Intestinal Structure and Function of Broiler Chickens on Wheat-Based Diets Supplemented With a Microbial Enzyme

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ABSTRACT : A study was conducted to assess the nutritive value of two diets based on a low-energy variety of wheat, RAC C1 and their effects on intestinal mucosal structure and function in broiler chickens. The diets were fed with or without microbial enzyme supplement to male and female broiler chickens. The digesta viscosity was reduced ($p<0.001$) through supplementation with a microbial enzyme in male and female chicks. Enzyme supplementation also improved the dietary apparent metabolizable energy content ($p<0.001$) and had slight but non-significant positive effects on chick growth and feed conversion ratio. Intestinal mucosal structure and enzyme function were not affected by microbial enzyme supplement. Male chicks consumed more feeds ($p<0.001$), attained higher final body weight ($p<0.001$) and were more efficient at feed utilization ($p<0.01$) than the female chicks. Except for duodenal villus surface area and ileal protein content, intestinal mucosal structure and enzyme activities were similar between the two sexes and dietary treatment groups. The study showed an improvement in the nutritive value of the diets in the presence of the microbial enzyme supplement. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 54-60*)

Key Words : Wheat, Intestinal Enzymes, Intestinal Structure, Non-Starch Polysaccharide, Microbial Enzyme Supplement

INTRODUCTION

Wheat is one of the principal cereals used in poultry diets. Previous studies (Annison, 1990; Annison, 1991) have revealed major differences in the performance of broiler chickens raised on diets containing different cultivars of wheat. These observations were traced to differences in the apparent metabolizable energy (AME) levels and the nature of polysaccharides (Mollah et al., 1983). Non-starch polysaccharides (NSP) do not succumb to enzymes of animal origin and therefore, tend to limit productivity through increased digesta viscosity and reduction in feed intake (Bedford et al., 1991; Choct and Annison, 1992).

Exogenous microbial enzymes enhance the digestion of NSP, leading to improvement in animal performance (Choct et al., 1995, 1996). The gross effects of antinutritive factors present in different cereals are known, as are the effects of microbial enzyme supplements but there is a dearth of published information on the impact of these treatments on intestinal structure and function in poultry. In the rat, viscous NSP have been shown to increase intestinal weight (Johnson and Gee, 1986; Brunsgaard et al., 1995) and mucosal cell proliferation rate (Johnson et al., 1984; Brunsgaard and Eggum, 1995).

The present study was therefore, conducted to assess the effect of low-AME wheat with or without a microbial enzyme supplement. The aim of the study was to examine changes in performance and determine if the variation was due to changes in intestinal structure and digestive enzyme function.

MATERIALS AND METHODS

Animals and diets

Forty-eight 3-week-old Steggles×Ross broiler chicks (Australian Poultry Ltd.) consisting of 24 males and 24 females were used for the study. The males were heavier than females at the onset of study. The chicks were randomly allocated to single-bird metabolic cages.

Two diets, identical in major ingredient composition were fed (table 1). The wheat included in the diets was a relatively low-energy variety (RAC CI; Roseworthy Agricultural College, Roseworthy, South Australia). One of the diets contained 1 g/kg of a microbial enzyme supplement (MES), Avizyme 1300 (Finfeeds International UK) which has predominantly xylanase (2500 IU/g) and protease (800 IU/g) activities. Each diet was fed to male and female chickens.

The experiment was designed for the classical AME determination and was run over a period of 7 days; consisting of 3 days of adaptation to the diets and 4 days of total faecal collection.

