knudsen (2001), with modifications.

Table 5.6: Correlation coefficients of fermentability (mL gas g^{-1} DM), swelling capacity (mL g^{-1} DM), soluble fibre (g kg^{-1} DM), water binding capacity (g g^{-1} DM) and feed intake (kg day^{-1}), weight gain (kg day^{-1}) and feed conversion ratio (feed/gain) in growing [1]pigs maize-soybean [2]diets

Mean live weight	Performance measures	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Swelling capacity		[5]Water binding capacity	
50	Feed intake	0.296	*	-0.287	*	-0.196	ns	-0.313	**
70		0.021	ns	-0.323	**	-0.078	ns	-0.178	ns
85		-0.010	ns	-0.344	**	-0.037	ns	-0.152	ns
95		-0.015	ns	-0.365	**	0.036	ns	-0.124	ns
50	Weight gain	0.378	**	-0.025	ns	-0.267	*	-0.212	ns
70		0.266	ns	-0.052	ns	-0.225	ns	-0.178	ns
85		0.106	ns	-0.077	ns	-0.149	ns	-0.079	ns
95		0.153	ns	-0.145	ns	-0.127	ns	-0.182	ns
50	Feed conversion ratio	-0.132	ns	-0.308	**	0.138	ns	-0.082	ns
70		-0.233	ns	-0.306	**	0.131	ns	-0.027	ns
85		-0.112	ns	-0.307	**	0.115	ns	-0.085	ns
95		-0.162	ns	-0.305	**	0.145	ns	0.005	ns

ns not significant.

*Significant at p≤0.05.

**Significant at p≤0.01.

[1]Male, intact, Large White X Landrace pigs.

[2]Standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted brewer's grains, lucerne hay, maize cobs, soy hulls, wheat bran to contain 246 g total dietary fibre kg^{-1} DM.

[3]Fermentation by pig faecal inoculum of washed residues remaining after the diets were digested by pepsin (porcine, 200 FIP-U/g, Merck No, 7190), followed by pancreatin (porcine, grade IV, Sigma No P-1750), according to Bindelle *et al.* (2007).

[4]AOAC (2006) method 991.43.

[5]Water binding by centrifugation and swelling capacity by bed volume technique, of the whole feed matrix, determined according to Canibe and Bach Knudsen (2001), with modifications.

5.4 Discussion

5.4.1 Effects of fibre on nutrient digestibility and animal performance

The generally negative impact of fibre on nutrient digestibility and on animal performance observed in this study was in agreement with findings reported in reviews by Noblet and Le Goff (2001), Bindelle *et al.* (2008), Aarnink, and Verstegen (2007). However, pigs, particularly those on the diets containing the more fermentable fibre, maintained growth and slaughter performance despite the more than twice the standard DF. This is important given the diets were formulated at lower cost than the standard, largely because there was no processing, no supplementary energy and limited synthetic amino acid supplements. Xu *et al.* (2010) and Yoon *et al.* (2010) reported similar findings. There were no negative effects on growth and carcass characteristics when growing-finishing pigs were fed maize-soybean diets containing up to 10-15% (Yoon *et al.,* 2010) and up to 30% (Xu *et al.,* 2010). dried maize distillers grain with solubles. However, Xu *et al.* (2010) reported negative effects on fat quality at inclusion levels above 20%. Poor meat quality will offset the cost benefit of the cheaper diets.

Pigs on the MC diet had numerically, but not statistically higher feed intake compared to the other diets, with statistically significant higher intakes compared to pigs on the LH diet. The low intake of the LH diet could be attributed to the poor palatability rather than metabolic effects, since nutrient digestibility and feed conversion ratio were not affected. The higher intake of the MC diet suggests limiting energy intake. High maintenance requirement may result due to the cost of physical processing of the highly lignified MC through the digestive system (Noblet and Le Goff, 2001). This hypothesis is supported by the low feed conversion ratio.

Noblet and Le Goff (2001) suggested that in general, although fibre may seem to contribute to digestible energy, due to its negative effect on other nutrient digestion, the energy cost of physical processing and the inefficient utilisation of metabolites, fibre may contribute negligible quantities of net energy in growing pig diets. In contrast, other researchers (Varel and Yen, 1997; Anguita *et al.,* 2006) reported significant energy contribution of fibre to the

animal requirement. SCFA provided up to 30% of the maintenance energy requirement (Varel and Yen, 1997), in excess of 10% of the total digestible energy (Anguita *et al.,* 2006), and contributed as much as 17.6% of total available energy of growing pigs (Anguita *et al.,* 2006). In this study, the similar growth performance by pigs on the highly fermentable fibre diets (LH, WB) at similar feed intakes to the STD diet suggests significant energy contribution form the DF of the fibrous diets.

The strong positive correlation between DF fermentability and the digestibility of ADF and NDF are consistent with findings by Chen *et al.,* (2013), which similarly validated the gas production test as a predictor of *in vivo* fibre degradability. Correlation analyses also suggested that the fermentability and hydration properties of DF tended to have a greater or beneficial impact on growth performance during growth in the early growth phase. The positive association between fermentability and growth performance particularly in the early growth phase can be due to different factors. The fermentation of fibre tends to be higher in the older animal (Noblet and Le Goff, 2001). In addition, in diets containing highly fermentable fibre such as soybean hulls, higher fermentation may have induced prebiotic effects, which promoted efficient growth (Williams *et al.,* 2001). On such diets, the trophic effects of short chain fatty acids on intestinal epithelia could also promote more efficient nutrient absorption (Martins *et al.,* 2010). In pigs on the BG and WB diets, swelling and water binding capacity of the soluble fibre may have limited feed intake, and consequently reduced weight gain, through stimulation of gastric mechano-receptors that signal satiety (Kristensen and Jensen, 2011). The greater negative correlation of swelling capacity to weight gain, and of water binding capacity to feed intake in pigs of live weight less than 50kg, and the negative correlation of soluble fibre to feed intake support this hypothesis.

5.4.2 Effects of RX on nutrient digestibility and animal performance

In this study, RX did not improve animal performance despite the high levels of DF, and across chemically diverse types of fibre. Similar to the findings of this study, a study by Kerr *et al.* (2013) showed that none of a range of enzyme products that included RX improved nutrient digestibility and growth performance of pigs fed nutritionally adequate maize-

soybean diets containing up to 30% maize distillers dried grains with solubles. However, when pigs of initial weight 14.7 kg were fed rye based diets, Roxazyme G2 increased the precaecal digestibility of total amino acids and increased the faecal digestibility of NSP (Nitrayova *et al.,* 2007). Mannanase improved the apparent total tract digestibility of protein, and increased daily gain (Yoon *et al.,* 2010) in pigs on soybean-maize diets including high levels of maize distillers' grain diets, with no effect on feed intake and feed efficiency. An enzyme cocktail containing galactosidase and mannanase activities improved the gain: feed ratio, energy and amino acid digestibility of nursery pigs on standard maize-soybean diets (Kim *et al.,* 2003). A glucanase-protease enzyme blend increased the faecal digestibility of DM, OM, CP, energy, fat, TDF, Ca, P and the apparent ileal digestibility of NDF and hemicellulose (Ji *et al.,* 2008).

In practical diets, the efficacy of NSPases depends on correct matching to the dietary NSP, and the enzymes should target the limiting physico-chemical properties of DF (Zijlstra *et al.,* 2010). The inconsistent results on the efficacy of enzymes may relate to failure to meet these conditions. Results from the *in vitro* study in Chapter 3 indicated that RX had different activities on DF from different sources, with particularly low activity on maize and soybean fibre. In the study in Chapter 4, the enzyme cocktail also did not substantially alter the fermentation characteristics of ileal digesta of pigs fed high fibre diets. The findings of the feeding trial therefore confirmed the observations in the *in vitro* evaluations.

5.5 Conclusion

The results of the study suggested that high fibre feed ingredients containing highly fermentable DF can substitute the conventional ingredients in growing pig diets to achieve up to twice the standard DF levels without affecting growth performance and carcass characteristics. The use of RX in growing pigs on maize-soybean diets containing high levels of commonly available high fibre feed ingredients was not justified.

CHAPTER 6

EFFECTS OF DIETARY FIBRE SOURCE AND ROXAZYME® G2 ON THE HISTO-MORPHOLOGY OF THE ILEAL EPITHELIUM OF GROWING PIGS

Abstract

The effects of dietary level, source of DF and Roxazyme® G2 on the histo-morphology of the ileal epithelium of pigs was investigated during the growth trial described in Chapter 5. At slaughter, ileal tissue samples were taken from each pig at a point 50cm above the ileo-caecal valve. *Villi* height, area and the corresponding crypt depth were measured by computerised image analysis and the *villi* height: crypt ratio was calculated. None of the measured parameters were affected ($p>0.05$) by the diet or the enzyme. However, diet tended ($p= 0.054$) to affect the crypt depth, whereby pigs on the LH and WB diets had similar crypt depth to the STD diet, while pigs on the other diets had higher values. There was a significant diet X enzyme interaction for *villi* height ($p=0.016$). The enzyme tended to reduce the *villi* height of pigs on the SH, STD and WB diets, with an opposite effect on pigs on the MC, BG, LH diets. There was a tendency ($p=0.086$) for interaction for the *villi* height: crypt depth ratio, whereby RX reduced the ratio in pigs fed the SH and WB diets, with an opposite effect on pigs fed the MC, BG, LH and STD diets. The dietary soluble DF content negatively correlated ($p=0.0498$) with the crypt depth. Fermentability of DF, water binding and swelling capacity of the diet matrix did not correlate with any of the measured parameters. In conclusion, adding high levels of fibrous co-product feeds to maize-soybean diets and supplementing RX to such diets had minimal effects on the morphology of the ileal epithelium of growing pigs over 30kg live weight.

***Keywords**; Growing pigs, fibre, ileal mucosa, morphology, enzymes, maize-soybean diets, grain-processing co-products*

6.1 Introduction

Morphologically, to maintain digestive efficiency and to maintain the barrier function of the intestinal epithelium (Kim *et al.,* 2012), long *villi* (Heo *et al.,* 2008), long *villi* and a high *villus* length: crypt depth ratio (Bach Knudsen *et al.,* 2012) are desirable. Dietary control of the morphology of intestinal epithelium to manage enteric disease may be possible. For instance, in a maize-soybean diet fed over two weeks to 14 kg pigs, wheat straw increased the depth of intestinal crypts and the width of *villi* in the jejunum and ileum (Jin *et al.,* 1994). In newly weaned piglets fed the treatment diets over 21 days, BG, and not WB were found to have increased jejunum *villus* depth and width, and jejunum and ileum crypt depth, and to increase jejunum *villus* width, jejunum, and ileum crypt depth when compared to pigs on a low fibre diet (Martins *et al.,* 2010). In soybean-maize-rice bran diets fed to 9kg pigs over 27 days, BG reduced crypt density, increased *villi* height in the ileum and in the duodenum and increased *villi* width in the ileum (Ngoc *et al.,* 2012). In a study where maize-soybean diets were fed over 28 days, added dried distillers grains tended to decrease ileal *villi* height the *villi* height/crypt depth ratio (Agyekum *et al.,* 2012). On barley diets fed to 7-8 kg pigs over 21 days, adding MC to the control diet increased proximal duodenal *villus* height and the *villi* height: crypt depth ratio (Van Nervel *et al.,* 2006) while WB or sugar beet pulp had no effect. On the other hand, on maize-wheat diets fed over approximately 5 weeks, WB increased *villus* length, width and area in both the jejenum and the ileum (Schedle *et al.,* 2008).

Overall, the nutritional management of “gut-health” targets the health defining equilibrium in the interaction of the diet, the host animal’s intestinal mucosa and its gut microbes (Montagne *et al.,* 2003). Short chain fatty acids have trophic effects on enterocytes, which may promote gut health and nutrient absorption (Jin *et al.,* 1994). On the other hand, soluble, viscous DF has detrimental effects on the intestinal morphology (McDonald *et al.,* 2001; Hedemann *et al.,* 2006). It is not clear if the bulking effect of DF, which is commonly quantified by its water binding and swelling capacities, affects intestinal morphology. It can be hypothesised that bulking factors may indirectly control of intestinal morphology by influencing feed, and energy intake (Pluske *et al.,* 1997),

The relevance of NSPases in managing gut health is unclear. Current NSPases are typically endolytic and largely target the soluble NSP to reduce the molecular size and branching (Choct, 2006; Paloheimo *et al.,* 2011). Depolymerisation will likely alter the physicochemical and fermentation properties. The consequent impact of DF on the intestinal morphology is unclear.

The aim of the study was to examine the effects of feeding maize-soybean diets containing high levels of different fibrous feedstuffs and supplemented with RX on the histo-morphology of the ileal epithelium of growing pigs, in relation to the solubility, water binding, swelling and fermentation properties of DF.

6.2 Methods and Materials

6.2.1 Histometry of the ileal epithelium

At slaughter of the animals at the termination of the growth trial described in Chapter 5, a 15 X 3 mm transverse strip of the ileum was removed approximately 50 cm above the ileo-caecal valve of each pig. The tissues were preserved in Clark's fluid for four weeks , after which they were soaked in 10% neutral buffered formalin, before fixing in paraffin wax. Samples were fixed using standard paraffin wax embedding procedures. *Villus* length, area and depth of the crypts were measured in one 5 µm thick transverse section of each sample, using computerised image analyses as illustrated in **Figure 6.1**. On each sample, measurements were taken on 24 well-oriented *villi* in 12 consecutive images per sample. The images were taken with overlap to avoid repeated measurements on the same *villi*. The images were captured using an Olympus BX43 light microscope fitted with an AxioCam ICc 3 Zeiss digital microscope camera. Images were analysed using AxioVision 4 Carl Zeiss Imaging Solutions software (AxioVs40 V 4.8.1.0).

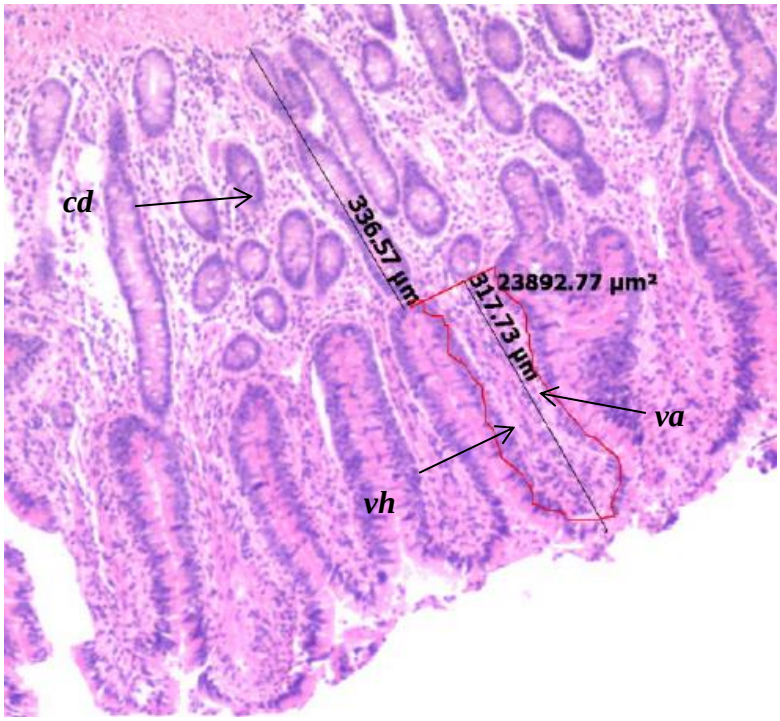


Figure 6.1: Histometry of the ileal epithelium in samples taken approximately 50 cm above the ileo-caecal valve of a growing pig fed a maize-soybean diet enriched in fibre from soybean hulls to 246 g kg^{-1} DM, illustrating the measurement of *vh*-*villi* height, *va*-*villi* area and cd-crypt depth)

6.2.2 Statistical Analysis

ANOVA of *villi* height, area, crypt depth, and *villi* height: crypt depth ratio was conducted using the PROC MIXED procedures of SAS software, version 9.3 (SAS, 2010), using Modell II, as described in Chapter 3.

Comparison of means was performed using the Bonferroni t-test at the α-level of 0.05. Pearson's product-moment correlation coefficients between the physicochemical properties of the diets and the ileal morphology, and the associated probabilities were calculated using the PROC CORR statement of SAS software, version 9.3 (SAS, 2010).

