

Effect of Non-starch Polysaccharides and Resistant Starch on Mucin Secretion and Endogenous Amino Acid Losses in Pigs

P. C. H. Morel*, J. Melai, S. L. Eady¹ and G. D. Coles¹

Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11-222, Palmerston North, New Zealand

ABSTRACT : Generally, dietary fibre (DF) includes lignin, non-starch polysaccharides (NSP) and resistant starch (RS). In monogastric species, low levels of dietary fibre in the diet are associated with various diseases and high levels reduce nutrient digestibilities. In this study, the effects of different types and levels of NSP (soluble: β -glucan, insoluble cellulose) and resistant starch on mucin secretion and endogenous nitrogen and amino acid losses in pigs were investigated. A total of 25 five-week-old weaner pigs (9.5 kg \pm 1.5 kg), were randomly allocated to each of five experimental diets. Different levels of purified barley β -glucan (BG) extract (5 or 10% of Glucagel[®] β -glucan, providing 4 or 8% of BG in the diet), and resistant starch (RS) (8.3 or 16.6% of Hi-MaizeTM, providing 5 or 10% RS in the diet) were substituted for wheat starch in a purified diet in which enzymatically-hydrolysed casein was the sole source of protein. The diets were fed for 21 days. No statistically significant difference between treatments ($p > 0.05$) was observed for growth performance and organs weights. No difference in ileal starch digestibility was observed between pigs on the cellulose or β -glucan diets. However, as the level of resistant starch in the diet increased the ileal starch digestibility decreased ($p < 0.05$). The inclusion of resistant starch in the diet (5 or 10%) did not increase mucin production when compared with the cellulose-only diet. However, as the level of beta-glucan in the diet increased, both crude mucin in the digesta dry matter and per kg dry matter intake increased ($p < 0.05$). Pigs fed the diet containing 8% of beta-glucan had higher endogenous loss flow than those fed the diets including 5 or 10% of resistant starch or 4% of β -glucan. In conclusion, dietary inclusion of resistant starch increased the level of starch reaching the large intestine without any effect on mucin secretion, or endogenous nitrogen or amino acid losses content in the small intestine. The addition of β -glucan to a diet containing cellulose increases both mucin secretion and endogenous amino acid and nitrogen losses in the small intestine. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 11 : 1634-1641)

Key Words : Non-starch Polysaccharides, Mucin, Endogenous Amino Acid Losses, Pigs

INTRODUCTION

Dietary fibre (DF) includes lignin, non-starch polysaccharides (NSP) and resistant starch (RS) (Trowell et al., 1976). In monogastric species, low levels (0.5 to 1%) of DF in the diet have been associated with different diseases such as colon cancer and coronary heart disease in humans (Anderson, 1986), and high levels of DF (10-15%) result in reductions in the apparent ileal digestibility of starch, crude protein, fat and minerals (Fernandez and Jorgensen, 1986; Graham et al., 1986; Yin, 1994; Jorgensen et al., 1996; Wang et al., 2002). These reductions may be associated with changes in the rate of absorption of nutrients, or increased endogenous secretions. In pigs, both the level and type of NSP in the diet influence losses of endogenous nitrogen and amino acid at the distal ileum (Sauer and Ozimec, 1986; de Lange et al., 1989; Leterme et al., 1998; Zebrowska and Kowalczyk, 2000; Souffrant, 2001). Moreover, Morel et al. (2003) found that endogenous amino acid flows in pigs fed mixed NSP diets (4% β -glucan and 3.5% cellulose) were higher than in pigs fed diets

containing 7.5% cellulose or 7.5% β -glucan. This result suggested that when different types of NSP are mixed together in a diet their effects are not always additive, so this finding was further investigated in the present study.

Endogenous ileal losses include digestive enzymes, sloughed epithelial cells, hair and mucus. Mucus is a high-molecular-weight glycoprotein that covers the entire luminal surface of the gastro-intestinal tract, protecting the underlying epithelium (Lien et al., 2001). Undegraded mucin in the ileal digesta is a significant proportion of the endogenous protein and carbohydrates found at this point (Lien et al., 2001). Diets high in fibre tend to induce structural, morphological and cytokinetic changes in the digestive tract related to a capacity for high mucin secretion (Jacobs, 1986; Vahouny and Cassidy, 1986).

Depending on how well it is digested in the small intestine of monogastric animals, starch can be classified into three categories: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1996). Undigested RS, and NSP that reaches the large intestine, will stimulate bacterial growth and thus the production of short-chain fatty acids (SCFA). It has been shown that SCFA, especially butyrate, have beneficial effects on hind gut health (Brouns et al., 2002). Thus, RS is often included in dietary fibre, but unlike other forms of fibre, has not been shown to stimulate intestinal mucin secretion.