Collection of tissue samples

At the end of the feeding trial, the birds were slaughtered through intravenous administration of

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sodium pentobarbital (1.0 ml/kg body weight). The carcass was weighed prior to removal of the gastrointestinal tract. Digesta were collected from different regions of the small intestine and stored on ice for about two hours prior to measurement of viscosity with a Brookfield DVIII viscometer (Brookfield Engineering Laboratories, Massachusetts, USA) at 25℃. Intestinal samples, about 10 cm long were taken from the proximal ends of the duodenum, jejunum and ileum and flushed with phosphate buffered saline (pH 7.4). One-cm pieces from each region were fixed in 4 % neutral buffered formalin for 24 hours for histology or snap-frozen in liquid nitrogen for preparation of mucosal homogenates. Intestinal samples were also embedded in strips of liver and snap-frozen in liquid nitrogen for enzyme cytochemistry.

Mucosal protein content

Mucosal homogenates were prepared according to the method described by Shirazi-Beechey et al. (1991). Fresh-frozen samples were cut into small pieces and defrosted in buffer (100 mM mannitol, 2 mM *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES)/Tris, pH 7.1). The mixture was vibromixed for 2×30 seconds and filtered through a Buchner funnel, 1 mm pore size. The filtrate was blended with a PCU-2 Polytron homogenizer for 30 seconds at high speed (setting 6). The protein content of this homogenate was measured as described by Bradford (1976).

Histology

Fixed tissues were stored in 70 % ethanol for 24 hours and processed over 16.5 hours in an automatic tissue processor (Shandon, Pittsburgh, USA). Processing consisted of serial dehydration in graded concentrations of ethanol and clearing with histoclear (Bayer Diagnostics (Aust.) Pty. Ltd.). The processed tissue was embedded in paraffin wax (melting point, 56-57℃).

Sections, 8 μm thick were cut using a Leitz 1512 microtome (Ernst Leitz Wetzler GmBH, Austria). The sections were stained in Lilee-Mayers hematoxylin, counter-stained with eosin yellow and mounted with DePeX (Bayer Diagnostics).

Table 1. Composition (g/kg) of the two feeds used in the experiment

Ingredient	Control	+Microbial enzyme
Wheat[1]	678	677
Meat & bone meal	76	76
Soybean meal	170	170
Poultry tallow	40	40
DL-methionine	3.2	3.2
Lysine	2.5	2.5
NaCl	2.5	2.5
Vitamin/mineral premix	5.0	5.0
Choline chloride	0.8	0.8
Avizyme 1300	0.0	1.0

[1] The wheat meal had an NSP content of 20 g/kg, its AME was 12.73 MJ/kg DM and extract viscosity was 4.8 mPa.

Enzyme cytochemistry

Fresh-frozen tissues were sectioned on a Riechert-Jung cryostat (Cambridge Instruments, GmBH, Germany) at a thickness of 8 μm.

The expression of two major membrane-bound enzymes, aminopeptidase N (APN; EC. 3.4.11.2) and α-glucosidase (AG; EC. 3.2.1.48) were assessed. The procedures were modifications of those already used and described in studies with mammalian tissues (Nachlas et al., 1957; Gutschmidt et al., 1979). The substrates used for the assays were L-alanine 4-methoxy-β-naphthylamide (0.749 mM) for APN and β-naphthyl-α-D-gluco-pyranoside (6.0 mM) for AG. The diazonium salts were Fast Blue BB and hexazonium-ρ-rosaniline for APN and AG, respectively.

The methods enabled quantitation of *in situ* enzyme expression as the density of a final reaction product formed by coupling of catalytic (primary) product with the diazonium salt. This is determined by measuring the absorbance of incident light through the tissue section on a slide. Preliminary time-course incubations were performed to establish initial rate conditions for these enzymes as expressed in the chicken. The tissues were subsequently incubated for 2 and 14 minutes for APN and AG, respectively. The incubation temperature was 39℃ in both cases.