Values of $0.05<p<0.1$ were considered tendencies towards significance.

6.3 Results

The ileal epithelial histometry is described in **Table 6.1**. Diet and enzyme did not affect (p>0.05) the *villi* height, area, crypt depth and *villi* height: depth ratio. However, diet tended (p= 0.054) to affect crypt depth, whereby pigs on the LH and WB diets had similar crypt depth to the STD diet, while pigs on the other diets had higher values. There was a significant (p=0.016) diet X enzyme interaction for *villi* height, whereby the enzyme numerically reduced the *villi* height in pigs on the soy-hulls, STD and WB diets, but increased *villi* height in pigs on the other diets. There was tendency (p=0.086) for diet X enzyme interaction for the *villi* height: crypt depth ratio, whereby the enzyme numerically reduced the ratio in soy-hull and WB diets, and increased the ratio on the other diets. Soluble DF content negatively correlated (P=0.0498) with crypt depth. Correlations between the histological parameters with fermentability, swelling or water binding capacity were not significant (p>0.05).

Table 6.1: Histometry of the ileal epithelium of growing [1]pigs fed maize-soybean based diets containing high levels of different fibrous feedstuffs and supplemented with Roxazyme® G2

[4]Parameter	[2]Diets						[3]Enzyme		SEM	P Value		
	STD	BG	LH	MC	SH	WB	+	-		Diet	Enzyme	Enzyme X Diet
Villi height (µm)	347.9	351.4	357.7	352.6	349.0	356.1	362.1	342.8	7.10	1.00	0.15	0.02
Villi area(μm^2)	39223.1	40731.5	45986.9	44327.7	42679.7	43597.1	41810.1	43705.3	1380.00	0.80	0.51	0.52
Crypt depth (µm)	235.5	268.0	235.6	294.0	256.7	239.0	248.5	261.0	6.01	0.05	0.30	0.36
Villi height/crypt ratio	1.47	1.33	1.43	1.23	1.38	1.51	1.44	1.34	0.04	0.35	0.22	0.09

[1]Male, intact, Large White X Landrace pigs.
[2]STD- standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet; diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).
[3]Roxazyme® G2.
[4]Tissue samples of the ileum taken approximately 50 cm above the ileo-caecal valve.

Table 6.2: Correlation coefficients of fermentability (mL gas g^{-1} DM), swelling capacity (mL g^{-1} DM), soluble fibre (g kg^{-1} DM), water binding capacity (g g^{-1} DM) and ileal *villi* height (µm), area ($µm^2$), crypt depth (µm) and *villi* height: crypt depth ratio in growing [1]pigs fed a standard and high fibre [2]diets containing high levels of different fibrous feedstuffs

[3]Parameter	[4]Fermentability		[5]Swelling		[5]Water binding		[6]Solubility	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
Villi height	-0.0362	ns	0.0004	ns	0.0614	ns	0.0023	ns
Villi area	0.0024	ns	-0.0114	ns	0.1143	ns	0.1160	ns
Crypt depth	0.2073	ns	0.1638	ns	-0.0170	ns	-0.2878	*
Villi height/crypt ratio	0.1331	ns	-0.1625	ns	-0.0127	ns	0.1631	ns

ns not significant (p>0.05).

[1]Male, intact, Large White X Landrace pigs.

[2]Standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted brewer's grains, lucerne hay, maize cobs, soy hulls, wheat bran to contain 246 g total dietary fibre kg^{-1} DM.

[3]Tissue samples of the ileum taken approximately 50 cm above the ileo-caecal valve.

[4]Fermentation by pig faecal inoculum of residues remaining after the diets were digested by pepsin (porcine, 200 FIP-U/g, Merck No, 7190), followed by pancreatin (porcine, grade IV, Sigma No P-1750), according to Bindelle *et al.* (2007).

[5]Water binding by centrifugation and swelling capacity by bed volume technique, of the whole feed matrix, determined according to Canibe and Bach Knudsen (2001), with modifications.

[6]AOAC (2006) method 991.43.

6.4 Discussion

The present study indicated minimal impact of source of DF and of RX on the histo-morphology of the ileal epithelium. This was not expected, given the high levels of DF, some of which, such as SH and WB, was highly fermentable, and given the differences among the fibres in SCFA production and composition. The trophic effects of SCFA on the intestinal epithelium, particularly of But, were discussed in Chapter 2.

Inconsistent findings on the effects of DF on intestinal histo-morphology are reported in literature. In a study on newly weaned pigs fed barley-wheat-animal protein diets over nine

days, added pectin reduced *villi* height and crypt depth, without altering the villous height/crypt depth ratio (Hedemann *et al.,* 2006). Barley hulls increased *villi* height and mucosal enzyme activity when compared with pigs fed pectin-containing diets. In wheat-maize-soybean diets fed to 20 and 30 kg pigs over 14 day periods, supplementary pectin and rye fibre differently affected *villi* height, area, crypt depth ratio in the duodenum, jejenum and ileum (Święch *et al.,* 2012).

In the current study, failure to detect any significant effects of DF on intestinal epithelial morphology may be because the histometry was conducted in relatively mature pigs. As is evident in previous studies cited above, such effects have been reported in pigs in the growth period from weaning to 30kg live weight. However, unlike the relatively low DF and short periods over which such studies are typically conducted, this study was conducted on pigs which had been subjected to prolonged intake of diets containing approximately twice the level of chemically diverse DF compared to the STD diet. For instance, the study was designed to include diets with substantial variation in the soluble DF (4.72-10.29 g kg^{-1} feed DM), swelling (1.65-2.38 mL g^{-1} DM), water binding capacity (g g^{-1} DM)) and water binding (3.67-5.17 g g^{-1} DM) capacities of the dietary matrix. The effect of viscosity (indicated by the soluble DF fraction in this study) on intestinal morphology depends on the level (Kim *et al.,* 2012). For instance, high levels of soluble DF increased the viscosity of the intestinal digesta, which lead to *villi* atrophy (McDonald *et al.,* 2001; Hedemann *et al.,* 2006). In contrast, low viscous DF increased *villi* height (Hedemann *et al.,* 2006). The contrasting effects suggest a positive-negative threshold in correlation between viscosity and *villus* height as viscosity increases (Bach Knudsen *et al.,* 2012). On the other hand, at similar viscosity, the effect may depend on the type of DF. Crypt depth was reduced by pectin (Hedemann *et al.,* 2006), but was increased by similarly low viscosity carboxymethylcellulose (McDonald *et al.,* 2001).

In addition to the variable hydration properties, in the present study, there was also substantial variation among the diets in the gas (1.26-205.3 mL g^{-1} DM DF) and SCFA (4.1-5.4 mMol g^{-1} DM DF) production, including that of But (10.9-13.7% of SCFA production). Supplementing But to weaned piglets increased the depth of the intestinal *villi* (Wang *et al.,* 2005). On supplementing But to neonatal piglets, Kotunia *et al.* (2004) reported reduction of

the *villi* height in the duodenum, while *villi* height increased in the jejunum and ileum. However, But did not influence intestinal morphology in piglets (Biagi *et al.,* 2007; Tonel *et al.,* 2010).

Correlation analyses showed that across all diets, only the dietary soluble DF content correlated negatively with the *villi* height: crypt depth ratio. It is hypothesised that the positive effect likely resulted from the high fermentability of soluble DF. However, the bulk of the fermentation in the small intestines is normally that of the soluble, readily fermentable fraction (Choct and Kocher, 2000). Interestingly, and in contradiction to the hypothesis above, there was no correlation between the fermentability of DF and the morphological parameters. It can therefore be hypothesised that the lack of correlation to fermentability was because fermentation was assayed on washed *in vitro* digesta, and therefore excluded the soluble, readily fermentable DF fraction.

Although RX did not affect ileal mucosal histometry across all diets, its effect tended to depend on the source of DF. As indicated in Chapter 4, the *ad-libitum* feeding regime could have limited the degradation of NSP due to reduced retention times in segments of the upper gut (Svihus, 2011).

In poultry, Bhat and Hazlewood (2001) and Choct (2006) suggested that it is unlikely that significant depolymerisation of NSP to monosaccharides could occur in the transit times of the upper gut. In pigs, the extent of depolymerisation of NSP is unclear, but could be more extensive, given the relatively longer retention times (Svihus, 2011). Choct and Kocher (2000) suggested that NSP hydrolysis could produce numerous and chemically diverse oligosaccharides. In consequence, depending on the structure and composition of NSP, and on the enzyme, substantial and variable alteration of the secondary structure of NSP, and consequently, of the physico-chemical properties of the DF, could occur. For instance, in poultry, xylanases from different micro-organisms with different affinities for soluble versus insoluble xylans resulted in different effects on digesta viscosity (Choct, 2006). *In vitro* experiments by Bindelle *et al.* (2011) suggested that depolymerisation of NSP in the upper tract of pigs could influence their fermentation characteristics.

Though weak, the diet X enzyme interactive effects on the histo-morphology observed in this study suggest that different affinities for glucans and xylans present in the fibrous feeds resulted in different effects on the physico-chemical properties of the DF (Choct, 2006). Unfortunately, it is not currently possible to target or strategically cleave specific NSP to stimulate beneficial epithelial cell function. Given the heterogeneity in the composition and physico-chemical properties of NSP among feedstuffs (Bach Knudsen *et al.,* 2001), multi-potent (Barletta, 2011) NSPases that are tailor-made (Choct, 2006) for specific DF are critical.

Surprisingly, few *in vivo* experiments evaluated the influence of NSPases on the intestinal epithelial histometry of growing pigs. In one such study by Agyekum *et al.* (2012), supplementing NSPases to dried distillers grain diets tended to decrease the ileal *villi* height/crypt depth ratio. In the current study, there was tendency to a similar effect of RX on the SH and WB diets, with the opposite effect on STD, BG, MC and LH diets.

6.5 Conclusion

Substantial variation in the composition and physico-chemical properties of the DF in diets enriched with different DF sources was not reflected in the histometry of the ileal epithelium of growing pigs. Supplementation with RX only resulted in a tendency for interaction with the type of DF in modulating intestinal epithelial morphology. The findings question the effectiveness of using practical, fibrous diets or supplemental RX to stimulate intestinal mucosal morphology as strategies to promote gut health in pigs over 30kg live weight.

CHAPTER 7

FIBRE FERMENTABILITY AND ENZYME EFFECTS ON NUTRIENT DIGESTIBILITY AND NITROGEN RETENTION BY GROWING PIGS FED MAIZE-SOYBEAN AND MIXED GRAIN CO-PRODUCT DIETS

Abstract

The aim of the study was to examine the effects of fermentability of DF and of RX on nutrient digestibility and nitrogen utilisation by growing pigs fed the two fermentability-contrasted, 319 g TDF kg^{-1} DM diets described in Chapter 4. Each diet was duplicated, and one mixture was supplemented with 0.270 g RX kg^{-1} DM of feed. The diets were fed *ad libitum* to eight ileum T-cannulated, intact Large White X Landrace male pigs weighing 65 ± 5.1 kg. The experimental design was a duplicate 4 x 4 Latin Square, with a 2 (enzyme) x 2 (fermentability) factorial arrangement of treatments. Each period consisted of two weeks adaptation followed by five days of sampling. Digestibility was estimated using 2% (as fed) chromium oxide as the indigestible marker. The HF diet had higher AID of DM (62.5 vs 58.6), organic matter (OM) (65.6 vs 62.1), energy (64.4 vs 61.0), fat (85.8 vs 81.7) and ash (41.8 vs 32.7). The AID of HO-Pro, Met and Val were higher for the LF diet. There was a significant diet X enzyme interaction on the AID of Met, whereby the RX reduced the AID of Met in the LF diet, and not that of the HF diet. The ATTD was higher for the HF diets for DM (74.2 vs 68.4), NDF (64.7 vs 57.4), and ADF (35.1 vs 21.0). Correlation analysis revealed significant positive associations between the fermentability of DF for AID DM, OM, ash, ash, fat and energy. Fermentability was negatively correlated with the apparent ileal digestibility of ash. Solubility was also negatively correlated with the AID of DM, OM, ash, fat, ADF and energy, to the ATTD of DM, OM, ash, fat, energy, NDF, and ADF, and to the partial apparent post ileal digestibility of ash. Correlations to Ser, Ala, Val, Iso-Leu, and His were positive. Swelling was positively correlated with the AID of protein, Trp and Lys. The results suggested that targeting rapidly fermenting insoluble DF could be important for efficient

nutrient digestion when growing pigs are fed high DF maize-soybean-grain processing co-product diets. The use of RX to improve nutrient digestibility of growing pigs on such diets was not justified.

***Keywords**; Growing pigs, dietary fibre, fermentability, enzymes, maize-soybean, co-product feedstuffs*

7.1 Introduction

Depending on the type and dietary level, DF tends to depress nutrient digestibility (Noblet and Le Goff, 2001, Wenk, 2001). The need for expensive supplements may therefore offset the cost advantage of cheaper, but fibrous feed ingredients. Much of the available information on the effects of co-product feeds and enzymes on nutrient digestibility and N retention is on conventional wheat, barley or rye-soybean diets, and increasingly, on diets enriched in DF from distillers' grains. There is limited information on maize-soybean diets containing high levels of alternative, commercially important grain-processing co-products

The variable effects of DF on nutrient digestion by growing pigs is attributed to the differences in the composition, and therefore, in the physiologically active physico-chemical properties of the constituent NSP (Zijlstra and Beltranena, 2013). Different mechanisms are proposed to explain the inhibitory effect on nutrient digestion. Adsorption to DF particles inhibits enzyme activity (Schneeman, 1978). The plant cell wall physically renders the cell content inaccessible to enzyme degradation (Bach Knudsen *et al.,* 1993a). High levels of soluble NSP increase the viscosity of digesta (Bach Knudsen, 2001). Viscosity inhibits the diffusion of both nutrients and enzymes (de Lange *et al*., 2000), which slows the digestion and absorption of nutrients. Bulking shortens transit times (Wilfart *et al.,* 2007; Solà-Oriol *et al.,* 2010) thereby shifting digestion to the colon (Anguita *et al.,* 2006). Water binding and bulking were linked to increased endogenous secretion of mucins (Libao-Mercado *et al.,* 2009). Incomplete recovery of the mucin amino acids resulted in lower apparent ileal digestibility (Święch *et al.,* 2012). Secretory mucins are particularly rich in Thr and Ser (Faure *et al.,* 2002; Zhu *et al.,* 2005; Libao-Mercado *et al.,* 2006), Met and Cys (Zhu *et al.,* 2007). Endogenous loss in mucins was reported to increase the requirement for threonine

(Zhu *et al.,* 2005) and for Met+Cys (Zhu *et al.,* 2007). The mechanism by which DF stimulates the secretion of mucins is unclear. Purified, soluble and highly fermentable pectin, and insoluble-DF-rich, poorly fermentable wheat shorts both increased endogenous protein losses (Libao-Mercado *et al.,* 2006). Mucin secretion was associated with the bulk forming properties of both soluble and insoluble DF (Lerteme *et al.,* 1996; Leterme *et al.,* 1998).

It can be hypothesised that rapid degradation of DF may alter its physico-chemical properties and thereby ameliorate the inhibitory effect on nutrient digestion. The rate of degradation of NSP by bacteria in the upper tract differs depending on the source. For example, the molecular weight of soluble arabinoxylans of rye was reduced by 25% compared to no change in wheat (Le Gall *et al.,* 2010). However, the reduction in the molecular size of the arabinoxylans did not eliminate the deleterious physicochemical properties.

The fermentative degradation of DF may be controlled by screening ingredients using *in vitro* quantified fermentation kinetic parameters (Bindelle *et al.,* 2007; Bindelle *et al.,* 2011). NSPases may also accelerate the degradation, and consequently, stimulate fermentation (Choct and Cadogan, 2001). Fermentation may in turn affect the apparent ileal digestibility (AID) of amino acids, either via fermentative catabolism (Columbus *et al.,* 2010) or by *de novo* synthesis (Torrallardona *et al.,* 2003; Libao-Mercado *et al.,* 2009; Zhu *et al.,* 2007). There is dearth of information on the importance of fermentative degradation on apparent ileal digestibility of nutrients.

