Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/j.1439-0396.2008.00852.x

ORIGINAL ARTICLE

Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation

M. D. Shakouri[1,2], P. A. Iji[1], L. L. Mikkelsen[1] and A. J. Cowieson[3]

1 School of Rural Science and Agriculture, University of New England, Armidale, NSW, Australia,
2 Department of Animal Science, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran, and
3 Danisco Animal Nutrition, Marlborough, Wiltshire, UK

Keywords
cereal grain, microbial enzyme, broiler chickens, feed intake, growth, microflora function, intestinal development

Correspondence
Paul A. Iji, School of Rural Science and Agriculture, University of New England, Armidale, NSW 2351, Australia. Tel: +61 2 6773 2082; Fax: +61 2 6773 3922; E-mail: piji@une.edu.au

Received: 13 February 2008; accepted: 10 June 2008
First published online: 12 August 2008

Summary

A study was conducted to investigate the effect of the key cereal grains and a microbial enzyme supplement on broiler chicken performance, gut microflora and intestinal function. Ingestion of the barley-based diet was associated with low 28-day body weight, decreased feed intake and high FCR. The supplemental enzyme increased feed intake and weight gain of the chickens on a wheat-based diet. The pH of the gizzard and caecal contents varied with the grain type. Enzyme supplementation reduced ileal viscosity, particularly in birds that received the diet based on wheat. The birds on the barley-based diet had lower ileal digestibility of dry matter, protein and energy than those given maize and sorghum-based diets. The ileal digestibility of starch was increased by enzyme supplementation. Enzyme supplementation increased the number of total anaerobic bacteria in the gizzard of birds fed on sorghum and increased lactobacilli in the gizzard of those fed both sorghum and wheat. The birds fed the sorghum-based diet had the lowest counts of caecal total anaerobic bacteria and lactobacilli. Jejunal villus height and villus:crypt ratio of birds fed the barley-based diet were the lowest when compared with those fed the other diets. Enzyme application induced an increase in villus height and villus:crypt ratio of birds on wheat, crypt depth on barley and a reduction in crypt depth of chickens on the sorghum-based diets. The highest activity of maltase and the lowest activity of sucrase were observed in tissue from birds fed on maize and sorghum-based diets respectively. The differences in the performance of broilers on cereal grains could be explained by changes in intestinal morphology, enzyme activities and gut microflora as well as nutrient digestibility. The improved performance by supplemental enzyme in wheat-fed chickens was associated with beneficial changes in intestinal morphology and digesta viscosity.

Introduction

Cereal grains are used in poultry diets mainly as a source of energy. Because these feedstuffs comprise the greatest proportion of the diets, they may affect gut function to a greater degree than other ingredients. The poor nutritive value of barley, for example, has been ascribed to the presence of viscous non-starch polysaccharides (NSPs) (Burnett, 1966). Bird performance has been shown to improve with the use of exogenous enzymes under such circumstances (Hesselman et al., 1981; Fengler and

Marquardt, 1988; Choct and Annison, 1990, 1992). The use of an NSP-degrading enzyme to eliminate the adverse effect of soluble NSPs of some cereals in poultry diets has also been reviewed (Bedford and Schulze, 1998). However, unlike gastrointestinal tract (GIT) digestive functions, there is limited information on the influence of cereal grains on absorptive function and the gut microbial populations that may directly affect productivity and health of the animal.

Ingested nutrients need to be digested by pancreatic and intestinal mucosal enzymes. The levels of pancreatic (Sklan and Noy, 2000) and intestinal mucosal (Uni et al., 1999) enzyme activities are well correlated with the body weight of birds. The activity of digestive enzymes of broiler chickens can be affected by factors such as age (Noy and Sklan, 1995; Uni et al., 1998; Iji et al., 2001b), exogenous enzymes (Mahagna et al., 1995; Engberg et al., 2004) and also the form (Gabriel et al., 2003) and type of cereal grains (Almirall et al., 1995) in the diet. A limited number of studies have examined the changes in intestinal morphometry caused by the type of cereal in the diet and results are equivocal. For example, there have been reports on the alteration in mucosal morphology of chickens on diets based on barley (Viveros et al., 1994) and rye (Mathlouthi et al., 2002a), compared with maize. A similar effect of microbial enzymes in cereal-based diets on intestinal morphometry have been reported by Mathlouthi et al. (2002a) and Wu et al. (2004). In contrast to these reports, Thomas et al. (2005) did not observe differences in intestinal structure on diets based on maize, wheat or sorghum.