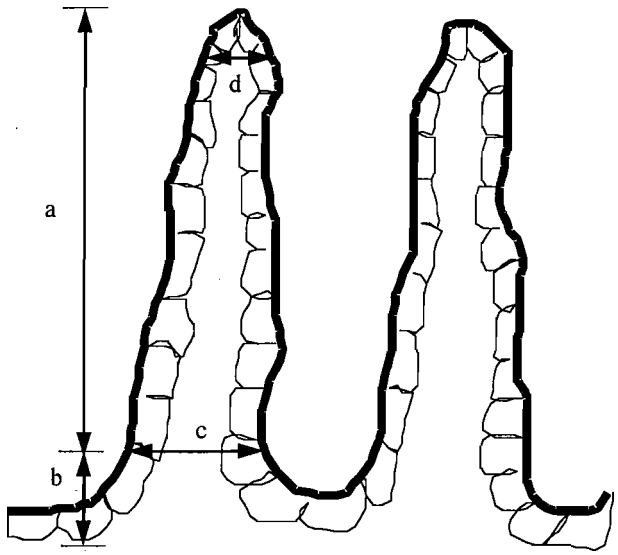
Slides were viewed on an Olympus BH-2 microscope and digitized using a computer software, Video Pro (Leading Edge, Bedford Park, South Australia). Measurements were made on 10-12 villi per replicate for histology and cytochemistry. For histology, the basal and apical widths as well as height of villus were measured. From these primary measurements, apparent villus surface area was derived as shown in figure 1.

Data analysis

All data collected were subjected to analysis of variance using the General Linear Model of Minitab (Minitab Inc., 1998). The experimental design was regarded as a 2×2 factorial, with diet and sex as main factors. Differences between mean values were identified by the least significant difference.

RESULTS

There were highly significant ($p<0.001$) differences



Morphometric measurements undertaken included villus height (a), crypt depth (b), villus basal width (c) and villus apical width (d). Apparent villus surface area was estimated as (c+d)/d × a.

Figure 1. Outline of whole villi, showing measurements involved in morphometric assessment of the intestinal mucosa

between the two sexes with regards to feed intake, growth rate and final body weight (table 2). Male chicks also converted feed more efficiently ($p<0.01$) than the female chicks. Enzyme supplementation improved ($p<0.001$) the AME of the diets as measured in both male and female chicks. The difference in AME between male and female chicks was not significant.

Enzyme supplementation significantly reduced ($p<0.001$) digesta viscosity in the three intestinal regions but there were no differences between the chicks on account of gender (table 3). Digesta viscosity also increased distally from the duodenum to the ileum.

Table 3. Effects of diet and sex on viscosity of digesta in the different intestinal regions

Diet	Sex	Digesta viscosity (cP)		
		Duodenal	Jejunal	Ileal
Control	Female	2.8±1.0	4.5±1.8	14.0±6.5
Control	Male	3.0±0.8	4.6±2.2	14.0±9.8
Enzyme	Female	1.6±0.2	2.2±0.3	3.8±0.9
Enzyme	Male	1.8±0.2	2.4±0.7	3.9±1.0
		Probability of greater F value		
Significance of diet effect		***	***	***
Significance of sex effect		NS	NS	NS

[1] Values are mean ± standard deviation.
Levels of significance indicated (NS: not significant ($p>0.05$); *** $p<0.001$).

There were no significant effects of the microbial enzyme supplement or of sex on the mucosal protein content, crypt depth or villus height in the duodenum (table 4). The apparent surface area of the duodenal villi was, however, reduced ($p<0.05$) in male chicks on diets supplemented with the enzyme. There was also a diet × enzyme interaction on villus surface area.

The mucosal protein content as well as morphometry of the jejunal mucosal was unaffected by dietary treatment and sex (table 5). The interaction between the two factors was significant ($p<0.05$) in the case of protein content. In the ileum, regardless of dietary treatment, male chicks had a higher ($p<0.001$) protein content than female chicks (table 6). The other variables were unaffected by diet or by sex.