The supply of fermentable energy to the lower gut influences the pattern of N excretion through faeces, relative to excretion via urine (Nahm, 2003). One mechanism by which the supply of fermentable energy reduces ammonia emission is by diversion of N from excretion as urea in urine, to stable bacterial protein in faeces (Canh *et al.,* 1997). It is hypothesised that, by depleting both exogenous and endogenous NH_3-N through *de novo* synthesis of microbial protein in the lower gut, the availability of fermentable DF reduces metabolic N absorbed from the colon, reducing the plasma urea, thus ultimately reducing urinary N excretion (Nahm, 2003; Zervas and Zijlstra, 2002). Canh *et al.* (1997) reported a ratio of

faecal to urinary N of 3.8 to 1.2 in pigs fed low DF, versus high DF, sugar-beet pulp diets, respectively Similarly, sugar beet pulp increased the faecal to urinary N ratio to 2.171, compared to 1.177 for oat hulls (Bindelle *et al.,* 2009). Similar results were reported when pigs were fed diets containing highly fermentable soybean hulls and/or sugar beet pulp (Mroz *et al.,* 2000).

The aim of the study was to examine the effects of fermentability of DF and of RX on the apparent ileal and total tract nutrient digestion, and on N retention and excretion patterns by pigs fed high DF, maize-soybean and mixed grain co-product diets.

7.2 Methods and Materials

The feeding experiment was conducted at the Animal Production Institute of the Agricultural Research Council. The experimental procedures used for the care and treatment of the animals were approved by the ethics Committees of both the University of South Africa and the ARC.

7.2.1 Experimental diets

The test diets were the high (HF) and low (LF) fermentability, high DF (319 g TDF kg^{-1} DM) formulations with (HF+, LF+) or without RX, as described in Chapter 4. In addition, swelling and water binding capacity of the complete diets, and of their ileal digesta were determined following the procedures described in Chapter 5, section 3.2.1. Water binding and swelling capacities of the diets and their ileal digesta are indicated in **Table 7.1**.

7.2.2 Animal management, experimental design and sample collection

Intact male Large White X Landrace pigs were weaned at 4 weeks and placed on a commercial weaner diet to a body weight of 20-25 kg. Ileal T-cannulae were placed in the ileo-caecal junction of 10 pigs. Pigs were restrained and an intra-venous catheter was placed into the ear vein. The pig was sedated with a bolus of 3 ml diluted pentobarbitone (1 in 3) after which it was shaved on the right abdominal wall which was then surgically

Table 7.1: Water binding (g g^{-1} DM) and swelling (mL g^{-1} DM) capacity of high fibre, maize-soybean plus co-product diets, and of the ileal digesta of growing pigs

Component	[1]Diets	
	High Fermentability	Low Fermentability
[2]Swelling capacity, whole feed	5.7	5.5
[2a]Swelling capacity, ileal digesta	5.7	5.6
[3]Water binding capacity, whole feed	2.3	2.3
[3a]Water binding capacity, ileal digesta	2.6	2.7

[1]Growing pig diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.
[2]Swelling capacity by the bed-volume technique, determined according to Canibe and Bach Knudsen (2002), with modifications.
[3]Water binding by centrifugation, according to Canibe and Bach Knudsen (2001), with modifications.
[a]Ileal digesta of intact Large White X Landrace male pigs fed high fibre, maize-soybean growing pig diets *ad libitum.*

prepared by washing with surgical soap and a triple disinfection with hibitane/alcohol preparation.

The drip was set-up and allowed to run at maintenance (10 drops per minute) for the duration of the surgery. A 5 cm vertical incision was made on the left abdominal wall in the lumbar area, and cuts made through the skin and abdominal muscular layers and lastly through the peritoneum. Once inside the peritoneal cavity the ileo-caecal junction was located. A purse-string suture through the ileal wall was placed 5 cm cranially from the ileo-caecal junction. The length of the purse-string suture was about 15 mm and the incision was made into the ileum to allow placement of the cannula. The cannula had the lip folded into itself to ease placement through the ileal wall.

Once in place the lip was opened by pushing the folded lip that was within the tubular part from the “outside” end by a long stainless steel rod. The lip of the cannula was opened into the ileum to prevent the cannula from slipping out into the peritoneal cavity. A second purse-string suture was placed in the ileal wall around the cannula to secure the placement. A second 15 mm incision was made 3 cm dorsally to the original incision. The incision

penetrated through all the muscular layers into the abdomen to allow the cannula to be pulled through.

The first layer included the peritoneum and the innermost muscle layer. After this layer was closed, 3 ml of Duplocillin® LA was injected intra-muscularly. The second layer of sutures just included the remaining two muscle layers and the last suture layer included the skin. A variation of suture patterns were used which included simple interrupted for the first layer, simple continuous for the two muscle layers, with cross mattress and horizontal mattress used on the skin. The cannula was secured with Elasto-plast™ on the outside of the pig. Three suture layers were used to close the wound. Supona® Aerosol wound spray was applied post-operation. All the patients recovered without problems.

After the surgery, the pigs were individually housed in pens with thick wood shavings bedding for recuperation. During the six week recuperation period, they were fed an 18% protein diet with water made available *ad libitum*. After recovering from surgery and at live weight 65.0 ± 5.1 kg, 8 experimental animals were housed individually in metabolism crates placed 85 cm above ground, measuring 75 cm high, 88 cm wide and 150 mm long, in an open house. The diets were randomly allocated to the 8 pigs in a duplicate 4X4 (animal X period) Latin square design. The pigs were fed *ad libitum.* Each period consisted of two weeks of adaptation followed by five days of sample collection.

7.2.3 Digestibility measurements

Digestibility was estimated using 2% chromium oxide (as fed) as the indigestible marker. The chromium diet was fed a week before, and during the collection period. Fresh faeces and urine were collected each day at 06:00. Fifty millilitres of 25% sulphuric acid were added to the urine collection vessels before each day's collection. An aliquot of 10% urine samples were collected daily. A minimum of 500 mL of ileal digesta were collected each day from 06:00 to 12:00. The daily samples were placed in a deep freezer at -18 ^{o}C and pooled at the end of the collection period. Ileal digesta and faeces samples were freeze-dried and milled through a 1mm screen.

At the end of the study the animals were humanely slaughtered at the ARC-Irene abattoir.

7.2.4 Statistical analyses

Apparent ileal (AID) and total tract (ATTD) nutrient digestibility, and nitrogen excretion and retention data were analysed using model III, as described in Chapter 4. Comparison of means was performed using ANOVA and treatment LS means were separated using the Bonferroni t-test at the α-level of 0.05.

Pearson's product-moment correlation coefficients between the physico-chemical properties of solubility, fermentability, swelling capacity, solubility, water binding capacity of ileal digesta and apparent digestibility coefficients and the associated probabilities were calculated using the PROC CORR statement of SAS software, version 9.3 (SAS, 2010).

Values of $0.05<p<0.1$ were considered tendencies towards significance.

7.3 Results

The apparent ileal (AID) and total tract digestibility (ATTD) of nutrients are indicated in **Table 7.2.** AID of DM, OM, energy, fat, ADF, ash, ATTD of DM, OM, energy, fat, ADF, NDF and ash were higher for the HF diet. There was a tendency for diet X enzyme interaction ($p=0.07$) on the AID of fat, whereby RX increased the AID of the fat in the low DF diet, and increased the AID in the HF diet.

Among the amino acids, fermentability reduced the AID of HO-Pro, Met and Val. There was a tendency for fermentability to reduce the AID of Ala (0.09), Pro ($P=0.07$), Iso-Leu ($p=0.08$) and His ($p=0.05$). RX marginally reduced the AID of Met. There was a significant diet X enzyme interaction on the AID of Met, whereby the RX reduced the AID of met in the LF diet, and not that of the HF diet. RX had no effect on the AID and ATTD of any other nutrients.

Correlation coefficients between AID, partial apparent post ileal digestibility, ATTD of nutrients and the physicochemical properties of solubility, swelling and water binding capacity, and fermentability of ileal digesta are summarised in **Table 7.3**. Correlation analyses revealed positive associations between the fermentability of dietary DF and the AID

of all the nutrients, which was significant for AID DM, OM, ash, ash, fat and energy. Solubility was negatively correlated with AID of DM, OM, ash, fat, ADF and energy, and to the ATTD of DM, OM, ash, fat, energy, NDF, and ADF. Swelling was positively correlated with the AID of protein, Trp and Lys. Solubility was positively correlated with Ser, Ala, Val, Iso-Leu and Histidine and tended to be positively correlated with Trp, Phe and total amino acids.

Nitrogen excretion in faces and in urine, and N retention are presented in **Table 7.4.** Neither diet nor enzymes affected N excretion and retention.

Table 7.2: Apparent ileal and total tract digestibility coefficients of nutrients by growing [1]pigs fed high fibre, maize-soybean and co-product diets contrasting in fermentability and supplemented with Roxazyme® G2

[2]Diets	HF		LF			P Value				
[2]Enzyme	(+)	(-)	(+)	(-)	SEM	Animal	Enzyme	Period	Diet	Diet*Enzyme
					Apparent Ileal digestibility					
DM	0.63[a]	0.62[ab]	0.58[b]	0.59[b]	0.007	0.24	0.56	0.07	<0.01	0.35
OM	0.66[a]	0.65[ab]	0.62[ab]	0.62[b]	0.006	0.20	0.69	0.06	<0.01	0.53
Energy	0.66[a]	0.63[ab]	0.61[ab]	0.61[b]	0.007	0.08	0.22	0.27	<0.01	0.45
Protein	0.77	0.75	0.76	0.77	0.007	<0.01	0.35	0.46	0.83	0.23
Fat	0.85[ab]	0.87[a]	0.83[ab]	0.81[b]	0.010	0.00	0.78	0.09	<0.01	0.07
NDF	0.55	0.53	0.55	0.53	0.013	0.13	0.44	0.53	0.73	1.00
ADF	0.22[b]	0.22[b]	0.24[ab]	0.25[a]	0.019	0.05	0.16	0.13	0.63	0.60
Ash	0.44[a]	0.40[ab]	0.32[b]	0.34[b]	0.013	0.20	0.56	0.91	<0.01	0.15
P	0.58	0.56	0.59	0.55	0.020	0.07	0.17	0.85	0.59	0.81
Cys	0.51	0.54	0.46	0.53	0.032	0.49	0.49	0.26	0.64	0.75
Trp	0.77	0.75	0.70	0.79	0.013	0.42	0.21	0.48	0.52	0.05
Arg	0.84	0.84	0.85	0.86	0.008	0.49	0.90	0.17	0.36	0.71
Ser	0.76	0.75	0.79	0.82	0.012	0.96	0.66	0.70	0.12	0.41
Asp	0.80	0.78	0.80	0.81	0.009	0.79	0.76	0.58	0.46	0.44
Glu	0.85	0.82	0.84	0.85	0.008	0.66	0.70	0.52	0.64	0.41
Gly	0.72	0.70	0.73	0.76	0.011	0.83	0.89	0.60	0.26	0.36
Thr	0.82	0.81	0.81	0.84	0.007	0.94	0.69	0.56	0.49	0.24
Ala	0.87	0.87	0.89	0.90	0.006	0.48	0.65	0.47	0.09	0.46
Tyr	0.84	0.82	0.79	0.82	0.007	0.34	0.51	0.36	<0.05	0.10
Prol	0.77	0.72	0.78	0.79	0.012	0.50	0.26	<0.05	0.07	0.17

[2]Diets	HF		LF			P Value				
[2]Enzyme	(+)	(-)	(+)	(-)	SEM	Animal	Enzyme	Period	Diet	Diet*Enzyme
HO-Prol	0.60[b]	0.59[b]	0.66[ab]	0.70[b]	0.019	0.15	0.16	<0.05	<0.01	0.61
Met	0.91[b]	0.90[b]	0.92[b]	0.95[a]	0.009	<0.01	<0.05	<0.01	<0.01	<0.01
Val	0.76	0.74	0.80	0.82	0.012	0.67	0.95	0.54	0.02	0.32
Phe	0.81	0.80	0.83	0.85	0.010	0.63	0.75	0.34	0.13	0.43
Iso Leu	0.77	0.76	0.80	0.82	0.011	0.58	0.79	0.46	0.08	0.36
Leu	0.78	0.77	0.79	0.82	0.010	0.78	0.66	0.50	0.35	0.46
His	0.68	0.71	0.87	0.87	0.036	0.60	0.86	0.83	0.05	0.87
Lys	0.80	0.84	0.86	0.85	0.014	0.46	0.63	0.30	0.25	0.52
Total amino acids	0.81	0.80	0.82	0.84	0.009	0.75	0.84	0.49	0.14	0.49
					Apparent total tract digestibility					
DM	0.74[a]	0.74[a]	0.69[b]	0.68[b]	0.007	0.85	0.53	0.41	<0.01	0.77
OM	0.76[a]	0.76[b]	0.71[b]	0.70[b]	0.007	0.88	0.53	0.43	<0.01	0.65
Energy	0.71[a]	0.71[a]	0.66[ab]	0.65[b]	0.007	0.58	0.39	0.19	<0.01	0.63
Protein	0.79	0.79	0.79	0.78	0.003	0.25	0.86	0.28	0.93	0.98
Fat	0.73	0.70	0.65	0.65	0.015	0.59	0.58	0.15	<0.05	0.49
NDF	0.67[a]	0.63[a]	0.58[b]	0.56[b]	0.009	0.72	<0.01	0.16	<0.01	0.49
ADF	0.35[a]	0.35[a]	0.23[b]	0.19[b]	0.020	0.98	0.34	<0.01	<0.01	0.46
Ash	0.53[a]	0.54[a]	0.45[b]	0.47[b]	0.009	0.07	0.32	0.08	<0.01	0.65
P	0.54	0.53	0.49	0.50	0.014	0.02	0.97	0.72	0.13	0.61

[1]Male, intact, Large White X Landrace, initial live weight 65.0 ± 5.1 kg fed the dietary treatments in a duplicate 4 (period) X 4 (animal) latin square design.

[2]Diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability, each with (+) or without (-) 0.270 g kg^{-1} (as fed basis) Roxazyme® G2.

[ab] For each factor, means within a row with different superscripts were significantly different ($p \leq 0.05$).