* Corresponding Author: Patrick C. H. Morel. Tel: +64-6-350-5338, Fax: +64-6-350-5684, E-mail: P.C.Morel@massey.ac.nz

¹ New Zealand Institute for Crop & Food Research Ltd, Lincoln, New Zealand.

Received December 16, 2004; Accepted June 3, 2005

Table 1. Ingredient composition (%; as fed basis) of the experimental diets

Ingredient	Diets				
	Cell	BG4	BG8	RS5	RS10
Wheat starch	67.55	57.55	57.55	59.22	50.89
Casein ¹ or enzymatically-hydrolyzed casein	15.0	15.0	15.0	15.0	15.0
Sugar	7.0	7.0	7.0	7.0	7.0
Cellulose ²	3.0	8.0	3.0	3.0	3.0
Soybean oil	3.0	3.0	3.0	3.0	3.0
Di-calcium phosphate	3.0	3.0	3.0	3.0	3.0
Titanium dioxide	0.4	0.4	0.4	0.4	0.4
DL-methionine	0.35	0.35	0.35	0.35	0.35
Vitamin and mineral premix	0.3	0.3	0.3	0.3	0.3
Threonine	0.25	0.25	0.25	0.25	0.25
NaCl	0.15	0.15	0.15	0.15	0.15
Hi-Maize TM Natural Maize Fibre 1043	0	0	0	8.33	16.66
Glucagel TM	0	5.0	10.0	0	0

¹ Casein was included in casein diets and enzymatically-hydrolyzed casein in EHC diets. Casein diets were fed from Day 1 to Day 15 of the experiment and the EHC diets from Day 16 to 22 of the experiment.

² Microcrystalline cellulose (Asahi Kasei Corporation, Osaka, Japan).

Table 2. Analyzed composition (%; as fed basis) of the experimental diets

g/kg DM	Diets				
	Cell	BG4	BG8	RS5	RS10
Dry matter	89.98	90.48	89.63	89.60	89.70
Nitrogen	2.09	2.14	2.33	2.22	1.74
Total starch	53.68	46.86	48.53	51.90	58.97
Resistant starch	0.33	0.14	0.13	4.69	8.58
Total NSP	3.11	8.95	9.59	2.90	2.90
Arabinose	0.07	0.19	0.21	0.07	0.06
Xylose	0.13	0.21	0.31	0.16	0.12
Mannose	0.10	0.10	0.13	0.12	0.10
Galactose	0.17	0.20	0.33	0.13	0.09
Glucose	2.45	7.96	8.37	2.23	2.23
Uronic acid	0.20	0.25	0.25	0.21	0.20
Amino acids ¹					
Aspartic acid	1.20	1.26	1.41	1.25	0.94
Threonine	0.63	0.62	0.73	0.61	0.44
Serine	0.69	0.73	0.80	0.72	0.55
Glutamic acid	3.20	3.40	3.76	3.28	2.55
Proline	1.49	1.58	1.73	1.52	1.14
Glycine	0.27	0.29	0.33	0.28	0.22
Alanine	0.47	0.50	0.56	0.48	0.38
Valine	0.90	0.96	0.11	0.93	0.71
Isoleucine	0.70	0.74	0.82	0.72	0.55
Leucine	1.24	1.32	1.44	1.28	0.99
Tyrosine	0.26	0.27	0.32	0.28	0.22
Phenylalanine	0.62	0.68	0.75	0.66	0.52
Histidine	0.38	0.45	0.49	0.43	0.31
Lysine	1.10	1.17	1.28	1.14	0.90
Arginine	0.50	0.55	0.61	0.51	0.41
Cysteine	0.06	0.06	0.08	0.06	0.05
Methionine	0.76	0.66	0.69	0.85	0.38

¹ Glutamic acid and aspartic acid values also included glutamine and asparagines, respectively.

In the present study, the effects of mixing different types and levels of NSP (soluble: β -glucan, insoluble cellulose) and also the effect of resistant starch on mucin secretion and

endogenous nitrogen and amino acid losses in pigs were investigated.

MATERIALS AND METHODS

Animals, diets and feeding regimen

A total of 25 Large White \times Landrace male and female weaner pigs (5 weeks old; 9.5 kg \pm 1.5 kg), selected from four litters, were used in this study. The animals were housed in individual smooth-edge metal cages under heat lamps, with access to fresh water at all times. Five piglets, at least one from each litter, were randomly allocated to each of five experimental diets. A purified diet (Control) based on wheat starch, sucrose and 3% cellulose was formulated with casein (CAS) or enzymatically-hydrolysed casein (EHC, New Zealand Pharmaceuticals Ltd., Palmerston North, New Zealand, molecular weight < 5,000 da) as the sole source of protein. The EHC-based diets contained titanium oxide (4 g/kg) as an indigestible marker. The ingredient and analysed composition of the experimental diets are presented in Table 1 and 2, respectively. The apparent ileal digestible amino acids content of the Casein and EHC-based diets were in excess of the NRC (1998) recommendations for 10 kg live weight piglets. The levels of NSP in the diets varied between 3% and 10% to allow comparison with previous studies. The β -glucan source was Glucagel[®] gelling barley β -glucan (Gracelinc Ltd, Lincoln, New Zealand) with 80% purity and the resistant starch was Hi-MaizeTM Natural Maize Fibre 1043 (Penford Australia Ltd., Tamworth, NSW, Australia) with 60% purity.