Mucosal protein content generally fell distally from the duodenum to the ileum. There was some similarity between duodenal and jejunal crypt depth but this was reduced distally towards the ileum. Villus height and

Table 2. Feed intake, weight gain, feed conversion and apparent metabolizable energy on various diets and sexes[1]

Diet	Sex	Feed intake (g/head/day)	Final weight (g)	Growth (g/head/day)	FCR (Feed:gain)	AME (MJ/kg DM)
Control	Female	114±18	1,510±176	56.5± 6	2.11±0.13	13.5±1.2
Control	Male	133±14	1,739±114	68.5±10	1.96±0.18	13.8±0.7
Enzyme	Female	116± 8	1,532± 69	58.3± 6	2.01±0.19	14.5±0.3
Enzyme	Male	131±12	1,755±143	70.1± 9	1.88±0.16	14.5±0.2
		Probability of greater F value				
Significance of diet effect		NS	NS	NS	0.09	***
Significance of sex effect		***	***	***	**	NS

[1] Values are mean ± standard deviation.
Levels of significance indicated (NS: not significant ($p>0.05$); ** $p<0.01$; *** $p<0.001$).

Table 4. Effects of sex and enzyme supplementation on duodenal mucosal protein content, crypt depth, villus height and villus surface area[1]

Diet	Sex	Protein (mg/g tissue)	Crypt depth (μm)	Villus height (μm)	Surface area (mm^2)
Control	Female	31.7±6.17	220.7±28.46	1,588.7±117.67	0.34±0.039
Control	Male	29.0±7.54	190.4±29.30	1,744.2±134.47	0.40±0.052
Enzyme	Female	26.2±3.51	202.5±22.20	1,701.8±126.31	0.35±0.056
Enzyme	Male	29.0±6.48	202.0±14.10	1,541.2±171.47	0.30±0.037
			Probability of greater F value		
Significance of diet effect		NS	NS	NS	*
Significance of sex effect		NS	NS	NS	NS
Diet×sex interaction		NS	NS	NS	*

[1] Values are mean±standard deviation.
Levels of significance indicated (NS: not significant ($p>0.05$); * $p<0.05$).

Table 5. Effects of sex and enzyme supplementation on jejunal mucosal protein content, crypt depth, villus height and villus surface area[1]

Diet	Sex	Protein (mg/g tissue)	Crypt depth (μm)	Villus height (μm)	Surface area (mm^2)
Control	Female	29.9±1.28	216.5±24.89	1,037.5±136.05	0.19±0.021
Control	Male	22.8±1.72	225.2±43.16	1,267.5±127.52	0.24±0.058
Enzyme	Female	20.7±1.76	200.2±28.92	1,229.4±168.41	0.24±0.043
Enzyme	Male	33.0±2.44	210.2±30.21	1,183.2±100.19	0.24±0.040
			Probability of greater F value		
Significance of diet effect		NS	NS	NS	NS
Significance of sex effect		NS	NS	NS	NS
Diet×sex interaction		*	NS	NS	NS

[1] Values are mean±standard deviation.
Levels of significance indicated (NS: not significant ($p>0.05$); * $p<0.05$).

apparent surface area were also reduced distally from duodenum to the ileum.

The activity of AG in the jejunum was lower ($p<0.05$) in male chicks on the enzyme-supplemented diet than in chicks on the other diets (figure 2). There were no effects of gender or MES on the activity of ileal AG. The activity of APN in both regions was also unaffected by gender or MES (figure 3).

DISCUSSION

The results obtained in the present study showed that the nutritive value of diets based on low-energy wheat can be enhanced through supplementation with appropriate microbial enzymes. These results are similar to those obtained in previous research (Pettersson et al., 1990; Campbell et al., 1993) except that feed intake was reduced by enzyme supplementation in the present study. The overall productivity could be improved through enzyme supplementation in a variety of ways. The microbial enzyme may act directly on NSP, which have been shown to be responsible for the reduced ME of certain wheat cultivars (Choct and Annison, 1990). The breakdown of NSP may also improve the digestion and absorption of other dietary components (Pettersson and Aman, 1989; Pettersson et al., 1990).