It is well known that the microbial community and its activity in the broiler intestinal tract is influenced by diet composition. In this respect, the effect of different cereal grains (Wanger and Thomas, 1978; Mathlouthi et al., 2002b; Apajalahti et al., 2004) and also exogenous microbial enzymes (Mathlouthi et al., 2002b; Engberg et al., 2004; Jozeflak et al., 2006) have been reported. It has been demonstrated that the antinutritional effects of soluble cereal NSPs for broilers is partially mediated via intestinal microbial activity (Choct et al., 1996, 1999). However, the increased interest in the study of broiler GIT microflora in recent years is due to the ban on antibiotic growth promoters in diets. Following this ban, the incidence of diseases associated with *Clostridium perfringens*, e.g. necrotic enteritis, is expected to increase (Porter, 1998; van Immerseel et al., 2004). There are some reports indicating that the incidence of this disease can be influenced by the type of cereal grains in the diet (Riddell and Kong, 1992; Branton et al., 1997).

The aim of the present study was to evaluate the effect of major cereal grains used in poultry diets; barley, maize, sorghum and wheat, and the corresponding influence of a commercially available microbial enzyme, Grindazym (Danisco Animal Nutrition, Marlborough, UK), in isocaloric and isonitrogenous diets on gut function with respect to intestinal physico-chemical condition, morphology, secretions, microflora, nutrient digestibility and broiler performance.

Materials and methods

Birds and experimental diets

A total of 288 day-old Cobb male broiler chicks were obtained from a local hatchery and distributed in groups of six into 48 cages with equal group weights (264.5 ± 2.3). The chickens were assigned to one of eight experimental diets that were based on barley, maize, sorghum and wheat, with or without microbial enzyme supplementation (Table 1). Therefore, a completely randomized design with a 4×2 factorial arrangement was employed, with six replicates for each treatment. Isocaloric and isonitrogenous diets were formulated to meet or exceed the nutrient requirements recommended by the National Research Council (1994). Grindazym^{TM} GPL 5000, a liquid feed enzyme (supplied by Danisco Animal Nutrition, Marlborough, Wiltshire, UK) was carefully sprayed on the diets containing enzyme at 0.05%. The guaranteed minimum activity of the supplemental enzyme was 5000 BGU of endo-1, 4-beta-glucanase (EC 3.2.1.4) and 12000 FXU of endo-1, 4-beta-xylanase (EC 3.2.1.8) per gram of product. Celite Corp. (Lompoc, CA, USA), a source of acid-insoluble ash (AIA), was included to all diets at 0.6% as a marker for the calculation of nutrient digestibility in the ileum. The chickens were raised under controlled conditions for 28 days. During this period, water and feed were supplied *ad libitum* and light was provided for 16 h/day, while the temperature was gradually reduced from 33 °C to approximately 25 °C. Body weight and feed intake of the chickens were assessed weekly. This study was approved by the Animal Ethics Committee of the University of New England.

Sampling of gastrointestinal contents and tissues

At the end of the fourth week of the experiment, two birds per cage (replicate) were randomly selected, weighed and killed by cervical dislocation.