The casein-based diets were fed from day 1 to 15 of the experiment and the EHC-based diets from day 16 to 22. The daily amount of feed offered was equal to 10% of the piglet's metabolic liveweight and was adjusted weekly. The

pigs were fed the experimental diets twice daily (09:00 and 17:00 hrs) for the first 16 days, and then five times daily (08:00, 10:00, 12:00, 14:00 and 16:00 h) for five days. On day 22, feed was offered five times at hourly intervals prior to the collection of digesta samples.

Sample collection and chemical analysis

On day 22, an hour after the last feed, the piglets were anaesthetized with a mixture of Fluothane (4%, Imperial Chemical Industries Ltd, Cheshire, England) and oxygen, and euthanased by intracardiac injection of sodium pentobarbitone. The gastrointestinal tract was exposed and digesta samples from the jejunum and ileum were collected, immediately frozen and freeze-dried. Diet samples were analyzed for soluble and insoluble NSP, nitrogen, amino acids and titanium. Jejunal digesta samples were analyzed for crude mucin, nitrogen and titanium. Ileal digesta samples were subjected to the centrifugation-ultrafiltration method (Moughan et al., 1992; Hodgkinson et al., 2000) and analysed for nitrogen, amino acids and titanium.

Nitrogen content was determined using a LECO FP-2000 analyzer (LECO Corporation, 3000 Lakeview Ave, St. Joseph 49085-2396 USA) by the Dumas process (Granger, 1997). Titanium contents were determined according to the method of Short et al. (1996). The total, soluble and insoluble non-starch polysaccharides (NSP) were analyzed using an assay kit (Englyst Fiberzym Kit GLC; Englyst Carbohydrate Services Limited, Cambridge, UK), which is based on the procedures described by Englyst et al. (1994). Resistant starch was analysed using the AOAC method 2002.02 assay kit (Megazyme; Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland).

Amino acids were determined following hydrolysis of duplicate samples (5-7 mg) in 1 ml of 6 mol/L glass distilled hydrochloric acid containing 0.1% phenol for 24 hours at $110 \pm 2^\circ\text{C}$ in glass tubes sealed under vacuum. Amino acid concentrations were measured using a Waters ion-exchange high performance liquid chromatography system calibrated against a reference amino acid mixture of known concentration. The peaks of the chromatogram were integrated using the dedicated software Maxima 820 (Waters, Millipore, Milford, MA), which identifies the amino acid by retention time against a reference amino acid mixture. Norleucine and lysozyme were used as internal and external standards, respectively, and the weights of each amino acid were calculated using free amino acid molecular weights. Cysteine and methionine were determined by oxidation of duplicate samples (3-4 mg) with 1 ml performic acid (1 part 30% hydrogen peroxide to 9 parts 88% formic acid) for 16 h at 0°C . The samples were then neutralized with 0.15 ml of 50% (w/w) hydrogen bromide prior to acid hydrolysis.

The method used to measure crude mucin was adapted from the method of Lien et al. (1997). Briefly, 3 g of freeze-

dried digesta were mixed with 25 ml of 0.15 M NaCl containing 0.02 M sodium azide, homogenised for 1 min and immediately centrifuged at $12,000 \times g$ at 4°C for 30 min. The aqueous layer was decanted into another 50 mL polystyrene tube and centrifuged at $12,000 \times g$ at 4°C for 30 min to ensure complete removal of insoluble material. Fifteen mL of the supernatant were mixed with ice-cold ethanol to a final concentration of 60% (v/v) and kept on an ice-bath. The samples were allowed to precipitate overnight at 220°C , then centrifuged at $1,400 \times g$ for 10 min and the supernatant discarded. The residue was solubilised into 15 mL of 0.15 M NaCl and cooled in an ice-bath. Ice-cold ethanol was added to a final concentration of 60% (v/v). The samples were allowed to precipitate overnight at -20°C , then centrifuged at $1,400 \times g$ for 10 min and the supernatant discarded. This process was repeated until the supernatant was clear. The final residue was solubilised in 10 ml RO water, freeze-dried and weighed.