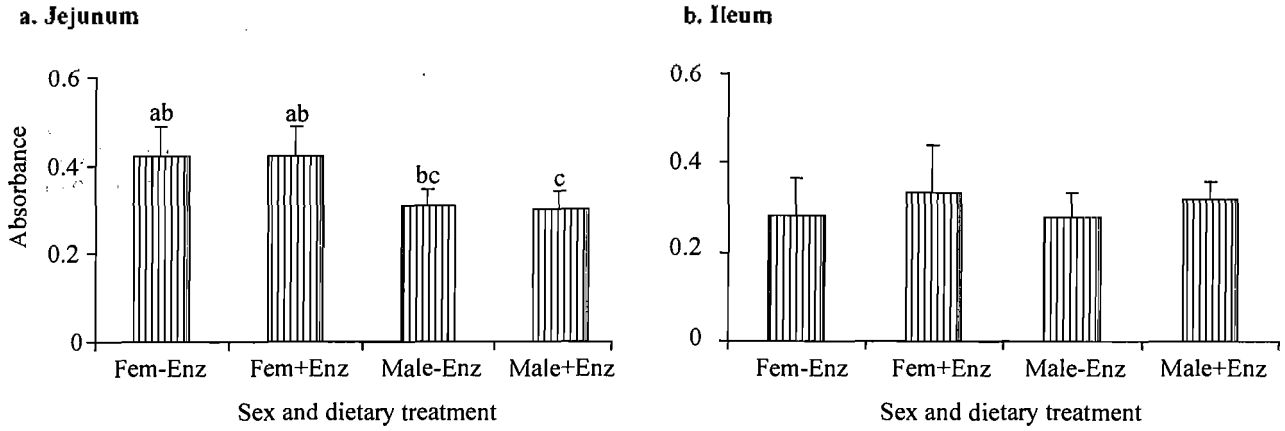
The high digesta viscosity in birds fed diets without enzyme supplementation may affect digesta passage rate and feed intake as well as prevent access of intestinal enzymes to the nutrients (Ikeda and Kusano, 1983). Digesta viscosity also tended to increase distally from the duodenum to the ileum. Intestinal structure and function at the ileum may therefore be more affected than at the other regions. In research with commercial NSP supplements (unpublished), we observed greater changes in ileal structure than jejunal structure over long-term feeding.

The impact of NSP in poultry diets on intestinal structure and enzyme function has been less studied and there are no reports on the effects of MES on these variables. An increase in cell proliferation as a result of NSP supplementation in the rat has been highlighted (Johnson et al., 1984; Brunsgaard and Eggum, 1995). Protein deprivation may be induced by reduced absorption of nutrients as has been noted by

Table 6. Effects of sex and enzyme supplementation on ileal mucosal protein content, crypt depth, villus height and villus surface area[1]

Diet	Sex	Protein (mg/g tissue)	Crypt depth (μm)	Villus height (μm)	Surface area (mm^2)
Control	Female	7.7 ± 1.21	170.3 ± 25.35	788.9 ± 114.86	0.16 ± 0.072
Control	Male	18.4 ± 4.93	167.8 ± 9.95	837.2 ± 148.89	0.13 ± 0.028
Enzyme	Female	8.8 ± 2.80	165.6 ± 20.16	788.3 ± 154.50	0.15 ± 0.018
Enzyme	Male	22.1 ± 3.57	164.3 ± 26.40	938.8 ± 141.09	0.16 ± 0.016
			Probability of greater F value		
Significance of diet effect		NS	NS	NS	NS
Significance of sex effect		***	NS	NS	NS
Diet × sex interaction		NS	NS	NS	NS

[1] Values are mean ± standard deviation.
Levels of significance indicated (NS: not significant ($p>0.05$); *** $p<0.01$.