Table 7.3: Correlation coefficients of fermentability (mL gas g^{-1} DM), swelling capacity (mL g^{-1} DM), soluble fibre (g kg^{-1} DM), water binding capacity (g g^{-1} DM) and apparent ileal and total tract nutrient digestibility coefficients of growing [1]pigs fed high fibre, maize-soybean and co-product [2]diets contrasting in fermentability

Nutrient	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Water binding capacity		[5]Swelling Capacity	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
				Apparent Ileal digestibility				
Dry matter	0.606	***	-0.459	**	-0.089	ns	-0.038	ns
Organic matter	0.564	**	-0.413	*	-0.115	ns	-0.034	ns
Ash	0.546	**	-0.619	***	0.105	ns	-0.143	ns
Crude protein	0.428	*	0.026	ns	0.172	ns	0.441	*
Fat	0.428	*	-0.309	*	0.025	ns	0.231	ns
Energy	0.580	**	-0.426	*	-0.139	ns	-0.002	ns
P	0.295	ns	0.030	ns	0.181	ns	0.218	ns
NDF	0.029	ns	-0.323	ns	0.215	ns	0.167	ns
ADF	0.086	ns	-0.459	**	0.182	ns	-0.181	ns
Cys	0.003	ns	-0.135	ns	0.428	≠	-0.182	ns
Trp	0.162	ns	-0.331	≠	-0.012	ns	0.237	ns
Arg	-0.370	ns	0.241	ns	0.002	ns	0.305	ns
Ser	-0.190	ns	0.520	*	0.177	ns	0.159	ns
Asp	-0.076	ns	0.243	ns	0.263	ns	0.060	ns
Glu	-0.188	ns	0.146	ns	0.209	ns	0.081	ns
Gly	-0.163	ns	0.371	ns	0.200	ns	0.108	ns

Nutrient	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Water binding capacity		[5]Swelling Capacity	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
Thr	-0.130	ns	0.222	ns	0.016	ns	0.066	ns
Ala	-0.073	ns	0.501	*	0.309	ns	0.304	ns
Tyr	-0.278	ns	0.475	≠	0.146	ns	0.621	**
Prol	-0.129	ns	0.398	ns	-0.145	ns	-0.218	ns
OH-Prol	-0.061	ns	0.591	ns	-0.332	ns	-0.281	ns
Met	-0.227	ns	0.405	ns	-0.325	ns	0.337	ns
Val	-0.284	ns	0.704	**	0.260	ns	0.201	ns
Phe	-0.266	ns	0.441	≠	0.045	ns	0.312	ns
Iso-Leu	-0.274	ns	0.518	*	0.166	ns	0.286	ns
Leu	-0.265	ns	0.295	ns	0.057	ns	0.272	ns
His	-0.160	ns	0.629	**	0.474	ns	0.327	ns
Lys	-0.023	ns	0.318	ns	0.222	ns	0.566	*
Total amino acids	-0.215	ns	0.465	≠	0.208	ns	0.275	ns
			apparent total tract digestibility					
Dry matter	0.470	**	-0.788	***	-0.036	ns	-0.173	ns
Organic matter	0.487	**	-0.770	***	-0.067	ns	-0.177	ns
Ash	0.230	ns	-0.633	****	0.150	ns	-0.093	ns
Crude protein	0.114	ns	-0.015	ns	-0.046	ns	0.048	ns
Fat	0.159	ns	-0.380	*	0.065	ns	0.130	ns
Energy	0.416	*	-0.729	***	0.034	ns	-0.160	ns

Nutrient	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Water binding capacity		[5]Swelling Capacity	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
P	0.204	ns	-0.262	ns	0.194	ns	0.209	ns
Neutral detergent fibre	0.320	ns	-0.489	**	0.085	ns	0.033	ns
Acid detergent fibre	0.330	ns	-0.636	***	0.065	ns	-0.302	ns

ns not significant.
≠ tendency to significance (0.05<p<1.0).
*****Significant at p≤0.05.
******Significant at p≤0.01.
*******Significant at p≤0.001.
[1]Male, intact, Large White X Landrace pigs.
[2]Diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.
[3]Fermentation by pig faecal inoculum of washed residues remaining after the diets were digested by pepsin, followed by pancreatin, according to Bindelle *et al.* (2007).
[4]AOAC (2006) method 991.43.
[5]Water binding by centrifugation and swelling capacity by the bed volume technique, of the whole feed matrix, determined according to Canibe and Bach Knudsen (2001), with modifications.

Table 7.4: Nitrogen intake, excretion patterns and retention (g day^{-1}) by growing [1]pigs fed high fibre, maize-soybean and co-product diets contrasting in fermentability and supplemented with Roxazyme® G2

[2]Diets	HF		LF			P Value				
[2]Enzyme	+	-	+	-	SEM	Animal	Enzyme	Period	Diet	Diet*Enzyme
N intake	118.4	122.8	123.2	123.3	3.05	<0.01	0.58	<0.01	0.51	0.59
Faecal N excretion	25.8	26.5	26.4	26.8	0.93	<0.01	0.64	<0.01	0.68	0.89
Urinary N excretion	44.9	52.3	47.5	49.5	1.68	0.03	0.07	<0.01	0.97	0.28
Retained N	47.7	44.0	49.3	47.0	2.24	0.57	0.45	<0.01	0.57	0.86
Retained N, % of absorbed	0.52	0.46	0.51	0.49	0.02	0.68	0.14	<0.01	0.85	0.64
Urine: Faecal N excretion ratio	1.8	2.0	1.8	1.9	0.08	0.51	0.20	<0.01	0.66	0.92

[1]Male, intact, Large White X Landrace, fed the dietary treatments in a duplicate 4 (period) X 4 (animal) Latin square design.
[2]Growing pig diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain diet.ary fibre of high (HF) versus low (LF), each with (+) or without (-) 0.270 g kg $^{-1}$ (as fed basis) Roxazyme® G2.

7.4 Discussion

The higher AID (DM, OM, fat, ash) and ATTD (DM, OM, fat, ash, ADF, NDF, energy) of nutrients in the HF, compared to the LF diet suggested that screening feeds for DF fermentabillity using *in vitro* methods could be an effective strategy to ameliorate the negative impact of DF on nutrient digestibility. This observation was further supported by the numerically positive correlation between fermentability and nutrient digestibility. The positive effect of fermentability of DF on the same nutrients' ATTD was similar to the findings reported in Chapter 5. It is important to note however, that the soluble DF fraction in PP and in ileal digesta is likely depleted through solubilisation during the extraction of the DF, and through fermentation *in vivo,* respectively. Therefore, positive correlation to the AID of nutrients could be underestimated since it largely reflected the effect of insoluble DF, and not the readily fermentable soluble fraction.

The inefficacy of RX in moderating the effects of DF on nutrient digestibility confirms the low activity of RX reported in the *in vitro* studies in Chapter 3 and 4. In the literature, the effects of NSPases on nutrient digestibility in high DF diets are variable and often conflicting, possibly reflecting the importance of matching the enzymes to the NSP substrates in the diet. For instance, supplemental xylanases and β-glucanases did not affect nutrient digestibility in pigs on wheat-barley-rye diets (Owusu-Asiedu *et al.* 2010).

Inclusion of exogenous βG to a wheat-based diet similarly had no effect on digestibility of DM, ash, neutral detergent fibre or nitrogen (Oshea *et al.,* 2010).

Kerr (2011) compared the efficacy of different commercial enzyme products on maize-soybean diets with 30% maize distiller's grains. The enzyme cocktails had varied and largely insignificant effects on nutrient digestibility. They concluded that the addition of NSPases to maize-soybean-30% DDGS diets had minimal effects on nutrient digestibility in both nursery and finishing pigs.

In contrast, in pigs on wheat based diets containing 20 and 40% wheat millrun, xylanase improved the AID and ATTD of energy (Nortey *et al.,* 2007). In growing pigs on standard wheat-barley-rye diets, supplemental NSPases also increased the AID of energy and caecal

digestibility of starch (Willamil *et al.,* 2012). However, the enzyme had no effect on maize based diets. Supplemental NSPases improved the AID of DM, OM and energy of barrows fed diets with 15 and 30% DDGS (Emiola *et al.* 2009). In growing pigs fed mixed grains and co-product based diets, supplemental NSPases increased AID of energy (Kiarie *et al.,* 2010). On standard maize soybean diets, Ao *et al.* (2010) reported improved AID of N due to supplemental enzymes. In finishing pigs fed wheat barley and co-product diets, supplemental NSPases increased the ATTD of DM, GE and N (Kiarie *et al.,* 2012).

Given the high cost of synthetic supplementary amino acids and of protein concentrates, of major concern to pig producers is the impact of DF on the utilisation of amino acids, and on overall N metabolism. In the current study, except for the higher AID of OH-Prol and Met in the LF diet, neither fermentability of DF nor RX affected the AID of amino acids, and the efficiency of utilisation of N. Interestingly, although not statistically significant, the relationship between the AID of amino acids and fermentability was negative. Dietary DF may influence amino acid availability via bacterial fermentative catabolism (Columbus *et al.,* 2010) or *de novo* synthesis (Torrallardona *et al.,* 2003; Libao-Mercado *et al.,* 2009; Zhu *et al.,* 2007). The tendency toward negative correlation of the AID of amino acids with fermentability therefore suggests a net increase in amino acid supply from higher *de novo* biosynthesis of amino acids in the pigs on the HF diets. This is consistent with previous research (Dierick *et al.,* 1983, cited by Bindelle *et al.,* 2008) which showed that, depending on the diet, up to 30% of N in distal ileal digesta is of microbial origin. There is dearth of information on how the fermentation characteristics of different types of DF influence the amino acid profile absorbed from the ileum.

In the current study, RX did not affect the AID of amino acids. In contrast, RX improved the AID of pigs on rye based diets (Nitrayova *et al.,* 2007). In pigs on wheat based diets containing 20 and 40% wheat millrun, xylanase combined with phytase to improve the AID of Ile and Phe, and tended to improve the AID of Leu, Thr, and Tyr (Nortey *et al.,* 2007). A cocktail containing β-glucase, cellulase and xylanase enzymes supplemented to 15% and 30% wheat DDGS-based diets improved the AID of Thr, Pro and Ser (Emiola *et al.,* 2009). On standard maize-soybean diets, Ao *et al.* (2010) reported improved the AID of all essential amino acids, and of total amino acids in response to β-Glucanase, xylanase, α-

galactosidase and galactomannase supplements. In growing pigs fed mixed grains and different co-product based diets, supplemental β- glucanase and xylanase NSPases increased the AID of amino acids, and improved the standardized ileal digestibility of Met and Thr (Kiarie *et al.,* 2010). Overall, it appears the efficacy of NSPases depends on enzyme activity level, and composition of dietary NSP (Emiola *et al*, 2009). Due to the low content of insoluble DF and the high fermentabilty of maize fibre, enzyme efficacy is more likely in wheat, rye or barley and oat based diets than in maize diets (Willamil *et al*., 2012).

In the current study, the large contrast in the fermentability of DF in the experimental diets did not result in different N excretion patterns and N retention. Ureolytic bacteria maintain a concentration gradient that favours a net transfer of BUN into the caecal lumen (Younes *et al.,* 1995; Mosenthin *et al.,* 1992). With increased fermentation, the assimilation of the BUN increases the amount of N present in the faeces and decreases N excretion as urea in urine (Younes *et al.,* 1995). Few studies have investigated the independent effect of DF fermentability on N excretion and retention using practical diets similarly contrasted in fermentability. A study by Hooda *et al.* (2011) used maize starch and casein diets supplemented with purified DF of different fermentability and viscosity. Neither DF viscosity nor its fermentability affected the pattern of N excretion, and N retention. However, DF fermentability tended to interact with viscosity to affect the faecal:urinary N ratio, whereby pigs fed a poorly fermented, high viscous DF diet had a lower faecal:urinary N ratio than those fed a highly fermentable, high viscous DF diet. Other previous research evaluated the effect of increased fermentation on N excretion by using high dietary levels of highly fermentable DF. For example, Canh *et al.* (1997) reported a ratio of faecal to urinary N of 3.8 to 1.2 on high, compared to low DF diets, respectively. Sugar beet pulp, containing highly fermentable soluble DF, also increased the faecal to urinary N ratio to 2.171, compared to 1.177 for less fermentable oat hulls (Bindelle *et al.,* 2009). Addition of beet pulp DF to high protein standard DF diets similarly increased fecal:urinary N ratio from 2.1 to 3.1 and increased overall N retention (Patrás *et al*, 2012).

In vitro studies by Bindelle *et al.* (2011) suggested that NSP-degrading enzymes can substantially alter fermentation in the lower gut. In the current study, it was hypothesised that by stimulating fermentation, RX could promote the pre-caecal *de novo* bacteria

synthesis of essential amino acids, and therefore improve N retention. Roxazyme could also increase the assimilation of BUN into microbial protein, and thereby increase the faecal:urinary N ratio. However, RX only numerically reduced the urinary/faecal excretion ratio, and only numerically increased retained N. Higher N retention could result from effects such as decreased endogenous wastage, increased AID or increased efficiency of utilisation of amino acids as a result of *de novo* bacterial biosynthesis of limiting amino acids in the ileum.

Despite similar hydration or bulking properties of the HF and LF diets, there was positive correlation between the AID of amino acids and DF solubility, water binding and swelling capacity of DF. The variation could reflect animal variance in the capacity to control the hydration properties of DF such that in pigs with poor control, high water binding by the soluble DF reduced the passage of fluid digesta (Wilfart *et al*., 2007; Solà-Oriol *et al*., 2010), and thereby promoted greater absorption of amino acids.

7.5 Conclusion

Fermentability has a positive effect on the digestibility of DM, OM, energy, fat and DF. Negative correlation between DF fermentability and the AID of amino acids suggested that formulating diets for fermentability of the insoluble DF fraction may increase the *de novo* biosynthesis of amino acids in the ileum. Fermentability did not affect N excretion pattern and retention, which suggested minimal impact on the overall efficiency of utilisation of N. The use of RX to improve nutrient digestibility of growing pigs on co-product-rich maize-soybean diets was not justified.

CHAPTER 8

BIOCHEMICAL EFFECTS OF FIBRE AND ROXAZYME® G2 IN GROWING PIGS FED MAIZE-SOYBEAN DIETS CONTAINING HIGH LEVELS OF CO-PRODUCT FEEDS.

Abstract

The biochemical influences of DF and RX were examined within the growth and balance trials described in Chapter 5 and 7, respectively. In the growth trial (Experiment 1), blood samples were collected directly from the severed *vena jugularis* at slaughter, 16 hours postprandial. In the balance trial (Experiment 2), blood was sampled via *vena jugularis* puncture on the last day of each sampling period, 15 hours (15-hour sample) postprandial, and again 3 hours (3-hour sample) after re-introduction to feed. Serum cholesterol, urea, glucose, triglycerides and creatinine were chemically analysed. Further, non-targeted metabolite analyses were performed using Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMRS). In Experiment 1, pigs on the WB diets had higher cholesterol compared to pigs on the MC and L diet. There was significant diet X enzyme interaction for urea, whereby RX increased the serum level in pigs fed the WB and L diets, with no or opposite effect in pigs on the other diets. ^{1}H-NMRS indicated Cys and His were higher in pigs on the WB diet, compared to pigs on the MC, LH, SH and STD diets. ^{1}H-NMRS also indicated that RX reduced formate but increased glucose and urea (all diets except (WB). There was significant diet X enzyme interaction for fructose, glucose, Arg and Cys, and possibly, Ser and Trp, whereby RX increased the levels in pigs on MC and WB, with opposite effect in pigs on the other diets. In Experiment 2, there was significant diet X enzyme interaction for chemically analysed urea in the 15-hour sample, whereby the enzyme reduced the serum level in pigs on the HF diets, with opposite effect in pigs on the LF diet. Fermentability and negatively correlated with plasma urea in the 15-hour sample and positively correlated with glucose in the 3-hour sample. In the 3-hour sample, ^{1}H-NMRS indicated higher fucose, Pro and cholesterol in the LF diet. ^{1}H-NMRS also indicated significant fermentability x RX interaction for Ser, Tyr, Lys, creatine, and possibly, glucose or

fructose, glycerol or Gly and His or Arg, whereby RX increased the metabolite concentrations in the LF diets, with opposite effect in the HF diet. There were no significant treatment effects in the 15-hour sample. In conclusion, feeding growing pigs maize-soybean diets containing high levels of fibrous feeds and supplemented with RX had minimal effect on the serum metabolome, which was restricted to sugars, cholesterol, formate, Pro, urea and some amino acids.

***Keywords**; Growing pigs, dietary fibre, fermentability, enzymes, maize-soybean, co-product feedstuffs, metabonomics.*

8.1 Introduction

Depending on the source, high levels of DF in growing pig diets influence the metabolism of energy (Noblet and Le Goff, 2001), amino acids (Zhu *et al.,* 2007; Columbus *et al.,* 2010; Libao-Mercado *et al.,* 2009), N (Święch *et al.,* 2010), lipids and glucose (Guillon and Champ, 2000). Alteration of nutrient metabolism may affect important production variables such as feed efficiency, animal performance, meat quality and the carbon and N footprints (Zijlstra and Beltranena, 2013).

Tracking the exogenous and endogenous metabolite fluxes in the circulatory system could further our understanding of the mechanisms that underpin the metabolic and physiological activity of DF, and the modifying effects of NPSases. Despite its chemical diversity, the digestive metabolic and physiological activity of DF is largely controlled by the shared properties of fermentability, viscosity, swelling, water binding and adsorption (Bach Knudsen *et al.,* 2001). Through its metabolites, in the growing pig, fermentation has arguably the most profound influence on the metabolic and physiological functions (Williams *et al.,* 2001; Bach Knudsen *et al.,* 2012). NSPases break down the complex structure of NSP and the cell wall matrix, and thereby modify the physicochemical properties (Willamil *et al.,* 2012).

Factors that complicate the DF question include its chemical diversity (Bach Knudsen *et al.,* 2001), matching the biochemical characteristics of NSPases to diverse NSP compositions and gut conditions (Paloheimo *et al.,* 2011) and the complex and dynamic animal and gut

microbial metabolic interaction (Bedford and Cowieson, 2012). In addition, homeostasis and its inter-animal variation mask the characteristically subtle diet-induced perturbations of the internal environment (Claus and Swan, 2013). Traditional chemical analyses for blood metabolites are therefore inadequate.