Table 1 Composition of experimental diets (g/kg diet)

Ingredient	Barley	Maize	Sorghum	Wheat
Barley	600.2	–	–	–
Maize	–	623.0	–	–
Sorghum	–	–	623.0	–
Wheat	–	–	–	623.0
Soyabean meal	193.8	248.1	238.5	244.8
Fish meal	52.7	22.3	22.3	21.3
Meat meal	51.8	63.0	66.8	30.4
Sunflower oil	82.1	21.6	26.7	45.5
Limestone	7.2	5.5	3.3	6.6
Dical. Phos.	0.0	3.6	5.6	13.6
Common salt	1.7	2.2	2.2	2.5
Premix†	2.0	2.0	2.0	2.0
DL-methionine	1.4	1.6	1.8	2.1
L-Lysine HCl	0.0	0.0	0.7	1.1
Choline Cl-70%	0.6	0.6	0.6	0.6
Marker‡	6.0	6.0	6.0	6.0
Enzyme	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000
Nutrient composition				
ME (MJ/kg)	13.0	13.0	13.0	13.0
Crude protein	222.8	222.8	222.8	222.8
Crude fibre	42.5	25.3	25.6	29.3
Crude fat	101.1	53.3	53.0	66.8
Lysine	12.0	12.0	12.0	12.0
Met	5.2	5.2	5.3	5.2
Met + Cys	8.7	8.7	8.7	8.7
Ca	9.7	9.7	9.7	9.7
Available P	4.8	4.8	4.8	4.8
Na	1.9	1.9	1.9	1.9

†The active ingredients contained in the vitamin–mineral premix were as follows (per kg of diet): vitamin A – 12000 IU, vitamin D_3 – 3500 IU, vitamin E – 30.0 mg, vitamin K_3 – 2.0 mg, thiamine – 2 mg, riboflavin – 6 mg, pyridoxine – 5 mg, vitamin B_{12} – 0.02 mg, niacin – 50 mg, pantothenate – 12 mg, biotin 0·01 mg, folic acid – 2 mg, Fe – 60 mg, Zn – 60 mg, Mn – 80 mg, Cu – 8 mg, Se – 0·1 mg, Mo – 1 mg, Co – 0·3 mg, I – 1 mg.
‡Celite (acid-insoluble ash) was obtained from Celite Corporation (Lompoc, CA, USA).

The birds were immediately dissected to obtain the samples of the intestinal digesta and tissues. The contents of gizzard, ileum, from Meckel's diverticulum to the ileocaecal junction, and caeca were collected and pooled per cage. Sub-samples were then taken for the enumeration of microbial populations and pH measurements. The remaining ileal digesta was used for measurement of viscosity and determination of nutrient digestibilities. The pancreas and a sub-sample of the proximal part of the jejunum were taken and snap-frozen in liquid nitrogen to measure tissue protein content and activities of some digestive enzymes. To determine the morphometric indices of the mucosa, another sub-sample of the proximal jejunum was fixed in 10% buffered formalin (pH 7.0). The jejunal tissue samples were washed with ice-cold phosphate buffered saline (pH 7.4) before snap-freezing for enzyme analysis or fixation in formalin for histology.

Physico-chemical analyses

To measure the ileal digesta viscosity, approximately 2 g of fresh digesta were centrifuged (12,000 *g* for 10 min at 20 °C) and viscosity of the supernatant (0.5 ml) was determined using a Brookfield DV III viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) at 25 °C with a CP40 cone and shear rate of 5–500 s^{-1}. The samples did not exhibit thinning over this range of shear rate. The pH of gizzard, ileal and caecal contents was measured immediately using a pH meter with a glass electrode (EcoScan 5/6 pH meter; Eutech Instruments, Singapore). Ileal samples were freeze-dried, followed by determination of dry matter content. The gross energy of the diets and ileal contents was determined using an adiabatic bomb calorimeter (IKA-WERKE®, C7000; Janke & Kunkel, Staufen, Germany). The crude protein content of the samples was measured by the Dumas' combustion method on an LECO analyzer (Leco, St Joseph, MI, USA). The starch content of the diets and ileal contents were determined enzymatically as glucose using a total starch assay kit (Megazyme International Ireland, Bray, Ireland). The amount of AIA in the feed and ileal digesta was determined by the method described by Choct and Annison (1990). Apparent ileal digestibility (AID) of nutrients was calculated using the following equation:

$$\text{AID} = 1 - [(\text{diet AIA}/\text{ileal AIA}) \times (\text{ileal nutrient}/\text{diet nutrient})]$$