[a,b,c] Mean values with unlike superscripts are significantly different ($p<0.05$)

Figure 2. Expression of α-glucosidase (mean absorbance ± SEM) in chicks on diets with or without microbial enzyme

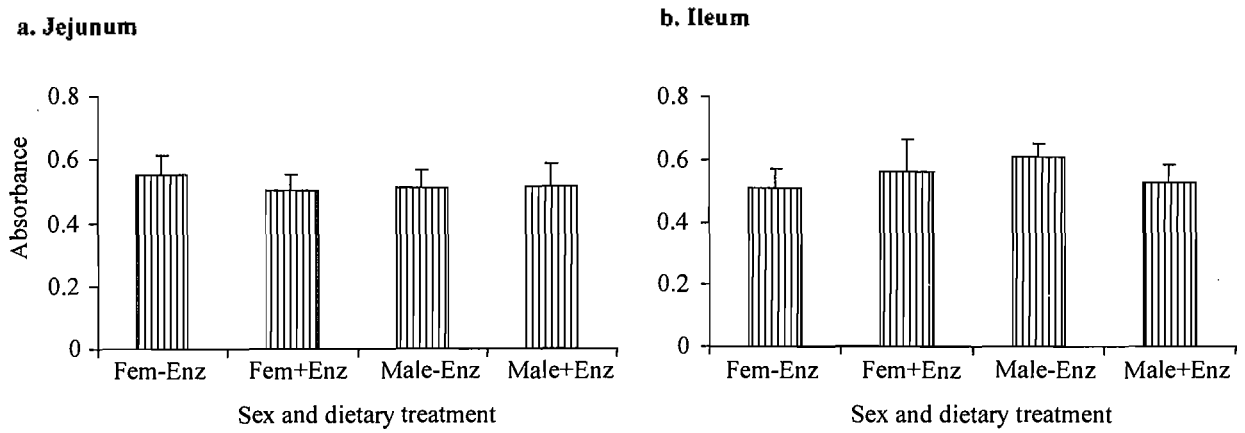


Figure 3. Expression of aminopeptidase N (mean absorbance ± SEM) in chicks on diets with or without microbial enzyme

Pettersson et al. (1990). Protein deprivation in the rat has been observed to reduce mucosal renewal (Syme and Smith, 1982; King et al., 1983).

In previous research, using immunohistochemical labelling with bromo-deoxyuridine (unpublished), intestinal cell formation in broiler chicks was completed within one hour. Cells migrated from the zone of proliferation (crypt) to the villus tip within 3-5 days, suggesting that relatively short periods of exposure to dietary factors may influence intestinal development and function. The response to NSP is dependent on duration of exposure, and although we have shown that cellular proliferation is rapid and completed over a short period of time, it has been reported that changes in intestinal structure and function could be transient or permanent (Brunsgaard and Eggum, 1995). This calls for closely spaced observations rather than single end-point assessment as was done in the current study.

The variation in performance between male and female chicks is not attributable to differences in intestinal structure or enzyme activity, as revealed by this study. The initial weight difference between male and female chicks conferred a general advantage on the former. In poultry, these differences are present pre-hatch and persist throughout life (Burke and Sharp, 1989; Bond et al., 1991). The underlying factors have not been fully investigated.

CONCLUSION

The improvement in nutritive value of low-energy wheat-based diets through microbial enzyme supplementation of the diet for a short period of time is not related to intestinal villus-crypt structure or expression of two major membrane-bound enzymes, amino-peptidase N and α-glucosidase in male or female chicks. These treatments need to be studied in greater detail over a longer period of feeding.

REFERENCES

Annison, G. 1990. Polysaccharide composition of Australian wheats and the digestibility of their starches in broiler chicken diets. Aust. J. Exp. Agric. 30:183-186.

Annison, G. 1991. Relationship between the levels of soluble non-starch polysaccharides and the apparent metabolisable energy of wheats assayed in broiler chicks. J. Agric. Food Chem. 39:1252-1256.

Bedford, M. R., H. L. Classen and G. L. Campbell. 1991. The effect of pelleting, salt and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. Poult. Sci. 70:1571-1577.