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) holistically explores changes in the plasma metabolome with greater sensitivity (Claus and Swan, 2013). In pig nutrition, interest in ^{1}H-NMRS based metabonomics is increasing, as nutritionists seek to expand the feed base to include unconventional feeds, whose nutrient and anti-nutrient composition is still largely not defined. For instance, naturally occurring metabolic modifiers have been identified by ^{1}H-NMRS in some co-product feedstuffs. Bertram *et al.* (2006) reported high levels of betaine in the urine of pigs fed high-DF rye bread. Bertram *et al.* (2009) also reported high levels of betaine in plasma of rye-DF fed pigs. Yde *et al.* (2011) reported high plasma levels of dimethyl sulfone in sows fed diets rich in sugar beet pulp and pectin residue, and high levels of betaine in sows on sugar beet pulp. Dimethyl sulfone is associated with a higher rate of intestinal fermentation. Betaine is linked to high protein accretion and depressed fat deposition, with positive effects on pork quality (Matthews *et al.,* 2001; Fernandez-Figares *et al.,* 2002; Lawrence *et al.,* 2002; Huang *et al.,* 2009).

The aim of the study was to examine the biochemical influences of DF and of RX in growing pigs fed maize-soybean diets containing high levels of different fibrous feed ingredients.

8.2 Methods and Materials

8.2.1 Blood sampling, chemical analyses and ^{1}H-NMRS

Experiment 1: During slaughter of the animals at the termination of the growth trial described in Chapter 5, blood samples were collected from the severed *vena jugularis,* 16 hours postprandial. To determine glucose, a sample was collected into BD Vacutainer® Fluoride Tubes containing a glycolytic inhibitor. To determine the other metabolites, a second sample was collected into BD Vacutainer® Plus Plastic Serum tubes. The blood was immediately centrifuged in a Hettich Universal 320 centrifuge (Labotec (PTY) LTD) at 4000 X g for eight

minutes. A Cobas Integra 400 Plus auto-analyzer (Roche, PVT LTD) was used to determine metabolite indicators of energy, lipid and amino acid metabolism which included glucose, triglycerides, total cholesterol, creatinine and blood urea N (BUN). Cobas Integra glucose, triglyceride, cholesterol, creatinine Jaffe, and blood urea nitrogen tests were sued, respectively. Serum for the ^{1}H-NMR analyses was stored at -80 ^{o}C until analyses after 30 days.

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) was conducted according to Yde *et al.* (2011). Aliquots (200 µl) of the serum samples were mixed with a solution of 400 µl 0.9% saline and 20% D_2O. These were transferred to a new NMR tube and analysed on a 600 MHz Agilent ^{unity}Inova NMR spectrometer operating at a ^{1}H frequency of 600 MHz and equipped with a dual channel 5-mm Inverse detection Pulsed Field Gradient probe. VnmrJ 2.1B software was used to operate the instrument. A standard s2pul pulse sequence to acquire a 1D-^{1}H dataset with a pre-saturation option (Gueron *et al.,* 1991) to suppress strong signals were utilized. The sequence features the option to suppress single peaks with a hard pulse and multiple peaks with a shaped pulse. The (H) PRESAT sequence was applied for acquisition of ^{1}H-NMR spectra, with a 2 second saturation delay utilized. A 2-step purge option was included to improve saturation efficiency for large water peaks. The spectra were acquired using 64 scans into 32k data points on a spectral width of 15 parts per million (ppm) at a temperature of 298 K. A fixed receiver-gain value was used for recording all spectra. An exponential line-broadening of 0.3 Hz was applied prior to the Fourier transformation. Each spectrum was manually phased, baseline corrected and referenced to a lactate doublet signal at 1.33 ppm.

Experiment 2: The experiment was conducted within the balance trial described in Chapter 7. On the last day of each sampling period, blood was sampled by puncture of the *vena jugularis,* into vacutainer tubes as described for Experiment 1. The first blood sample was collected 15 hours postprandial (15-hour sample). A second sample was collected three hours after reintroduction to feed (3-hour sample). Chemical and ^{1}H-NMRS analyses for serum metabolites were as described for Experiment 1.

8.2.2 Statistical Analysis

8.2.2.1 Chemically analysed serum metabolites

ANOVA on the chemically analysed serum metabolites was performed using the PROC MIXED procedures of SAS software, version 9.3 (SAS, 2010). Data from Experiment 1 were analysed using mode II, as described in Chapter 3. Data from Experiment 2 were analysed using the model III, as described in Chapter 4.

Treatment LS means were separated using the Bonferroni t-test at the α-level of 0.05. Values of $0.05<p<0.1$ were considered tendencies towards significance. Pearson's product-moment correlation coefficients between the physico-chemical properties of solubility, fermentability, swelling capacity, solubility, water binding capacity of ileal digesta and the chemically analyses serum metabolites were calculated using the PROC CORR statement of SAS software, version 9.3 (SAS, 2010).

8.2.2.2 ^{1}H-NMR data analysis

The ^{1}H PRESAT spectra were subdivided into bins of 0.013 ppm and integrated. The resulting data sets were then imported into SIMCA-P+ version 12.0 (Umetrics, Umea, Sweden). The unsupervised method of principal component analysis (PCA) was applied on the spectra to determine the clustering behaviour. The analysis was carried out using mean-centred data and Pareto scaling. Multivariate analysis was performed excluding the water signal in the region 4.4–5.0 ppm. ANOVA was further performed to identify NMR spectra where there were significant treatment effects, to quantify the treatment effects and to analyse variances. The ANOVA of the NMR data was performed as described for the chemical analytes.

8.3 Results

8.3.1 Experiment 1

8.3.1.1 Chemically analysed metabolites

The chemical analyses for serum metabolites are indicated in **Table 8.1.** Correlation coefficients of the physico-chemical properties of DF and the serum metabolite concentrations are indicated in **Table 8.2**.

Pigs on the WB diets had higher cholesterol compared to pigs on the MC and LH diet. Correlation analyses revealed a tendency (p=0.097) towards significant negative correlation between fermentability and total cholesterol. The enzyme did not affect any of the chemically analysed metabolites. However, there was significant diet X enzyme interaction for urea, whereby RX tended to increase the serum level in pigs fed the WB and L diets, with no or opposite effect in pigs on the other diets.

8.3.1.2 ^{1}H-NMR evaluation

Figure 8.1 shows typical ^{1}H-NMR spectra of serum samples taken from pigs on the STD and on the test diets. **Figure 8.2** shows PCA plots based on all the ^{1}H-NMR spectral data, illustrating weak clustering behaviour. Results of the ANOVA on the NMR data are summarised in **Table 8.3**. The probable spectral assignments were according to Lindon *et al.* (1999). Only the data with significant diet, RX or diet X RX effects and which could be assigned are indicated. The means are presented as the ratio between the control and the test diets. Spectra indicating Cys and His were higher in pigs on the WB diet, compared to pigs on the MC, LH, SH and STD diets. RX reduced spectra indicating formate but increased the spectra indicating glucose and urea (all diets except (WB).

There was significant diet X enzyme interaction for spectra indicating fructose, glucose, Arg and Cys, and possibly, Ser and Trp, whereby RX increased the levels in pigs on MC and WB, with opposite effect in pigs on the other diets.

Table 8.1: Chemically analysed sixteen-hour postprandial serum metabolite composition (mMol L^{-1}) of growing [1]pigs fed maize-soybean- co-product diets with Roxazyme® G2

Component	[2]Diets						[3]Enzyme			P Value		
	STD	BG	LH	MC	SH	WB	-	+	SEM	Diet	Enzyme	Enzyme X Diet
Urea N	5.0	5.0	5.4	6.1	6.1	6.7	5.8	5.7	0.23	0.07	0.92	<0.05
Total Cholesterol	2.0^{abc}	2.2^{ab}	1.8^{c}	2.0^{bc}	2.0^{abc}	2.3^{a}	2.1	2.0	0.18	<0.01	0.49	0.11
Creatinine	144.2	146.3	132.5	140.1	144.1	153.4	143.2	143.6	0.16	0.48	0.94	0.49
Glucose	5.8	5.8	5.7	5.1	5.0	4.9	5.4	5.3	0.20	0.20	0.63	0.93
Triglycerides	0.425	0.455	0.449	0.466	0.476	0.554	0.475	0.465	0.321	0.56	0.76	0.35

[1]Male, intact, Large White X Landrace growing pigs.

[2]STD- standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran). Diets were fed over a 10 week period

[3]Roxazyme® G2.

[ab]For each factor, means in a row which do not share a common superscripts are significantly different ($p \leq 0.05$).

Table 8.2: Correlation coefficients of fermentability (mL gas g^{-1} DM), swelling capacity (mL g^{-1} DM), soluble fibre (g kg^{-1} DM), water binding capacity (g g^{-1} DM), and serum metabolite concentrations of growing [1]pigs fed maize-soybean [2]diets

Serum Metabolites	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Swelling capacity		[5]Water binding capacity	
	Correlation Coefficent	p	Correlation Coefficent	p	Correlation Coefficent	p	Correlation Coefficent	p
Urea N	-0.103	ns	0.067	ns	-0.117	ns	0.142	ns
Creatinine	-0.050	≠	0.047	ns	-0.081	ns	-0.064	ns
Total Cholesterol	-0.211	Ns	0.173	ns	-0.063	ns	-0.011	ns
Triglycerides	-0.119	Ns	0.148	ns	-0.067	ns	0.115	ns
Glucose	0.040	Ns	0.026	ns	0.122	ns	-0.065	ns

ns not significant (p>0.05).

≠Tendency towards significance (0.05<p<0.1)

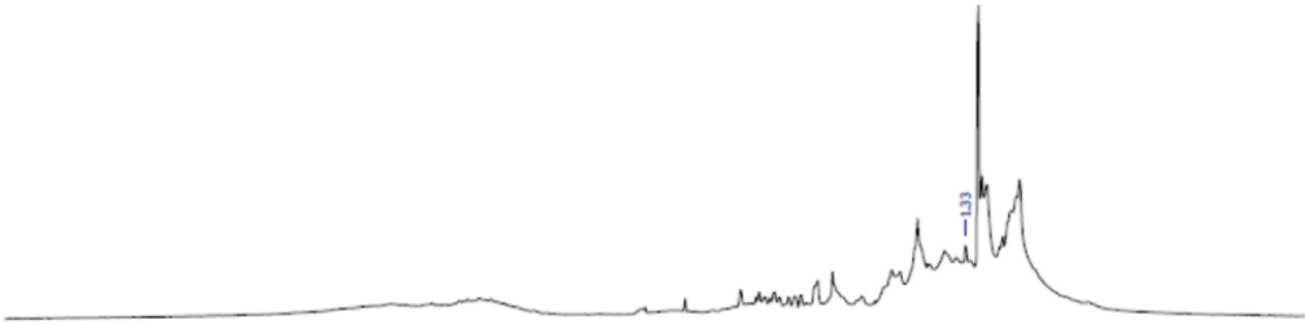
[1]Male, intact, Large White X Landrace growing pigs.

[2]Standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted brewer's grains, lucerne hay, maize cobs, soy hulls, wheat bran to contain 246 g total dietary fibre kg^{-1} DM. Diets were fed over a 10 week period.

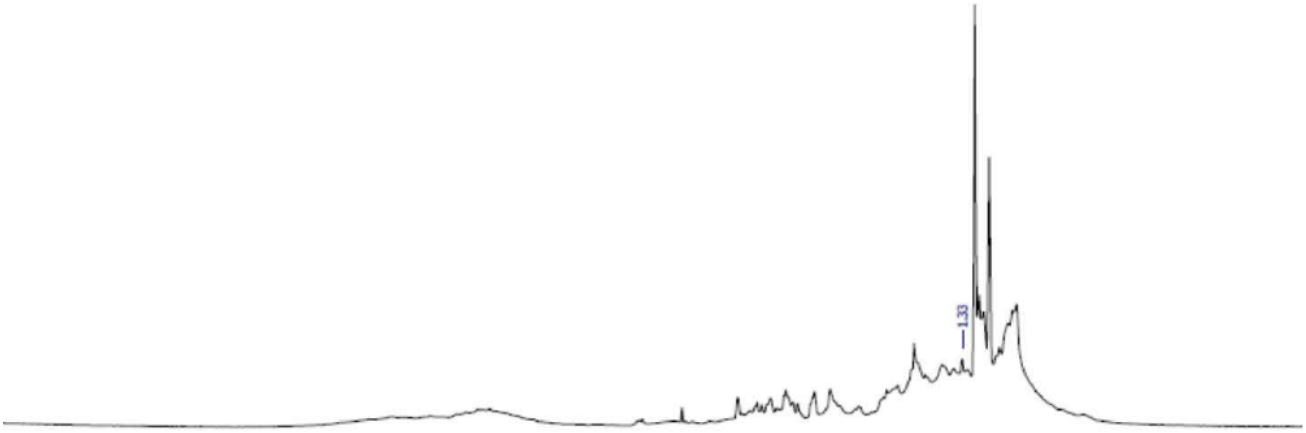
[3]Fermentation by pig faecal inoculum of washed residues remaining after the diets were digested by pepsin (porcine, 200 FIP-U/g, Merck No, 7190), followed by pancreatin (porcine, grade IV, Sigma No P-1750), according to Bindelle *et al.* (2007).

[4]AOAC (2006) method 991.43.

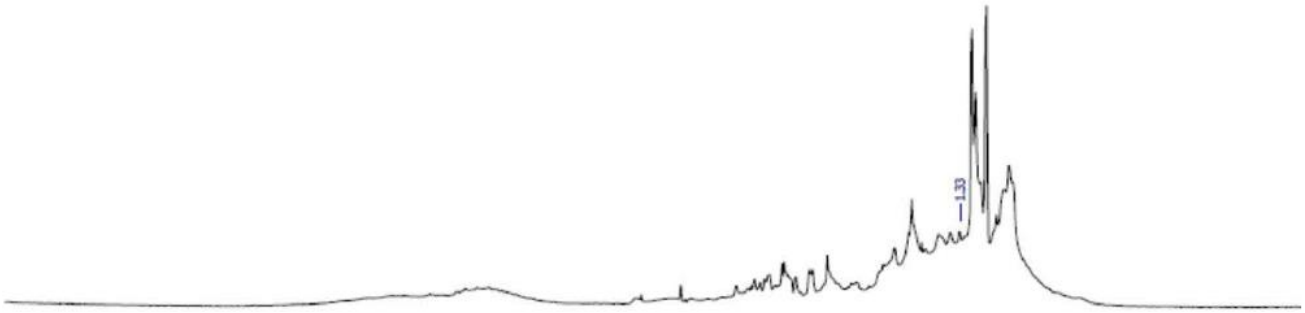
[5]Water binding by centrifugation and swelling capacity by the bed volume technique, of the whole feed matrix, determined according to Canibe and Bach Knudsen (2001), with modifications.