Bacterial enumeration

Approximately 1 g of each gizzard, ileum and caeca content was rapidly transferred into sterile McCartney glass bottles containing 10 ml of a pre-reduced salt medium (Holdeman et al., 1977). The suspension was quickly poured into a CO_2-flushed plastic bag and homogenized using a stomacher laboratory blender (Interscience, St. Nom, France) for 2 min. Then 10-fold dilutions were serially made in pre-reduced salt medium according to the technique of Miller and Wolin (1974). The total culturable anaerobic bacteria counts were determined using anaerobic roll tubes containing Wilkins–Chalgren anaerobe agar (CM0619; Oxoid Australia, Adelaide, Australia) anaerobic incubated at 39 °C for 7 days. The number

of lactobacilli was enumerated on Rogosa agar (CM0627; Oxoid) after incubation in an anaerobic cabinet at 39 °C for 48 h. Coliform bacteria and lactose-negative enterobacteria were counted as red and white colonies, respectively, on MacConkey agar (CM0115; Oxoid) incubated aerobically at 39 °C for 24 h. Numbers of *C. perfringens* were determined on Tryptose Sulphite Cycloserine (TSC) agar (Oxoid, Agar base CM0587, TSC supplement SR0088 and egg yolk emulsion SR0047) using pour-plating technique and after anaerobic incubation at 39 °C for 24 h. The number of microbial colony forming units (CFUs) was expressed as logarithmic ($\log_{10}$) transformation per gram of intestinal digesta.

Jejunal morphometry

The fixed tissue samples in formalin solution were processed and embedded in paraffin. Sections were prepared at a thickness of 5 μm and stained with haematoxylin and eosin. The morphometric indices were determined using computer-aided light microscope image analyses (SPOT 3.1; Diagnostic Instruments, Sterling Heights, MI, USA). The measurements of villus height (from the tip of villus to the villus–crypt junction), villus width, crypt depth, villus height:crypt depth ratio and thickness of muscle layer were made. Apparent villus surface area was calculated from the villus height and width, as described by Iji et al. (2001a). The values of means from 12 adjacent and vertically orientated villi and crypts were used for further analysis.

Digestive enzyme and tissue protein assay

To assay the digestive enzyme activities and protein content, the jejunal tissue was homogenized as described previously by Shirazi-Beechey et al. (1991). The tissue was cut into an ice-cold buffer (100 mM mannitol, 2 mM Tris/HEPES, pH 7.1) and the mucosa was then stripped into the buffer using a swirl mixer at high speed for 1 min. The mixture was homogenized at medium speed (3/6 setting) for 30 s on an Ultra Turrax T25 Basic homogenizer (IKA® Works, Wilmington, NC, USA). Sub-samples of the homogenate were taken into Eppendorf tubes (Eppendorf South Pacific, North Ryde, Australia), frozen in liquid nitrogen and stored in a deep freezer (−20 °C) for enzyme analysis. For the pancreas, the entire tissue was homogenized through a similar process for the jejunum except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used. The homogenate tissue was centrifuged (30,000 *g* for 10 min) to obtain a supernatant on which the analysis was done (Nitsan et al., 1974).

The specific activity of jejunal brush-border and pancreatic enzymes were assessed by incubation with fixed substrate concentrations as standardized for poultry by Iji et al. (2001b). On the jejunal homogenates, the assays were conducted for the activities of maltase (EC 3.2.1.20), sucrase (EC 3.2.1.26) and alkaline phosphatase (EC 3.1.3.1). For the pancreas, an assay was conducted for chymotrypsin (EC 3.4.21.1). The specific activities of enzymes were measured according to the methods previously described for other species (Miller et al., 1960; Dahlqvist, 1964; Holdsworth, 1970; Serviere-Zaragoza et al., 1997) after standardization for poultry. All assays were conducted at 39 °C. The protein content of both the jejunal mucosa and pancreatic tissue was measured using the Coomassie dye-binding method described by Bradford (1976).