Calculations

The ileal endogenous flows of mucin, nitrogen and amino acids at the terminal ileum were calculated as grams lost per kilogram of feed dry matter (DM) ingested, and were calculated using the following formula (Moughan et al., 1992).

$$\text{Endogenous flow (g/kg DM intake)} = \frac{\text{Concentration of mucin, nitrogen or amino acid in ileal digesta}}{\text{Diet titanium}} \times \frac{\text{Ileal digesta titanium}}{\text{Ileal digesta titanium}}$$

Statistical analysis

A linear model with litter of origin as random effect and diet as fixed effect was fitted to the data (GLM procedure, SAS Institute 2002). Empty body weight was used as a covariate for the analysis of the organ weight data. Differences between diets were tested using Fisher's least significant difference test (LSD).

RESULTS

Growth parameters and organ weights

Data from one pig were excluded from the analysis due to its poor growth rate, which was found to be due to a defective ileo-caecal valve. The growth performance of pigs fed different types of diets is presented in Table 3. Neither litter of origin nor dietary treatment had a statistically significant effect on any of the parameters measured.

The organ weights of weaner pigs fed β -glucan (BG) or resistant starch (RS) are presented in Table 4. All organ weights, except the lung weights, were influenced by the pig empty body weight. The litter of origin had an effect on the weight of the stomach ($p < 0.05$), liver ($p < 0.05$), kidney

Table 3. Least-square means for liveweight, carcass weights and growth parameters of pigs fed different level of β -glucan and resistant starch

	Diets					SE	Litter	Diet
	Cell	BG4	BG8	RS5	RS10			
N	5	5	5	5	4			
LW start (kg)	9.46	9.58	9.32	9.96	8.62	0.63	NS ¹	NS
LW end (kg)	12.90	12.95	12.82	12.83	11.36	0.82	NS	NS
Carcass weight (kg)	10.24	9.99	10.22	10.04	8.79	0.65	NS	NS
DFI (g/d)	345	329	348	338	331	16.3	NS	NS
FCR (kg)	2.2	2.1	2.2	2.2	2.6	0.16	NS	NS
Daily gain (g/d)	161	156	164	156	127	14	NS	NS
Gutfill (g)	587	778	476	662	621	110	NS	NS
Gutfill (%)	4.4	5.9	3.6	5.2	5.6	0.74	NS	NS

¹ NS: Not significant; LW: Liveweight; DFI: Daily feed intake; FCR: Feed conversion ratio.

Table 4. Least-square means for empty body weight (EBW) and organ weights (adjusted for EBW) of weaner pigs fed different levels of β -glucan (BG) and resistant starch (RS)

Organs (g)	Diets					SE	EBW	Litter	Diet
	Cell	BG4	BG8	RS5	RS10				
N	5	5	5	5	4				
EBW ¹	11,751	11,614	11,772	11,631	10,292	710	-	NS	NS
Stomach	90.5	103.0	100.5	94.2	90.2	4.1	**	*	NS
Caecum	37.2	38.2	38.2	34.6	38.2	2.6	**	NS	NS
Colon	169.8	170.2	187.9	193.5	226.5	13.9	**	NS	NS
Small intestine	558.5	604.6	526.5	574.2	538.5	26.4	*	NS	NS
Heart	56.6	59.9	58.5	53.8	51.7	1.9	***	NS	NS
Lung	143.5	192.1	183.8	190.8	241.2	29.2	NS	NS	NS
Liver	321.8	333.6	325.5	328.1	328.3	14.1	***	*	NS
Kidney	80.2 ^c	70.1 ^{ab}	68.6 ^a	76.7 ^{bc}	67.9 ^a	2.6	***	**	*
Spleen	40.72	45.0	38.3	37.7	36.1	3.6	*	**	NS

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS: Not significant.

^{a, b, c} Rows with different letter superscript are significantly different (LSD, $p < 0.05$).

¹ EBW = Empty body weight.

($p < 0.01$) and spleen ($p < 0.01$). The dietary treatment had an effect on the kidney weight only ($p < 0.05$), in that pigs fed the cellulose diet had the heaviest kidneys and those fed the diet with 10% RG or 8% BG had the lightest kidneys.

Ileal starch digestibility

The ileal starch digestibilities of pigs fed diets containing different levels of cellulose, BG or RS are presented in Table 5. No difference in ileal starch digestibility was observed between pigs on the cellulose diet or the β -glucan diets. However, as expected, as the percentage of resistant starch in the diet increased the starch ileal digestibility decreased.

Crude mucin content and flow

The digesta crude mucin yield of two pigs was not determined because of insufficient sample. The crude mucin content (CM) in the jejunum expressed per kilogram of digesta dry matter (DDM) or per kilogram of dry matter intake (DMI) of pigs fed different types and levels of dietary fibre is presented in Table 5. The inclusion of resistant starch in the diet (5% or 10%) did not increase mucin production when compared with the cellulose-only

diet. However, as the level of β -glucan in the diet increased, both crude mucin measurements (DDM and DMI) increased significantly ($p < 0.05$).

Endogenous nitrogen and amino acid flow

The endogenous losses of two pigs were not determined because of insufficient digesta.