Standard diet



Maize cob diet



Soy hulls diet

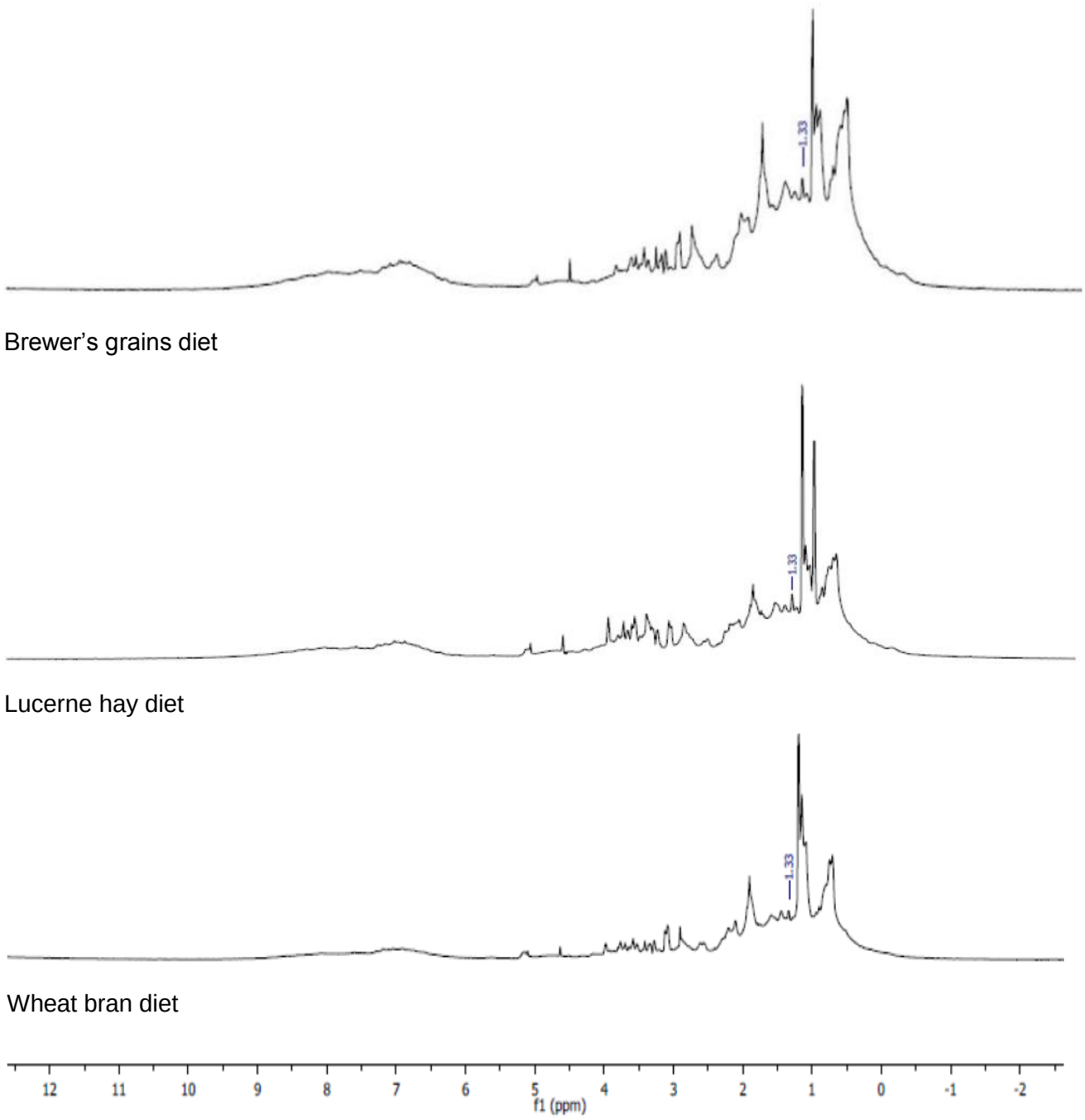
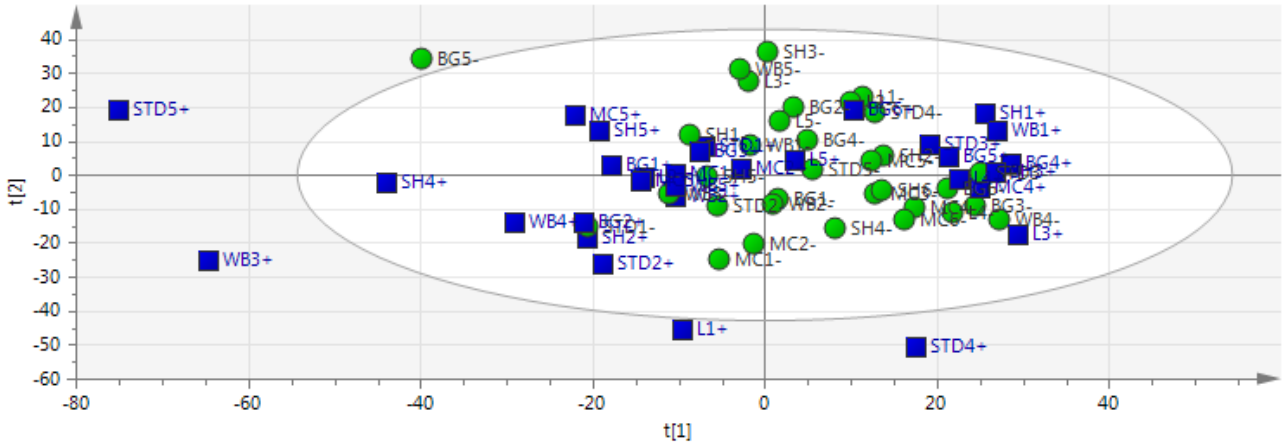


Figure 8.1: Typical ^{1}H-NMR spectra of 16-hour postprandial serum from pigs fed a standard, 141 g total dietary fibre kg^{-1} DM maize-soybean growing pig diet, and diets in which the basal ingredients were partially substituted with different feeds to contain 246 g total dietary fibre kg^{-1} DM. The lactate standard is indicated at 1.33 ppm



R^2 [1] = 0.497 R^2 [2] = 0.309 Ellipse: Hoteling's T2 (95%)

Figure 8.2: Score scatter plots for PCA models of ^{1}H-NMRS spectra of the 16-hour postprandial serum from growing pigs fed a standard (STD), 141 g total dietary fibre kg^{-1} DM maize-soybean growing pig diet, and from high fibre diets in which the basal ingredients of the STD diets were partially substituted with brewer's grains (BG), maize cobs (MC), Lucerne hay (LH), soy hulls (SH) or wheat bran (WB) to contain 246 g total dietary fibre kg^{-1} DM. The diets were fed with (+, ●) or without (-, ■) Roxazyme® G2. The numbers (1-6) on the dietary treatments denote replicates.

Table 8.3: ANOVA of [1]H-NMRS data of 16-hour postprandial serum samples from growing [1]pigs showing possible assignments to spectra with significant diet, enzyme or diet X enzyme interaction effects

δ[1]H	Assignment	Metabolite	Multiplicity	[2]Diet						[3]Enzyme		P Values		
				STD	BG	LH	MC	SH	WB	+	-	Diet	Enzyme	Diet X Enzyme
8.47	CH	Formate	s	1.00	-0.05	0.09	0.76	0.22	1.00	13.0[a]	1.00[b]	0.53	<0.05	0.53
5.78	NH2	Urea	s	1.00	0.95	0.69	0.83	0.85	0.95	1.30[a]	1.00[b]	0.95	0.04	0.95
5.23	CH1	α-Glucose	d	1.00	0.65	0.60	0.76	0.75	0.80	1.45[a]	1.00[b]	0.80	<0.05	0.80
3.85	H6	Fructose (β-furanose)	m	1.00	1.13	1.30	1.18	1.34	1.28	0.99	1.00	0.83	0.93	0.03
	α-CH	Serine	ABX											
3.82	H4	Fructose (β-furanose)	m	1.00	1.22	1.49	1.20	1.37	1.41	0.97	1.00	0.59	0.80	<0.05
3.77	α-CH	Arginine	t	1.00	0.98	1.09	1.07	1.14	1.34	1.07	1.00	0.67	0.57	0.03
3.64	H3	Fructose (β-pyranose)	m	1.00	1.08	1.41	1.18	1.31	1.49	1.02	1.00	0.68	0.86	0.04
3.49	CH3	β-Glucose	t	1.00	1.16	1.37	1.20	1.34	1.29	1.05	1.00	0.54	0.62	0.02
	CH2	Tryptophan	ABX											
3.47	C-H5	β-Glucose	dd	1.00	1.19	1.51	1.19	1.34	1.37	1.00	1.00	0.29	0.97	0.03
3.14	β-CH2	Histidine	ABX	1.00[b]	1.34[b]	1.69[ab]	1.22[b]	1.24[b]	2.87[a]	1.10	1.00	0.58	0.22	0.22
3.12	CH2	Cysteine	ABX	1.00[b]	1.06[b]	1.14[ab]	0.99[b]	1.07[b]	1.71[a]	1.07	1.00	0.54	0.08	0.08

[1]Male, intact, Large White X Landrace growing pigs.

[2]STD- standard, 141 g total dietary fibre kg^{-1} DM maize-soybean diet and diets in which the basal ingredients were partially substituted with the fibrous feeds to contain 246 g total dietary fibre kg^{-1} DM - BG (brewer's grain), LH (lucerne hay), MC (maize cobs), SH (soy hulls), WB (wheat bran).

[3]Roxazyme® G2.

[ab]For each factor, means in a row which do not share a common superscripts are significantly different (p≤0.05).

8.3.2 Experiment 2

8.1.1.1 Chemically analysed metabolites

The chemically analysed serum metabolite concentrations in the fasting and absorptive states are indicated in **Table 8.4** Correlation coefficients of the physico-chemical properties of DF and the serum metabolite concentrations are indicated in **Table 8.5**.

Neither fermentability nor RX affected the chemically analysed serum metabolites. However, there was significant diet X enzyme interaction for serum urea in the 15-hour postprandial serum, and only a tendency (p=0.0629) in the 3-hour postprandial serum sample, whereby RX tended to increase the serum urea in the LF diet, while it decreased that of the HF diet. There was also tendency (p=0.072) towards significant diet X enzyme interaction for serum cholesterol in the 3-hour postprandial serum, whereby the enzyme tended to increase total cholesterol on the LF, and not on the HF diet. Water binding positively correlated (p=0.0075) with glucose concentration in the 15-hour postprandial serum. Swelling positively correlated with glucose (p=0.0087) and total cholesterol (p=0.0296) in the 15-hour postprandial serum, and tended (p=0.057) to positively correlate with glucose in the 3-hour postprandial serum. Fermentability and negatively correlated with serum urea N (p=0.0020) in the fasting state, with only a tendency (p=0.066) in the 3-hour postprandial serum. Fermentability positively correlated (p=0.0133) to serum glucose in the 3-hour postprandial serum3-hour postprandial serum.

8.1.1.2 ^{1}H-NMR profiles

Figure 8.3 shows ^{1}H-NMR spectra from the 15 and 3-hour samples after feeding on the different dietary treatments. The spectra are from the 1st period in the Latin square experimental design, and from pigs on diets without RX. **Figure 8.4** shows PCA plots based on all the ^{1}H-NMR spectral data form the 3-hour postprandial serum, illustrating the weak clustering behaviour of the data by the treatments. The results of the ANOVA of the spectral data are summarised in **Table 8.6.** Only the data with significant diet, RX or diet x RX effects and which could be assigned are indicated. The probable spectral assignments were also according to Lindon *et al.* (1999). The means for the diets are presented as the ratio of

Table 8.4: Chemically analysed serum metabolite concentrations in growing [1]pigs fed high fibre, maize-soybean and co-product diets contrasting in fermentability and supplemented with Roxazyme® G2

[2]Diets	High Fermentability		Low fermentability			P Value				
[3]Enzyme	(+)	(-)	(+)	(-)	SEM	Animal	Enzyme	Period	Diet	Diet * Enzyme
15 hours postprandial										
Urea N	6.65	6.83	7.29	6.78	0.223	<0.001	0.580	0.000	0.119	0.047
Creatinine	14.25	103.95	110.85	108.88	3.382	0.007	0.815	0.034	0.246	0.862
Glucose	4.86	4.85	4.84	5.00	0.035	0.035	0.653	0.794	0.613	0.627
Total cholesterol	1.98	1.92	1.92	1.82	0.094	0.008	0.128	0.410	0.103	0.710
Triglycerides	0.28	0.25	0.33	0.29	0.019	0.249	0.317	0.267	0.616	0.887
3 hour postprandial, following 15 hour fast										
Urea N	5.12	5.44	5.37	4.90	0.167	0.010	0.985	0.627	0.569	0.063
Creatinine	99.69	100.88	107.47	104.25	2.968	0.001	0.913	0.002	0.248	0.676
Glucose	4.23	4.33	4.13	4.25	0.086	0.017	0.362	0.082	0.465	0.967
Total cholesterol	1.83	1.87	2.02	1.84	0.035	0.054	0.297	0.534	0.219	0.074
Triglycerides	0.32	0.31	0.33	0.30	0.010	0.291	0.366	0.177	0.910	0.585

[1]Male, intact, Large White X Landrace growing pigs.

[2]Diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.

[3]Each diet was supplement with (+) or without (-) 0.270 g kg $^{-1}$ (as fed basis) Roxazyme® G2.
[ab] For each factor, means within a row with different superscripts were significantly different ($p \leq 0.05$).

Table 8.5 Correlation coefficients of fermentability (mL gas g^{-1} DM), swelling capacity (mL g^{-1} DM), soluble fibre (g kg^{-1} DM), water binding capacity (g g^{-1} DM) and serum metabolites of growing [1]pigs fed high fibre, maize-soybean and co-product [2]diets contrasting in fermentability and supplemented Roxazyme® G2

Metabolite	[3]Fermentability of dietary fibre		[4]Solubility of dietary fibre		[5]Water binding capacity		[6]Swelling Capacity	
	Correlation Coefficent	p	Correlation Coefficent	p	Correlation Coefficent	p	Correlation Coefficent	p
				15 hours postprandial				
Urea N	-0.541	**	0.118	ns	0.163	ns	0.233	ns
Creatinine	-0.238	ns	0.159	ns	-0.093	ns	0.159	ns
Glucose	0.231	ns	0.082	ns	0.478	**	0.470	**
Total cholesterol	0.233	ns	-0.207	ns	0.195	ns	0.398	*
Triglycerides	0.145	ns	0.209	ns	0.158	ns	0.099	ns
				3 hours postprandial, following 15 hour fast				
Urea N	-0.352	≠	-0.056	ns	0.128	ns	0.021	ns
Creatinine	0.061	ns	0.142	ns	-0.025	ns	0.104	ns
Glucose	0.139	**	0.195	ns	0.163	ns	0.364	≠
Total cholesterol	0.076	ns	-0.018	ns	0.212	ns	0.179	ns
Triglycerides	0.447	ns	-0.093	ns	-0.015	ns	-0.157	ns

ns not significant.

≠Tendency towards significance ($0.05<p<0.1$)

*Significant at $p \leq 0.05$.

**Significant at $p \leq 0.01$.

[1]Male, intact, Large White X Landrace growing pigs.

[2]Diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.

[3]Fermentation by pig faecal inoculum of washed residues remaining after the diets were digested by pepsin (porcine, 200 FIP-U/g, Merck No, 7190), followed by pancreatin (porcine, grade IV, Sigma No P-1750), according to Bindelle *et al.* (2007).

[4]AOAC (2006) method 991.43.

[5]Water binding by centrifugation and swelling capacity by bed volume technique, of the whole feed matrix, determined according to Canibe and Bach Knudsen (2001), with modifications.

the high, to that of the LF diet, and of the no RX diet, to the RX treated diet. Spectra indicating fucose, Pro and cholesterol were higher in the LF diet. There was significant fermentability x RX interaction for spectra indicating Ser, Tyr, Lys, creatine, and possibly, glucose or fructose, glycerol or Gly and His or Arg, whereby RX increased the levels in the LF diets, with opposite effect in the HF diet. There were no significant treatment effects in the 15-hour sample.