Statistical analysis

The experiment was regarded as a 4 × 2 general linear model, with grain type and microbial enzyme supplementation as factors. Data were subjected to statistical analysis using the General Linear Model procedures of SAS® (SAS Institute, 2003). The significance of differences ($p < 0.05$) between mean values was examined by Duncan's multiple range test.

Results

Growth performance

The results on the biological response of the broiler chickens fed on the different experimental diets are presented in Table 2. There were significant interactions between cereal type and enzyme supplement on feed intake in the first week ($p < 0.001$) and up to 14 days ($p < 0.05$), 21 days ($p < 0.05$) and 21 days ($p < 0.01$). There were also separate significant effects of each of the two factors. The birds on maize diet supplemented with enzyme consumed more ($p < 0.05$) feed than those on non-supplemented diet up to the second week, and this trend continued ($p < 0.05$) for birds fed the wheat-based diet to the end of experiment.

The weight gain of broilers was affected ($p < 0.05$) by the cereals and following the first week of experiment, by enzyme supplementation too ($p < 0.05$). During 0–21 days and 0–28 days, a significant ($p < 0.01$) interaction was found between cereal and the enzyme supplement. Enzyme supplementation of the wheat diet improved ($p < 0.01$) weight gain of

Table 2 The effect of cereal grain type and microbial enzyme on feed intake, body weight gain and feed conversion ratio

	Dietary treatments									Probability		
Cereal	Barley		Maize		Sorghum		Wheat					Cereal ×
Enzyme		−	+	−	+	−	+	−	SEM	Cereal	Enzyme	enzyme +
Feed intake (g/bird)												
0–7	132.9^{abc}	122.6^{c}	130.1^{bc}	143.9^{a}	130.2^{bc}	130.1^{bc}	120.9^{c}	139.7^{ab}	3.75	0.099	0.430	0.001
0–14	427.1^{d}	435.6^{cd}	466.9^{bc}	507.2^{a}	483.4^{ab}	477.5^{ab}	417.8^{d}	476.0^{ab}	12.20	0.000	0.005	0.048
0–21	1032.1^{cd}	1047.4^{bcd}	1121.7^{ab}	1161.8^{a}	1111.3^{abc}	1155.7^{a}	989.4^{d}	1160.6^{a}	27.45	0.001	0.001	0.031
0–28	1831.6^{b}	1837.9^{b}	2067.6^{a}	2006.3^{a}	1987.7^{a}	2020.1^{a}	1794.8^{b}	2077.0^{a}	49.89	0.001	0.073	0.008
Weight gain (g/bird)												
0–7	89.6	83.8	94.3	101.0	93.3	87.3	88.6	95.3	3.54	0.029	0.873	0.113
0–14	282.5	291.2	339.5	359.4	348.0	337.7	291.2	340.8	11.24	0.000	0.047	0.077
0–21	660.9^{b}	674.9^{b}	775.5^{a}	800.8^{a}	768.9^{a}	780.4^{a}	663.9^{b}	809.2^{a}	18.81	0.000	0.0007	0.002
0–28	1063.7^{b}	1055.9^{b}	1329.3^{a}	1334.3^{a}	1305.6^{a}	1302.6^{a}	1154.9^{b}	1377.5^{a}	35.63	0.000	0.037	0.005
Feed conversion ratio												
0–7	1.48	1.46	1.38	1.43	1.40	1.49	1.38	1.46	0.03	0.139	0.013	0.188
0–14	1.51	1.50	1.38	1.41	1.39	1.41	1.43	1.40	0.02	0.000	0.783	0.427
0–21	1.56	1.55	1.45	1.45	1.44	1.48	1.49	1.43	0.02	0.000	0.641	0.294
0–28	1.73	1.75	1.56	1.50	1.52	1.55	1.56	1.51	0.04	0.000	0.585	0.559

[ab]Means in the same row with different superscripts are significantly different at levels indicated for factors.

the birds in relation to the birds receiving the control diet. Addition of enzyme to the wheat diet resulted in a final weight gain similar to the maize-fed birds without enzyme supplementation (1377.5 g vs. 1329.3 g respectively). Birds fed on the barley-based diet had impaired ($p < 0.001$) FCR, compared with the birds fed diets based on the other cereal grains.