Endogenous nitrogen and amino acid flow measured at the terminal ileum of pigs fed diets containing different levels of cellulose, BG or RS are presented in Table 5. Litter of origin had a statistically significant effect ($p < 0.05$) on the endogenous flow of asparagine, threonine, serine, proline, glycine, valine, isoleucine, leucine and total amino acids. A statistically significant effect ($p < 0.05$) of the dietary treatment on endogenous loss flows was observed for nitrogen, total amino acid and all individual amino acids, with the exception of glutamic acid, proline and histidine. Overall, pigs fed the diet containing 8% BG had higher endogenous amino acids loss flow than the pigs fed the other diets (4% BG, 5% RS or 10% RS). The endogenous flow level measured in the pigs fed the cellulose diet was between those observed for the 8% BG group and the other groups.

Table 5. Least-square means for ileal starch digestibility, crude mucin and ileal endogenous flow in pigs fed different type and levels of dietary fibres

	Diets					SE
	Cell	BG4	BG8	RS5	RS10	
Ileal starch digestibility (%)	98.96 ^b	97.66 ^b	98.86 ^b	96.48 ^{ab}	93.67 ^a	1.21
Crude mucin (CM)						
CM (g/kg DDM) ¹	40.1 ^{ab}	65.1 ^{bc}	77.4 ^c	45.1 ^{ab}	38.0 ^a	9.0
CM flow (g/kg DMI) ²	12.6 ^a	32.3 ^{bc}	44.8 ^c	15.3 ^{ab}	13.3 ^a	6.1
Endogenous flow (g/kg DMI)						
Nitrogen	4.92 ^{ab}	3.02 ^a	7.14 ^b	3.79 ^a	3.30 ^a	0.74
Aspartic acid	2.00 ^a	1.57 ^a	2.89 ^b	1.79 ^a	1.35 ^a	0.27
Threonine	1.58 ^a	1.19 ^a	2.52 ^b	1.16 ^a	1.05 ^a	0.31
Serine	1.44 ^{ab}	1.04 ^a	2.18 ^b	1.03 ^a	0.90 ^a	0.24
Glutamic acid	3.47	2.55	3.92	3.20	1.84	0.46
Proline	1.48	1.16	2.07	1.27	0.95	0.24
Glycine	1.95 ^a	1.28 ^a	4.55 ^b	1.38 ^a	1.87 ^a	0.60
Alanine	1.35 ^{ab}	0.94 ^a	1.86 ^b	1.05 ^a	0.84 ^a	0.18
Valine	1.24 ^{ab}	0.86 ^a	1.80 ^b	0.97 ^a	0.82 ^a	0.19
Methionine	0.30 ^{ab}	0.19 ^a	0.38 ^b	0.26 ^a	0.19 ^a	0.04
Isoleucine	0.83 ^{ab}	0.56 ^a	1.15 ^b	0.67 ^a	0.52 ^a	0.10
Leucine	1.41 ^{ab}	0.93 ^a	2.06 ^b	1.04 ^a	0.93 ^a	0.22
Tyrosine	0.70 ^{ab}	0.44 ^a	1.05 ^b	0.47 ^a	0.44 ^a	0.12
Phenylalanine	0.73 ^a	0.46 ^a	1.12 ^b	0.53 ^a	0.48 ^a	0.12
Histidine	0.73	0.59	1.37	0.60	0.44	0.20
Lysine	0.99 ^{ab}	0.66 ^a	1.27 ^b	0.81 ^a	0.58 ^a	0.13
Arginine	0.91 ^{ab}	0.55 ^a	1.20 ^b	0.63 ^a	0.55 ^a	0.14
Total amino acids	21.12 ^a	14.96 ^a	31.37 ^b	16.86 ^a	13.75 ^a	3.15

^{a,b,c} Rows with different letter superscript are significantly different (LSD, $p < 0.05$).

¹ DDM: Digesta dry matter; ² DMI: Dry matter intake.

DISCUSSION

Growth parameters and organ weights

In contrast to a previous study (Morel et al., 2001), dietary inclusion of different levels of β -glucan and cellulose at the expense of wheat starch did not cause any significant effects on growth performance. In the present study, inclusion of resistant starch similarly showed no significant effects. However, it should be noted that due to the small number of animals investigated, the loss of an animal due to an undetectable birth defect, and the short experimental period (22 days), it was unlikely that statistically significant differences in growth parameters between dietary treatment groups would be detected.

The different dietary treatments had no effects on the organ weights of weaner pigs, with the exception of the kidney. These results are similar to those of Anugwa et al. (1989) and Morel et al. (2001) where increased levels of dietary fibre fed over a short period (2–4 weeks) failed to alter organ weight in weaner pigs. However, when diets high in dietary fibre are fed to growing-finishing pigs, a significant increase in the weight of stomach, caecum, and colon are observed (Jorgensen et al., 1996). Moreover, Pluske et al. (1998) demonstrated a positive linear relationship between the weight (full or empty) of the large intestine at 55 kg liveweight and the daily intake of NSP

and resistant starch during the growth period (18 to 55 kg liveweight). Martinez-Puig (2003) reported an increase in colon weight for pigs fed a barley-based diet containing 25% potato starch instead of corn starch for 38 days.