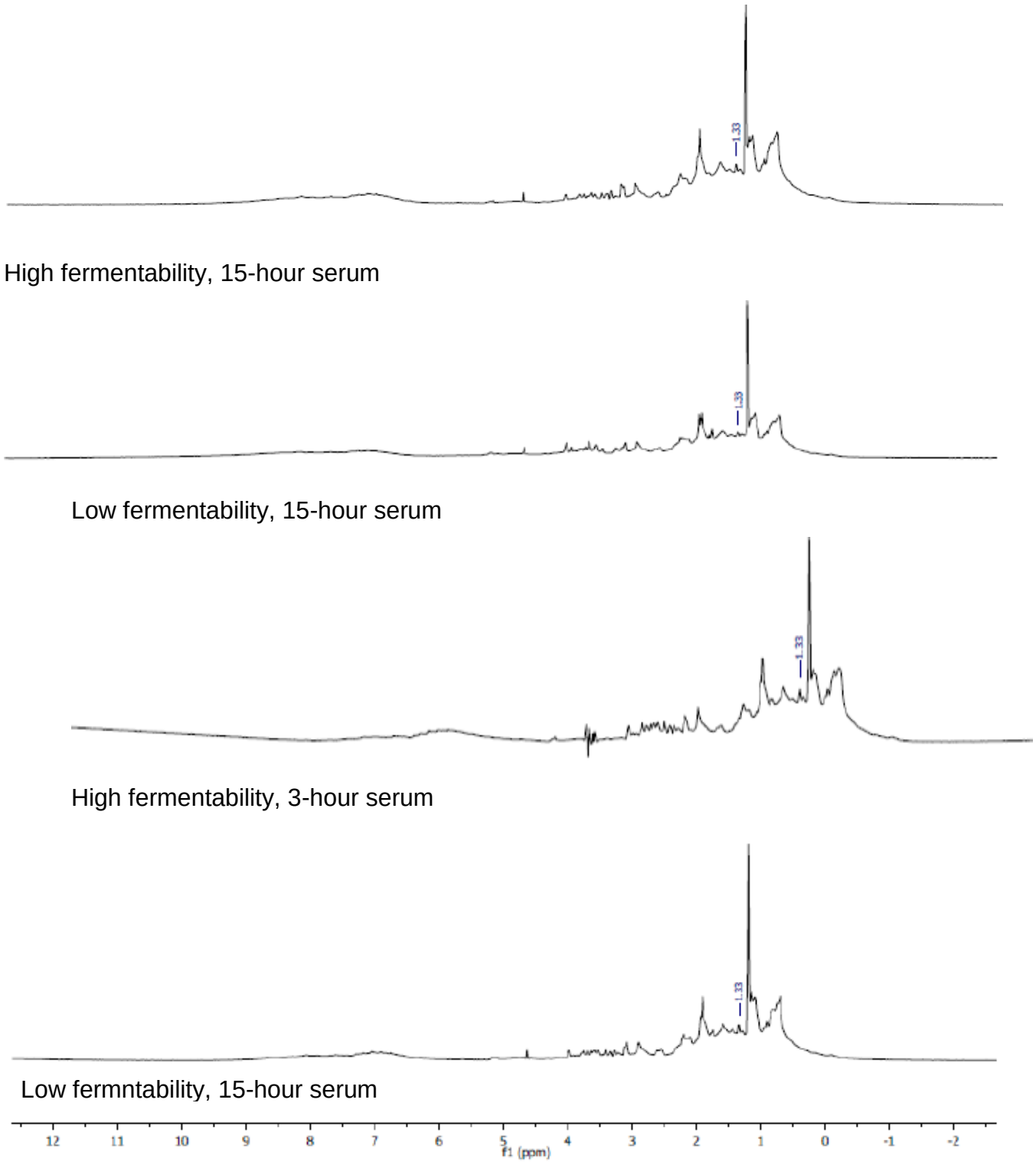
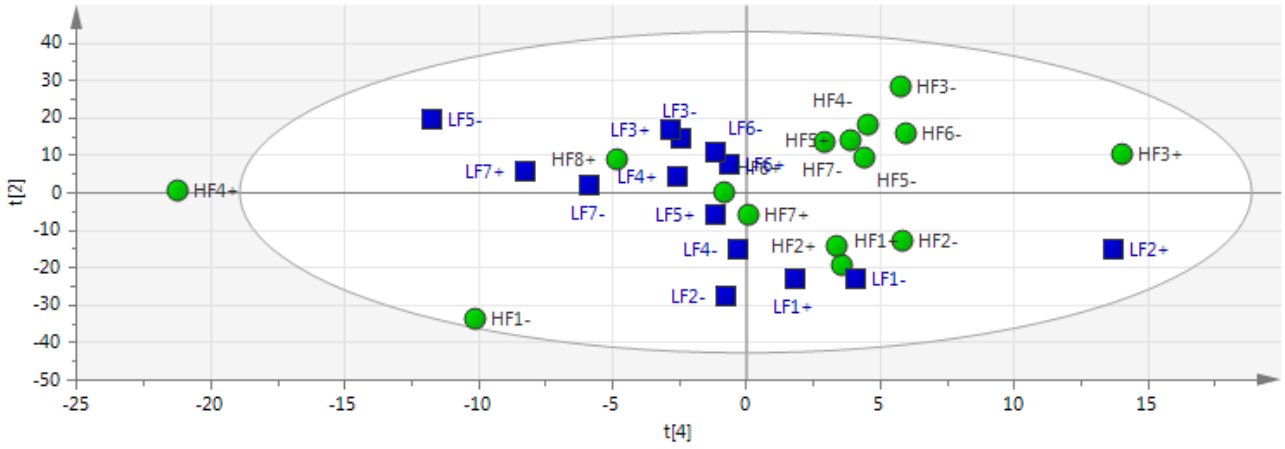
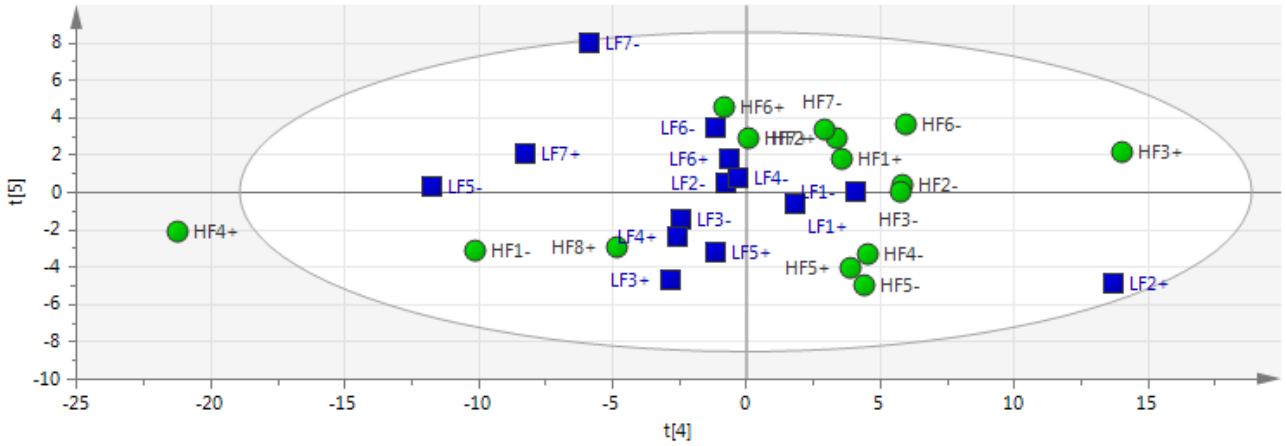


Figure 8.3: Typical ^{1}H-NMR spectra of 15 and 3 hour postprandial serum from pigs fed diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high versus low) fermentability. The lactate standard is indicated at 1.33 ppm.



R^2 [2] = 0.285 R^2 [4] = 0.055 Ellipse: Hoteling's T2 (95%)



R^2 [5] = 0.055 R^2 [4] = 0.011 Ellipse: Hoteling's T2 (95%)

Figure 8.4: Score scatter plots for PCA models that included ^{1}H-NMRS metabolite profiles of 3-hour postprandial serum from growing pigs fed diets containing 319 g total dietary fibre kg^{-1} DM and formulated to contain dietary fibre of high (HF, ●) versus low (LF, ■) fermentability. The diets were fed with (+) or without (-) Roxazyme® G2. The numbers (1-6) on the diet X enzyme treatments denote replicates.

Table 8.6: ANOVA of ^{1}H-NMRS data of serum samples from growing ^{1}pigs showing possible assignments to spectra with significant diet, enzyme or diet X enzyme interaction effects

δ^{1}H	Assignment	Metabolite	Multiplicity	Diet		Enzyme		P Values				
				HF	LF	+	-	Animal	Diet	Enzyme	Period	Diet X Enzyme
3.99	α-CH	Histidine	ABX	0.70	1.43	0.90	1.00	0.22	0.70	0.21	0.01	0.05
3.95	β-CH	Serine	ABX	0.63	1.59	0.90	1.00	0.25	0.79	0.24	0.12	0.03
3.94	CH	Tyrosine	ABX	0.49	2.05	0.01	1.00	0.21	0.88	0.19	0.19	0.05
3.93	CH2	Creatine	s	0.51	1.97	1.38	1.00	0.26	0.95	0.16	0.19	0.05
3.90	CH6 H4	β-Glucose Fructose (β-pyranose)	dd m	0.64	1.56	0.92	1.00	0.26	0.78	0.17	0.01	0.05
3.76	α-CH	Lysine	t	0.71	1.40	1.18	1.00	0.56	0.39	0.10	0.02	0.03
3.71	H6’	α-Glucose	dd	0.68	1.47	1.08	1.00	0.74	0.73	0.11	0.03	0.03
3.56	CH2	Glycine	ABX	0.75	1.33	1.01	1.00	0.87	0.94	0.56	0.02	0.03
3.25	β-CH2 δ-CH2	Histidine Arginine	ABX t	0.67	1.50	0.96	1.00	0.73	0.87	0.09	0.13	0.04
3.24	CH2	α-Glucose	dd	0.65	1.55	0.89	1.00	0.73	0.70	0.18	0.36	0.05
1.31	CH3	Fucose	d	0.93^{b}	1.07^{a}	1.15	1.00	0.08	0.04	0.25	<0.001	0.87
1.12	CH3	Propionate	t	0.99^{b}	1.01^{a}	1.12	1.00	0.42	0.05	0.89	<0.001	0.95
0.91	C21	Cholesterol	m	0.89^{b}	1.12^{a}	1.17	1.00	0.14	0.03	0.31	<0.001	0.83

[1]Male, intact, Large White X Landrace growing pigs.

[2]Diets containing 319 g total dietary fibre kg^{-1} DM, formulated to contain dietary fibre of high (HF) versus low (LF) fermentability.

[3]Each diet was supplement with (+) or without (-) 0.270 g kg $^{-1}$ (as fed basis) Roxazyme® G2.

[ab] For each factor, means within a row with different superscripts were significantly different (p≤0.05).

8.2 Discussion

8.2.1 Chemically analysed metabolites

In Experiment 1, growing pigs were fed a standard and five test diets containing substantially high DF of variable physicochemical and fermentation properties. Wheat bran increased cholesterol compared to MC and LH. Differences in the effect of DF on cholesterol could be due to variation in the absorption or excretion in the GIT. For instance, in growing pigs, rye fibre reduced plasma cholesterol (Laerke *et al.,* 2008). It was hypothesised that rye DF impaired the absorption of dietary cholesterol, and the reabsorption of bile acids. In rats, Chen *et al.* (1984) reported negative control of the cholesterol by SCFA, particularly Pro.

Reduction of serum glucose due to high DF, particularly in the diets containing high soluble DF was expected due to slow absorption and the satiating effect of bulking. In a previous study (Hooda *et al.,* 2010), soluble, viscous oat βG reduced peak glucose absorption. The explanatory mechanisms were impaired absorption due to viscosity and or early satiety due to slowed gastric emptying resulting from water binding. This was consistent with the hypoglycaemic effect of DF (Bach Knudsen *et al.,* 2005). In the present study, the insignificant effect of both level and source of DF on serum glucose could be explained by the low dietary level of soluble, viscous NSP. However, the site and postprandial delay in blood sampling could be a main factor. For instance, portal-vein blood of growing pigs (Bach Knudsen *et al.,* 2005) and sows (Serena *et al.,* 2009) showed peak glucose absorption 0.5–2 hours after feeding, which rapidly diminished.

Surprisingly, correlation analyses did not link the fermentation and hydration properties of the diets to the serum concentration of any of the chemically measured metabolites. It is possible that due to the dominance of maize and soybean fibre in the diet, the dietary variations in fermentation and physicochemical properties were not sufficiently large to stimulate different animal responses.

In Experiment 2, the test diets were formulated for similarity in nutrient composition and hydration properties, with as much contrast as possible in the fermentation properties of DF. However, neither fermentability nor the enzymes influenced the chemically analysed serum metabolites, in both the 3-hour and the 15-hour postprandial serum samples. Correlation analyses suggested negative association between serum urea and fermentability. Reduction in serum urea could reflect more efficient protein or amino acid metabolism (He *et al.,* 2012). Increased fermentation of NSP may also deplete endogenous urea by promoting its assimilation by gut microbes (Libao-Mercado *et al.,* 2009), or by sparing protein from degradation for gut microbial energy (Macfarlane and Macfarlane, 2003).

Correlation analysis showed a positive association of fermentability to serum glucose in the 3-hour postprandial sample. The correlation diminished but remained positive 15 hours postprandial. While the correlation could be attributed to the higher starch content of the HF diet, the effects must also be interpreted in relation to the metabolic status of the animal. The 3-hour postprandial blood sampling occurred after 15 hours of fasting, which complicates the glucose homeostasis. For instance, whereas the liver of pigs has a net uptake of glucose in the absorptive state, there is net release during the fasting state (Nafikov and Beitz*,* 2007). Pigs fed after 24 hour feed deprivation had low glucose clearance compared to pigs fed three meals per day (Bach Knudsen *et al.,* 2005). The effects of DF on glucose homoeostasis are also linked to colonic fermentation. For instance, in a study on pigs in the absorptive state (Theil *et al.,* 2011), fatty acids produced during fermentation, particularly But, were linked to insulinaemia. Negative feedback control of blood glucose was reported for Pro (Boillot *et al.,* 1995) and Ace (Sakakibara *et al.,* 2006).

Despite the dietary similarity in the hydration properties, serum glucose also positively correlated with the water binding and swelling of ileal digesta. This was not consistent with

the known negative effects of water binding and bulking on the glycaemic response (Bach Knudsen *et al.,* 2005). It is possible that both water binding and swelling were associated with higher sugar content in the diet, which was not quantified.

Overall, the effects of DF on chemically analysed blood metabolites reported in literature are variable and depend on the source and level of DF. In diets enriched with pectin, potato or sugar beet pulp to 30 per cent DF, Yde *et al.* (2011) reported positive correlation of serum short chain and non-esterified fatty acids to the DF level. Including 6% DDGS did not affect BUN or creatinine in growing pigs on maize-soybean diets (Wang, 2009), suggesting limited effect on N efficiency. In growing pigs, rye DF (23.5% TDF) reduced the excretion of creatine and creatinine in urine (Bertram *et al.,* 2006).

In Experiment 1, RX increased the urea level in pigs fed the WB and L diets, with no or opposite effect in pigs on the other diets. RX may improve amino acid digestion by altering the physical properties of DF. RX may also stimulate fermentation through depolymerisation of NSP to highly fermentable oligosaccharides (Choct, 2006). The effects of fermentation on urea metabolism in the lower gut were discussed above. In growing pigs fed standard maize-soybean diets, NSPases increased chemically analysed serum urea (Ao *et al.,* 2010). In growing pigs fed maize-soybean- distiller's grain with solubles diets (Wang, 2009), enzymes did not alter chemically serum urea.

8.2.1.1 ^{1}H-NMRS

^{1}H-NMRS revealed influences of the level and source and fermentability of DF on few metabolites, which did not provide sufficient insight into the overall metabolic impact of DF or RX on energy and N metabolism. There are limited comparable experiments in which ^{1}H-NMRS was used to evaluate practical, growing pig diets formulated to increase the supply of insoluble NSP of different fermentability. In an experiment which used sows (Yde *et al.,* 2010a), 40% DF mixtures of pectin, sugar beet and potato pulp (high soluble DF) and brewer's spent grain, pea hull and seed residue (high insoluble DF) reduced plasma creatine compared to a standard DF diet. The high soluble DF, characterised by high water binding and swelling capacities, reduced glucose, and increased Ace and lipid signals. The

fermentability of the diets was not quantified. In sows, pectin increased LDL, potato pulp reduced Val, potato and sugar beet pulp DF reduced glycine and creatine, and all three sources of DF increased VLDL, Ace and formate (Yde *et al.,* 2011). It was suggested that SCFA other than formate and Ace were not detectable due to rapid clearance in the liver. Changes in creatine concentration were related to the level of glucose–glycogen interchange and its production in the liver. They attributed the effects on lipoprotein metabolism to increased synthesis of triglycerides in the liver resulting from clearance of absorbed SCFA and non-esterified fatty acids.