Intestinal pH and ileal digesta viscosity

The pH of the digesta from the gizzard ($p < 0.05$) and caeca ($p < 0.001$) differed on account of cereal type (Table 3). Birds on the sorghum-based diet had the same pH value in their gizzard and caecal contents as those on maize diet, with values higher than those on barley and wheat diets.

There was a significant ($p < 0.001$) interaction between cereal and enzyme on the ileal digesta viscosity of the chickens. The wheat-based diet resulted in the highest ($p < 0.0001$) digesta viscosity among the diets. The microbial enzyme reduced the ileal digesta viscosity of birds on all cereals, but this reduction was only significant ($p < 0.001$) in birds on wheat diet.

Digestibility

The ileal digestibility coefficient of dry matter, energy and protein was higher ($p < 0.0001$) in the chickens on maize and sorghum diets than those on barley and wheat diets (Table 4). Birds fed the barley-based diet had the lowest ($p < 0.0001$) ileal digestibility of dry matter, energy and protein (Table 4). The microbial enzyme significantly ($p < 0.05$) increased ileal digestibility of starch. There was no significant interaction between cereal and enzyme supplement.

Table 3 The effect of cereal grain type and microbial enzyme on pH in the intestinal contents and ileal digesta viscosity

Dietary treatments			pH		
Cereal	Enzyme	Gizzard	Ileum	Caeca	Viscosity (mPa.s)
Barley	−	3.35	6.69	6.80	3.18^{bc}
	+	3.34	7.00	6.81	2.66^{c}
Maize	−	3.30	6.22	7.10	2.38^{c}
	+	3.55	6.74	7.18	2.22^{c}
Sorghum	−	3.55	7.38	7.19	2.22^{c}
	+	3.66	6.76	7.46	2.17^{c}
Wheat	−	3.28	6.42	6.61	7.32^{a}
	+	3.38	6.62	6.30	4.15^{b}
SEM		0.096	0.327	0.133	0.407
Source of variation					
Cereal		0.029	0.241	0.000	0.000
Enzyme		0.099	0.650	0.799	0.017
Cereal × enzyme		0.578	0.337	0.125	0.000

[ab]Means in the same row with different superscripts are significantly different at levels indicated for factors.

Microbial population

The number of total anaerobic bacteria and lactobacilli in the gizzard was lower ($p < 0.05$) in chickens fed the sorghum- and wheat-based diets, when compared

Table 4 The effect of cereal grain type and microbial enzyme supplement on apparent ileal digestibility of dry matter, energy, protein and starch

Cereal	Enzyme	Dry matter	Energy	Protein	Starch
Barley	–	0.66	0.68	0.69	0.93
	+	0.68	0.71	0.71	0.98
Maize	–	0.79	0.80	0.83	0.95
	+	0.81	0.83	0.84	0.97
Sorghum	–	0.80	0.83	0.83	0.97
	+	0.77	0.79	0.81	0.96
Wheat	–	0.70	0.72	0.80	0.93
	+	0.73	0.75	0.82	0.96
SEM		0.020	0.021	0.016	0.013
Source of variation					
Cereal		0.000	0.000	0.000	0.470
Enzyme		0.324	0.263	0.491	0.022
Cereal × enzyme		0.379	0.337	0.512	0.166

with barley and maize, without the enzyme supplement (Table 5). However, adding the enzyme to the sorghum- and wheat-based diets increased the number of total anaerobic bacteria and lactobacilli in the gizzard to the same level as observed for the diets containing maize and barley. In the caeca, significantly lower numbers of total anaerobic bacteria and lactobacilli were observed in chickens fed the sorghum diet with or without enzyme supplementation than in chickens on the other diets. The numbers of total anaerobic bacteria or lactobacilli in the ileum were not affected either by the type of cereal or by the enzyme supplementation.