Ileal starch digestibility

The substitution in the diet of wheat starch, a rapidly digestible starch, by different of types of NSP (BG4: 5% cellulose and 4% β -glucan; BG8: 8% β -glucan) did not reduce ileal starch digestibility (99%). However, when RS (5% and 10%) was substituted for wheat starch, a linear decrease in ileal starch digestibility was observed. It can be calculated that as the RS in the diet (Table 2) increases by 1% the total ileal starch digestibility (Table 5) decreased by 0.64 percentage points. Similarly, Wang et al. (2002) found that the inclusion of potato starch (type B starch) instead of cooked rice (type A starch) reduced starch ileal digestibility in cannulated pigs. The same authors observed no effect on ileal starch digestibility when sugarbeet pulp (soluble NSP) or wheat bran (insoluble NSP) were substituted for cooked rice in the diet. This suggests that ileal starch digestibility is mainly dependent on the type of starch in the diet and independent of the dietary NSP. Similarly, ileal starch digestibility decreased when pigs were fed a barley-based diet containing 25% potato starch instead of corn starch for 38 days (Martinez-Puig et al., 2003).

Crude mucin

Until now, the effect of resistant starch on mucin production in the small intestine has not been extensively researched. In this study the inclusion of 5 or 10% resistant starch in the diet had no impact on the production of crude mucin. This is in contrast to the results of Morita et al. (2004) who found that the inclusion of 30% of high-amylose starch in the diet of rats elevated the mucin content in the small intestine.

However, when β -glucan was added to the diet, both the crude mucin per kilogram of digesta dry matter (CM_{DDM}) and the crude mucin flow per kilogram of dry matter intake (CMF_{DMI}) increased. Several authors have reported, in different species, that some dietary fibre increases the mucin content in the small intestine (see Montagne et al., 2003 for a review). In pigs, Morel et al. (2003) showed that both CM_{DDM} and CMF_{DMI} in the jejunum increase when corn arabinoxylan or barley β -glucan were exchanged for cellulose in diets. Increased levels of mucin in the ileum of pigs have also been reported after the dietary inclusion of a mixture of cellulose, maize cobs and wheat straw (Mariscal-Landin et al., 1995) or pea fibre (Lien et al., 2001). In contrast, the addition of soya fibre to the diet of ileostomized human did not increase the daily mucin output (Lien et al., 1996).

Endogenous losses

In this study, the inclusion of resistant starch in the diet did not affect the endogenous flow of nitrogen and amino acids at the terminal ileum. To our knowledge no data have been published on the effect of resistant starch on ileal endogenous nitrogen or amino acids flows (EL_I) in monogastric animals.

In our study a statistically significant 50% increase in EL_I was observed when 10% Glucagel[®] barley β -glucan was substituted for wheat starch, however when wheat starch was substituted by a mixture of 5% Glucagel[®] barley β -glucan and 5% cellulose no difference was observed. The opposite was observed in a previous study: when 7.5% cellulose was replaced by a mixture of 3.5% cellulose and 4% Glucagel[®] β -glucan, an increase of EL_I was observed, whereas no increase was observed when 7.5% cellulose was substituted by 7.5% Glucagel[®] β -glucan (Morel et al., 2003).

Increasing the dietary level of cellulose has not been shown to significantly increase EL_I (Furiya and Kaji, 1992; Leterme et al., 1992). When barley endosperm fibre was added to a protein-free diet, an increase in ileal endogenous nitrogen loss was observed but this increase was not related to the β -glucan level in the diets (Leterme et al., 2000). These data suggest that when β -glucan or cellulose are the sole sources of dietary fibre, they have only a minimal effect on secretions in the small intestine. However, when

they are fed together, an increase in small intestine secretion is observed. This increase in EL_I seems to be related to the ratio between β -glucan and cellulose (soluble/insoluble NSP) in the diet. Using the dietary treatment BG4 and BG8 in this study and the dietary treatment 4% BG in our previous study (Morel et al., 2003), a linear increase in the nitrogen endogenous flow (3.02, 4.92 and 7.94 g/kg DMI) was observed as the ratio BG/Cellulose in the diet increased (0.5, 0.91 and 2.67 for the BG4, 4% BG and BG8 diets, respectively).