Bond, P. L., T. W. Sullivan, J. H. Douglas and L. G. Robeson. 1991. Influence of age, sex and method of rearing on tibia length and mineral deposition in broilers. Poult. Sci. 70:1936-1942.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Brunsgaard, G. and B. O. Eggum. 1995. Small intestinal tissue structure and proliferation as influenced by adaptation period and indigestible polysaccharides. Comp. Biochem Physiol. 112A:365-377.

Brunsgaard, G., B. O. Eggum and B. Sandstrom. 1995. Gastrointestinal growth in rats as influenced by indigestible polysaccharides and adaptation period. Comp. Biochem Physiol. 111A:369-377.

Burke, W. H. and P. J. Sharp. 1989. Sex differences in body weight of chicken embryos. Poult. Sci. 68:805-810.

Campbell, G. L., B. G. Rossnagel and R. Bhatty. 1993. Evaluation of hull-less barley genotypes varying in extract viscosity in broiler chick diets. Anim. Feed Sci. Technol. 41:191-197.

Choct, M. and G. Annison. 1990. Antinutritive activity of wheat pentosans in broiler diets. Br. Poult. Sci. 31:811-821.

Choct, M., R. J. Hughes, R. P. Trimble and G. Annison. 1994. The use of enzymes in low-ME wheat broiler diets:effects on bird performance and gut viscosity. Aust. Poult. Sci. Sym. 6:83-87.

Friesen, O. D., W. Guenter, B. A. Rotter and R. R. Marquardt. 1991. The effects of enzyme supplementation on the nutritive value of rye grain (*Secale cereale*) for the young broiler chick. Poult. Sci. 70:2501-2508.

Gutschmidt, S., W. Kaul and E. O. Riecken. 1979. A quantitative histochemical technique for the characterization of α-glucosidases in the brush-border membrane of rat jejunum. Histochem. 63:81-101.

Ikeda, K. and T. Kusano. 1983. *In vitro* inhibition of digestive enzymes by indigestible polysaccharides. Cereal Chem. 60:260-263.

Johnson, I. T., J. M. Gee and R. R. Mahoney. 1984. Effect of dietary supplements of guar gum and cellulose on intestinal cell proliferation, enzyme levels and sugar transport in the rat. Br. J. Nutr. 52:477-487.

Johnson, I. T. and J. M. Gee. 1986. Gastrointestinal adaptation in response to soluble non-available polysaccharides in the rat. Br. J. Nutr. 55:497-505.

King, I. S., J. Y. F. Paterson, M. A. Peacock and M. W. Smith. 1983. Effect of diet upon enterocyte differentiation in the rat jejunum. J. Physiol. 344:465- 481.

Minitab Inc. 1998. Minitab Release 12.1. Minitab Inc., State College, PA 16801-3008, USA.

Mollah, Y., W. I. Bryden, I. R. Wallis, D. Balnave and E. F. Annison. 1983. Studies on low metabolizable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. Br. Poult. Sci. 24:81-89.

Nachlas, M. M., D. T. Crawford and A. M. Seligman. 1957. The histochemical demonstration of leucine amino-peptidase. J. Histochem. Cytochem. 5:264-278.

Pettersson, D. and P. Aman. 1989. Enzyme supplementation of a poultry diet containing wheat and rye. Br. J. Nutr. 62:139-149.

Pettersson, D., H. Graham and P. Aman. 1990. Enzyme supplementation of broiler chicken diets based on cereals

with endosperm cell walls rich in arabinoxylans or mixed-linked beta-glucans. Anim. Prod. 51:201-207.
Shirazi-Beechey, S. P., M. W. Smith, Y. Wang and P. S. James. 1991. Postnatal development of lamb intestinal digestive enzymes is not regulated by diet. J. Physiol. 437:691-698.
Syme, G. and M. W. Smith. 1982. Intestinal adaptation to protein deficiency. Cell Biol. Int. Rep. 6:573-578.