In both Experiment 1 and 2, ^{1}H-NMRS indicated amino acid metabolism was probably most affected by the treatments. The influence of DF on amino acid metabolism is too complex to link the observed effects of DF on serum amino acids to any one mechanism. The mechanisms by which DF inhibits nutrient digestion and absorption were discussed in chapter 7. An elevated serum concentration of amino acids was attributed to decreased synthesis of proteins to favour lipogenesis (He *et al.,* 2012). In addition, DF may reduce the dietary supply of some amino acids via fermentative catabolism in the upper gut (Columbus *et al.,* 2010). Dietary DF may also increase the supply through *de novo* synthesis by microbes (Torrallardona *et al.,* 2003; Libao-Mercado *et al.,* 2009; Zhu *et al.,* 2007). Catabolism of amino acids may affect their requirement (Libao-Mercado *et al.,* 2006). Amino acids may also be lost in secreted mucins. The mucins are particularly rich in threonine and serine (Faure *et al.,* 2002). Endogenous secretions increased the requirement for threonine (Zhu *et al.,* 2005) and for Met and Cys (Zhu *et al.,* 2007). Blank (2009), cited by Susenbeth *et al.* (2011), reported increased requirement of pigs for ileal digestible threonine with increase in ingested NDF from WB.

Purified pectin, a soluble, highly fermentable non-starch polysaccharide (NSP), and wheat shorts, rich in insoluble, poorly fermentable DF, both increased specific endogenous protein losses (Libao-Mercado *et al.,* 2006). Apparently, the soluble DF increased the endogenous secretion through the increased production of mucin proteins in the colon, and not in the ileum (Libao-Mercado *et al.,* 2007). Mucins were secreted in proportion to the bulk forming properties of DF (Leterme *et al.,* 1996; Leterme *et al.,* 1998). Libao-Mercado *et al.* (2007) suggested that mucin protein synthesis could be mediated via hormones, controlled by the

viscosity of digesta, or indirectly through the release of components such as galacturonic acids and or other products of bacterial fermentation, such as short chain fatty acids or ammonia.

^{1}H-NMRS revealed metabolic effects of RX that were not apparent in the *in vitro* assays, and in the feeding trials. A weakness of gravimetric quantification of enzyme activity as applied in the present study is that it does not account for the impact of NSP depolymerisation on the physicochemical and fermentation properties of DF. For instance, NSPases altered the composition of SCFA (Reilly *et al.,* 2010; Bindelle *et al.,* 2011) produced during the fermentation of different pig feeds. I*n vitro*, enzymes also reduced water-holding capacity, but increased the viscosity of NSP (Courtin and Delcor, 2001).

In this study, ^{1}H-NMRS did not detect any metabolically active compounds. In contrast, Yde *et al.* (2011) reported that sugar beet pulp elevated serum scyllo-inositol, betaine, and dimethyl sulfone. Elevation of betaine by rye DF was also reported by Betram *et al.* (2006) and Betram *et al.* (2009).

8.3 Conclusion

In experiment 1, ^{1}H-NMRS evaluation suggested that including high levels of WB in growing pig diets increased serum His and Cys compared to a standard DF diet, and to the iso-fibrous BG, BG, MC and SH diets RX increased formate and urea, with diet X enzyme interaction for urea, fructose, glucose, Arg and Try. Chemical analyses indicated that WB also elevated serum cholesterol compared to the pigs on the MC and LH diets, with diet X enzyme interaction for urea N. In experiment 2, ^{1}H-NMRS evaluation revealed that highly fermentable insoluble DF increased serum fucose, Pro, and cholesterol, with diet X fermentability interaction for His, Ser, Tyr, creatine, glucose, and fructose. Chemical analyses revealed negative correlation between fermentability and Urea N 3 hour and 16 hour postprandial, and between water binding capacity and glucose 16 hours postprandial. Overall, the findings suggested that the co-product ingredient, the fermentability of its DF and supplemental RX may influence sugar, lipid, amino acid, and overall N and energy metabolism in growing pigs.

CHAPTER 9

GENERAL DISCUSSION

9.1 Overview of the current state of knowledge

The research was conceptualised targeting the current production scenario in which high feed costs are threatening the viability of the pig industry. Globally, substitution of the conventional pig feed ingredients with typically fibrous grain processing co-products is considered the most viable option. The challenge is how to minimise the negative impact of DF on productivity, while maximising the beneficial influences. The complex three-way interaction of the DF source, animal homeostasis and lower gut microbial responses to feeding fibrous diets complicates the design of strategies to manipulate dietary characteristics to achieve these goals. Accordingly, the research design recognised that no single study can address the complex factors involved, and was therefore structured to contribute to on-going global research on the nutrition of DF, and ultimately, to the possibility of a modelling approach to the DF question.

Because of the biofuel industry, the bulk of global research on expanding the role of fibrous grain processing co-products has focussed on cereal DDG or DDGS, with limited information on alternative feedstuffs that may be abundant in different production systems. Much effort has also gone into research to characterise the fermentative properties of DF in unconventional fibrous feed ingredients. The objective is to control fermentation in the GIT through diet formulation. NSPases have the potential to expand the range of feedstuffs that can be used for growing pigs. They might also be an important tool to control fermentation. However, there is uncertainty on the efficacy of enzymes in growing pigs, particularly on maize-soybean diets.

The *in vitro* methods that were applied to evaluate the efficacy of RX and to characterise the fermentation properties of pig feeds are increasingly accepted as routine laboratory procedures. However, they have not been sufficiently validated for commercial application in the feed industry. Although ^{1}H-NMRS is increasingly applied in pig nutrition studies, the

approach has so far been largely applied in studies targeting the metabolic health benefits of DF in humans, using the pig as a model. There is limited application in growing pig nutrition.

9.2 Physico-chemical and fermentation properties of dietary fibre

9.2.1 Variability and correlation to animal responses

The DF sources used in the research had substantial variation in the physico-chemical properties of DF that are known to control its metabolic and physiological activity in pigs. The heterogeneity in the composition of the DF fraction, its fermentability, solubility, water binding and swelling capacity were evident among the growth-trial diets. For the balance trial, diets were formulated for uniformity in the physicochemical properties, with contrast in just the fermentation properties. Statistical analyses on the ileal digesta of each pig confirmed that swelling and water binding capacity were not different between the diets, and that the contrast in fermentability was achieved.

The dietary treatments were therefore contrasted in the key properties sufficiently to effectively test a number of hypotheses, and to evaluate the extent to which the physicochemical properties of DF control the physiological activity of DF. In both feeding trials, significant correlations of the physicochemical and fermentation properties of DF to the animal's metabolic and physiological responses were observed. The correlations were overall consistent with conventional hypotheses on the underlying control of these parameters of the physiological activity of DF. Inexplicable correlations were attributed to possible association of the parameters with unmeasured variables, such as sugar composition or viscosity.

9.2.2 Implications of dietary variation in fermentation characteristics

The profound influence of fermentation on the pig's physiology is well document. Examples reported in the literature include prebiotic (Williams *et al.,* 2001), bactericidal (Verstegen and Williams, 2002) and enterotrophic (Tonel *et al.,* 2010) effects of SCFA products. SCFA acids are also known to control satiety (Sleeth *et al.,* 2010) and are involved in glucose (Theil *et al.,* 2011) and lipid (Fushimi *et al.,* 2006) homeostasis. SCFA are an important source of energy for the pig (Anguita *et al.,* 2006). The current production scenario presents

opportunity to exploit the fermentation-induced beneficial influences of DF on the physiology and metabolism of the growing pig. Fibre fermntability is therefore an important concept in growing pig nutrtion.

Fermentation in the gut of pigs can be increased by raising the levels of DF, screening feeds for the fermentability of DF, and possibly, by application of NSPases. In pigs, fermentation studies have been largely restricted to the characterisation of DF in feed ingredients, with hardly any studies to test these effects.

Depending on the source, at high levels, the relationship between DF level and total SCFA production in the gut may not be linear and will reach different thresholds for different sources of DF. For instance, in rats, increasing dietary inulin from 10 to 20% reduced SCFA production in the lower gut by almost 50% (Levrat *et al.,* 1991). The findings of the current study suggested that, at high dietary levels, DF fermentability and its restriction of feed intake may limit fermentation in the gut. To illustrate the effect, in the growth trial, based on the estimated daily feed intake (**Table 5.3**) and the *in vitro* prediction of fermentation activity using the proportion of fibrous mass extracted by pepsin-pacreatin digestion (**Table 3.5)** and its SCFA production (**Table 3.7**), relative to the STD diet, the fermentation activity (total SCFA production per day) predicted for the high DF diets was variable. Relative fermentation activity was low for BG (99%), MC (93%) and WB (89%), and was high for LH (109%) and SH (123%). On the other hand, in the balance trial, based on the same predictions, the HF diet had 148% fermentation activity relative to the LF diet.

It is informative also that, because of the high fermentability of maize and soybean diets, formulating diets for high fermentability based insoluble DF-rich sources did not increase the fermentability of DF beyond that of the standard maize-soybean diet. Therefore, for a high DF diet, without NSPases to increase DF fermentability, the flow of fermentable NSP to the lower gut can only increase depending on the extent to which the DF limits feed intake. The effects of such changes in DF levels and of DF-induced changes in feed intake on patterns of fermentation are unknown.

9.3 Performance of pigs fed high fibre diets

High levels of DF, particularly the poorly fermented DF, tended to reduce nutrient digestibility. This was consistent with previous research, as reported in reviews by Noblet and Le Goff (2001), Bindelle *et al.* (2008), Aarnink, and Verstegen (2007). Pigs on the diets containing highly fermentable DF maintained productivity and attained similar carcass attributes at more than twice the standard DF. This is important given that diets were formulated at lower cost than the standard, largely because there was no processing, no supplementary energy, and limited synthetic amino acid supplements. The similar growth performance at similar feed intakes to the standard diet suggested significant energy contribution from the DF.

Correlations between the physico-chemical properties and animal responses in the early phase of the growth trial suggested that DF had greater physiological activity in the young (50kg live weight), compared to the older animal. The generally positive relationship between fermentability and apparent ileal nutrient digestibility in the balance trial suggests that selecting feedstuffs with highly degradable NSP, or the application of technology to accelerate small intestinal degradation of NSP, might be beneficial to nutrient digestion and absorption.

9.4 Effect of high fibre on the histometry of the ileum

The lack of effect of DF on the intestinal morphology was surprising, given the greater and variable supply of SCFA by the high DF diets and the variation in SCFA composition from the high DF diets, compared to the STD. Therefore, the findings of the study question the strategy to promote intestinal epithelial development in growing pigs using practical, insoluble DF-rich diets. Comparison of findings to similar studies is complicated by variables such as feed intake (Pluske *et al.,* 1997), animal age (Cera *et al.,* 1988), and the period of DF consumption (Montagne *et al.,* 2003). Comparative analyses of different reports are further limited by inconsistent characterisation of diets for the properties of DF that are critical to explaining the variable effects

9.5 ^{1}H-NMRS and the biochemical influence of fibre in growing pigs

In the present study, whereas chemical analyses of metabolites did not detect substantial influence of the source of DF and of RX, ^{1}H-NMRS exposed salient diet, enzyme, and diet X enzyme interactive effects. The sensitivity and holistic explorative capacity of ^{1}H-NMRS is therefore an opportunity to deepen our understanding of the metabolic influence of DF in growing pigs.

Similar, limited influences of high dietary levels of co-product feeds have been reported in sows (Yde *et al.,* 2010a; Yde *et al.,* 2010b; Yde *et al.,* 2011). The reason could be the typically subtle and transient metabolic responses to dietary manipulation due to homeostatic regulation, and its inter-animal variability (Claus and Swan, 2013). To the researcher's knowledge, ^{1}H-NMRS based nutrimetabononomic studies have not previously been employed to explore the biochemical influence of enzyme supplements to growing pigs fed maize-soybean diets containing high levels of the range of co-product feeds used in the present study.

9.6 Effects of RX

The *in vitro* assays indicated that the NSPases in RX have low affinities for the largely insoluble NSP in the range of feedstuffs used in the experiment, particularly in maize and soybean. This could explain its inefficacy on the fermentability of ileal digesta, and on the nutrient digestibility and animal performance. However, the findings of the *in vitro* assay must be interpreted with caution, given that gravimetric (DM disappearance) measurement of enzyme activity does not account for possible alterations in important physicochemical properties such as swelling, viscosity, and fermentation. For instance, ^{1}H-NMRS evaluation of serum metabolites indicated weak but significant enzyme effects on some of the DF sources. It was hypothesised that there was sufficient alteration of the chemistry of DF by RX to cause the biochemical effects detected through ^{1}H-NMRS.

To standardise the procedure, the inoculum used in the fermentation tests was collected from mature sows. Mature inoculum may not be representative of microbes in the lower gut of growing pigs. The lower gut of mature pigs harbours bacteria better adapted to DF than in

the growing pig (Noblet and Le Goff, 2001). The use of the mature pig faecal inoculum could therefore have masked the effects of the enzyme on fermentation.

Overall, the extent to which NSPases can exert significant biological effect in the upper tract of pigs is unclear. The *ad libitum* feeding regime used in the feeding trials possibly reduced digesta retention time in the stomach and in the small intestines (Wilfart *et al.,* 2007; Solà-Oriol *et al.,* 2010). Faster passage may limit the action of NSPases (Svihuis, 2011). Indications of possible additive or synergistic effects when different NSPases were combined in the *in vitro* assays suggest that, subject to cost, there could be beneficial if Roxazyme ® G2 is cocktailed with other gut NSPases.

A comprehensive review on the inconsistency in the effects of NSPases on the performance of pigs, particularly on maize-soybean diets was published by Kerr and Shurson (2013). The inconsistency can be attributed to the fact that the efficacy of NSPases depends on correct matching to the dietary NSP, and positive results can only result if the enzymes target the limiting physico-chemical properties of DF (Zijlstra *et al.,* 2010). Therefore, for the future, there is need to develop multi-potent enzymes tailor-made for such diets.

9.7 Overall conclusions

Dietary fibre from different sources differed in the hydration and fermentation properties. The differences were reflected in the high DF diets in which they partially substituted the basal ingredients of the standard growing pig diet. Correlation analyses suggested that DF solubility, swelling and water binding capacity were important explanatory variables for the variances of the metabolic and physiological responses of the pigs to the different sources of DF. Application of *in vitro* methods to screen the DF sources was effective in altering the fermentation characteristics of DF. Predictions based on the *in vitro* fermentability of DF and the feed intake suggested that a strategy whereby pig diets are enriched in DF after the feedstuffs are screened on DF fermentability could substantially increase fermentation in the lower gut. Due to poor fermentability and or restriction of feed intake, relative to a standard DF diet, high dietary levels of MC, WB and BG may reduce fermentation, while SH and LH will likely substantially increase fermentation in the lower gut. The same predictions

suggested that in the balance trial, feeding the HF diet would likely result in one and half times more fermentation in the lower gut relative to the LF diet.

Depending on the DF source and on the nutrient, high levels of DF depressed nutrient digestibility, with minimal effects on growth and slaughter performance. The substantial dietary differences in physico-chemical and fermentation properties did not result in the expected impact on blood metabolites, and on ileal epithelial morphology. The results suggested that targeting highly fermentable insoluble DF substantially increased fermentation in the gut, and is important to improve nutrient digestion and growth performance, particularly in pigs below 50 kg live weight. The use of RX to stimulate fermentation, to improve nutrient utilisation, and the performance of growing pigs on high DF, maize-soybean diets, was not justified.

9.8 Recommendations for future research

The following recommendations arise from the findings of the research;

- There is need test the hypotheses tested in the study in the early weaning stage, during which the metabolic and physiological activities of DF are known to be more pronounced.
- Similarly, there is need to test the effects of RX on other DF sources, in the early weaning stage and in the small-scale systems, where feed is often quantitatively and qualitatively restricted.
- The inactivity of RX enzymes across a wide range of fibrous feeds challenge research to develop multi-potent enzymes tailor-made for maize-soybean-co-product growing pig diets.
- The use of the co-product feeds that maintained productivity at the levels used in the study need to be extensively tested in the different, normal production systems, starting from the early weanling phase.
- Although the pigs on high DF diets were able to maintain productivity and carcass quality, the effects on meat quality need to be investigated.

- There is need to investigate the reported (Rideout *et al.,* 2004; Aarnink and Verstegen, 2007) beneficial effects of fermentation on ammonia and odour compound production, which were not investigated in the study
- There is also need to investigate the effects of fermentation on mineral fluxes in the lower and upper gut which have been previously reported by (Metzler-Zebeli *et al.,* 2010a; Metzler-Zebeli *et al., 2010b*).

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