The endogenous nitrogen flows of 4.92 g/kg DMI, 3.79 g/kg DMI and 3.30 g/kg DMI for pigs fed the EHC diet containing 3% cellulose (CELL, RS5 and RS10) were higher than the value of 2.85 g/kg DMI and 2.27 g/kg DMI for EHC diets containing 5 and 7.5% cellulose as reported by Hodgkinson et al. (2000) and Morel et al. (2003), respectively. Butts et al. (1993a, b) reported similar values (2.7–3.7 g/kg DMI) for EHC diets containing 3 to 5% cellulose. The endogenous flow of 7.14 g/kg DM with the BG8 diets is comparable to the flows measured by Leterme et al. (1996, 1998) when pea endosperm fibre was added to a protein free-diet.

The data presented in this paper show that dietary inclusion of resistant starch increased the level of starch reaching the large intestine, thus allowing the manipulation of the short chain fatty acid profile in the hind gut, without any effect on secretion of mucin or endogenous nitrogen and amino acid losses content in the small intestine (Coles et al., unpublished). In this study, the addition of β -glucan to a diet containing cellulose increases both mucin secretion and endogenous amino acid and nitrogen losses in the small intestine. More research is needed to better understand the dynamics of different type and level of dietary fibre in the small and large intestines. This will ultimately enable the development of dietary fibre blends, which will be beneficial in term of gastro-intestinal secretion and health.

ACKNOWLEDGEMENTS

We are grateful to the New Zealand Foundation for Research, Science and Technology for funding this work.

REFERENCES

- Anderson, J. W. 1986. Fibre and health: an overview. *Am. J. Gastroenterol.* 81:892–897.
- Anugwa, F. O. I., V. H. Varel, J. S. Dickson, W. G. Pond and L. P. Krook. 1989. Effects of dietary and protein concentration on growth, feed efficiency, visceral organ weights and large intestine microbial population in swine. *J. Nutr.* 119:879–886.
- Brouns, F., B. Kettlitz and E. Arrighi. 2002. Resistant starch and the "butyrate revolution". *Trends Food Sci. Tech.* 13:251–261.
- Butts, C. A., P. J. Moughan, W. C. Smith and D. H. Carr. 1993a.

- Endogenous lysine and other amino acid flows at the terminal ileum of the growing pig (20 kg bodyweight): The effect of protein-free, synthetic amino acid, peptide and protein alimentation. *J. Sci. Food Agric.* 61:31-40.
- Butts, C. A., P. J. Moughan, W. C. Smith, G. W. Reynolds and D. J. Garrick. 1993b. The effect of food dry matter intake on endogenous ileal amino acid excretion determined under peptide alimentation in the 50 kg liveweight pig. *J. Sci. Food Agric.* 62:235-243.
- de Lange, C. F. M., W. C. Sauer, R. Mosenthin and W. B. Souffrant. 1989. The effects of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and faeces of pigs. *J. Anim. Sci.* 67:746-754.
- Englyst, H. N., M. E. Quigley and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 119:1497-1509.
- Englyst, H. N., S. M. Kingsman, G. J. Hudson and J. H. Cummings. 1996. Measurement of resistant starch *in vitro* and *in vivo*. *Br. J. Nutr.* 75:749-755.
- Fernandez, J. A. and J. N. Jorgensen. 1986. Digestibilities and absorption of nutrients as affected by fibre content in the diet of the pig. Quantitative aspects. *Livest. Prod. Sci.* 15:53-71.
- Furuya, S. and Y. Kaji. 1992. The effects of feed intake and purified cellulose on the endogenous ileal amino acid flow in growing pigs. *Br. J. Nutr.* 68:463-472.
- Graham, H., K. Hesselman and P. Amman. 1986. Influence of wheat bran and sugar-beet pulp of the digestibility of dietary components in a cereal-based pig diet. *J. Nutr.* 116:242-251.
- Granger, D. 1997. Kjeldahl versus Dumas-which gives the right answer? *Chem. New Zealand*, 7.
- Hodgkinson, S. M., P. J. Moughan, G. W. Reynolds and K. A. C. James. 2000. The effect of dietary peptide concentration on endogenous ileal amino acid loss in the growing pig. *Br. J. Nutr.* 83:412-430.
- Jacobs, L. R. 1986. Dietary fiber and gastrointestinal cell proliferation. In: (Ed. G. V. Vahouny and D. Kritchevsky), *Dietary Fiber: Basic and Clinical Aspects*. Plenum Press, New York. pp. 211-228.
- Jorgensen, H., H. Q. Zhao and B. Eggum. 1996. The influence of dietary and environmental temperature on the development of the gastro-intestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. *Br. J. Nutr.* 75:365-378.
- Leterme, P., E. Froidmont, F. Rossi and A. Thewis. 2000. Effect of barley fibres and barley intake on the ileal endogenous nitrogen losses in piglets. *J. Cereal Sci.* 31:229-239.
- Leterme, P., E. Froidmont, F. Rossi and A. Thewis. 1998. The high water-holding capacity of pea inner fibres affects the ileal flow of endogenous amino acids in pigs. *J. Agric. Food Chem.* 46:1927-1934.
- Leterme, P., A. Thewis, P. van Leeuwen, T. Monmart and J. Huisman. 1996. Chemical composition of pea fibre isolate and their effect on endogenous amino acid flow at the ileum of the pig. *J. Sci. Food Agric.* 72:127-134.
- Leterme, P., L. Pirard and A. Thewis. 1992. A note on the effect of wood cellulose level in protein-free diets on the recovery and endogenous protein collected from ileum of pigs. *Anim. Prod.* 54:163-165.
- Lien, K. A., M. I. McBurney, B. I. Beyde, A. B. R. Thomson and W. C. Sauer. 1996. Ileal recovery of nutrients and mucin in humans fed total enteral formulas supplemented with soy fiber. *Am. J. Clin. Nutr.* 63(4):584-595.
- Lien, K. A., W. C. Sauer and J. M. He. 2001. Dietary influence on the secretion into and degradation of mucin in the digestive tract of monogastric animals and humans. *J. Anim. Feed. Sci.* 10(2):223-245.
- Lien, K. A., W. C. Sauer and M. Fenton. 1997. Mucin output of ileal digesta of pigs fed a protein-free diet. *Z. Ernährungswiss.* 36:182-190.
- Mariscal-Landin, G., B. Sève, Y. Colléaux and Y. Lebreton. 1995. Endogenous amino nitrogen collected from pigs with end-to-end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. *J. Nutr.* 125:136-146.
- Martinez-Puig, D., J. F. Perez, M. Catillo, A. Andaluz, M. Anguita, J. Morales and J. Gasa. 2003. Consumption of raw potato starch increases colon length and fecal excretion of purine bases in growing pigs. *J. Nutr.* 133:134-139.
- Montagne, L., J. R. Pluske and D. J. Hampson. 2003. A review of interaction between dietary fibre and intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Tech.* 108:95-117.
- Morel, P. C. H., R. M. Padilla and G. Ravindran. 2003. Effect of Non-Starch Polysaccharides on Intestinal Mucin Production and Amino Acid Endogenous Losses in Pigs. *Asian-Aust. J. Anim. Sci.* 16(9):1332-1338.
- Morel, P. C. H., R. M. Padilla, Y. H. Cottam and G. D. Coles. 2001. Influence of soluble and insoluble dietary fibre on growth, organ weights and blood metabolites in weaner pigs. *Recent Advances in Animal Nutrition in Australia* 13:22A.
- Morita, T., H. Tanabe, K. Takahashi and K. Sugiyama. 2004. Ingestion of resistant starch protects endotoxin influx from the intestinal tract and reduces D-galactosamine-induced liver injury in rats. *Journal of Gastroenterology and Hepatology* 19:303-313.
- Moughan, P. J., G. Schutttert and M. Leenaars. 1992. Endogenous amino acid flow in stomach and small intestine of the young growing pigs. *J. Sci. Food Agric.* 60:437-442.
- Nutrient Requirements of Swine. 1998. 10th Ed. National Academic Press: Washington, DC.
- Pluske, J. R., D. W. Pethick and B. P. Mullan. 1998. Differential effects of feeding fermentable carbohydrate to growing pigs on performance, gut size, and slaughter characteristics. *Anim. Sci.* 67:147-156.
- SAS Institute. 2002. SAS for Window, version 8.02, SAS Institute. Cary, NC.
- Sauer, W. C. and L. Ozimec. 1986. Digestibility of amino acids in swine: results and their practical application. A review. *Livest. Prod. Sci.* 15:367-388.
- Short, F. J., P. Gorton, J. Wiseman and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Tech.* 59:215-221.
- Souffrant, W. B. 2001. Effect of dietary fibre on ileal digestibility

- and endogenous nitrogen losses in pig. *Anim. Feed. Sci. Tech.* 90:93-102.
- Trowell, H., D. A. Southgate, T. M. Wolever, A. R. Leeds, M. A. Gassull and D. J. Jenkins. 1976. Letter: dietary fibre refined. *Lancet* 1:967.
- Vahouny, G. V. and M. M. Cassidy. 1986. Dietary fibre and intestinal adaptation. In: *Dietary Fiber: Basic and Clinical Aspects*. (Ed. G. V. Vahouny and D. Kritchevsky), Plenum Press, New York. pp. 181-209.
- Wang, J. F., B. B. Jensen, H. Jorgensen, D. F. Li and J. E. Lindberg. 2002. *Anim. Feed Sci. Tech.* 102:125-136.
- Yin, Y. L. 1994. Nutrients value of feedstuff and diets for pigs: I. Apparent post-ileal digestibility and interrelationships between dietary constituents and faecal and ileal digestibility. *Anim. Feed Sci. Tech.* 45:243-255.
- Zebrowska, T. and J. Kowalczyk. 2000. Endogenous nitrogen losses in monogastrics and ruminants as affected by nutritional factors. *Asian-Aust. J. Anim. Sci.* 13:210-218.