The number of coliform bacteria was increased ($p < 0.05$) in the caeca of birds because of enzyme supplementation, while the reverse was observed for the caecal population of lactose-negative enterobacteria. Furthermore, lower ($p < 0.0001$) numbers of caecal lactose-negative enterobacteria were observed for the barley and wheat diets compared with maize and sorghum. In the ileum, a significantly higher number of lactose-negative enterobacteria was observed in birds fed the maize diet without enzyme supplementation, in relation to birds on the other diets. However, the population of these bacteria decreased as a result of enzyme addition to the maize-based diet.

Jejunal mucosal morphometry

The influence of cereal grains on jejunal morphometry was much greater than that of the microbial

Table 5 The effect of cereal grain type and microbial enzyme supplement on bacterial counts (log 10 CFU/g) in the contents of different intestinal segments

	Dietary treatments								Probability			
Cereal	Barley		Maize		Sorghum		Wheat					
Enzyme	–	+	–	+	–	+	–	+	Cereal	SEM	Enzyme	Cereal × enzyme
Total anaerobic bacteria												
Gizzard	7.84^{ab}	7.87^{ab}	8.03^{a}	7.80^{ab}	7.16^{c}	7.89^{a}	7.28^{bc}	7.88^{ab}	0.189	0.118	0.041	0.045
Ileum	9.12	8.92	9.39	9.15	8.70	9.16	8.91	9.20	0.168	0.246	0.515	0.110
Caeca	9.71	9.78	9.80	9.50	9.37	9.53	9.79	9.71	0.093	0.008	0.568	0.087
Lactobacilli												
Gizzard	7.68^{ab}	7.61^{ab}	7.97^{a}	7.73^{ab}	6.80^{c}	7.74^{ab}	7.10^{bc}	7.85^{a}	0.207	0.051	0.024	0.015
Ileum	9.07	9.02	9.21	9.13	8.61	9.19	8.83	9.15	0.182	0.519	0.140	0.219
Caeca	9.60	9.61	9.68	9.28	9.32	9.27	9.64	9.62	0.103	0.009	0.118	0.189
Coliform bacteria												
Gizzard	3.16	3.03	3.00	3.20	3.33	3.14	3.25	3.08	0.114	0.581	0.383	0.290
Ileum	4.75	4.38	4.40	4.96	4.82	4.19	4.26	4.25	0.401	0.748	0.699	0.490
Caeca	7.98	8.44	7.65	8.30	8.06	8.39	8.47	8.53	0.221	0.142	0.021	0.603
Lactose-negative enterobacteria												
Gizzard	3.08	3.03	3.00	3.00	2.98	3.02	3.05	3.22	0.061	0.096	0.333	0.321
Ileum	3.01^{b}	2.97^{b}	3.40^{a}	2.93^{b}	3.26^{ab}	3.01^{b}	2.97^{b}	3.15^{ab}	0.114	0.432	0.082	0.047
Caeca	6.74	6.45	7.09	6.96	7.04	6.61	6.43	6.02	0.165	0.000	0.011	0.806
Clostridium perfringens												
Gizzard	3.18	3.23	3.00	3.00	3.15	3.07	3.00	3.08	0.095	0.161	0.831	0.864
Ileum	5.50	5.96	3.44	3.38	4.28	3.55	3.33	3.26	0.363	0.000	0.704	0.452
Caeca	5.64	5.81	4.29	4.21	4.47	4.33	4.47	4.02	0.388	0.001	0.654	0.888

[ab]Means in the same row with different superscripts are significantly different at levels indicated for factors.

Table 6 The effect of cereal grain type and microbial enzyme supplement on jejunal morphometry parameters

Cereal	Enzyme	Villus height (μm)	Crypt depth (μm)	Villus surface area (mm^2)	Villus height/crypt depth	Muscle layer thickness (μm)
Barley	–	899.7^{c}	155.0^{bc}	0.27	5.81^{c}	243.3
	+	984.2^{bc}	177.5^{a}	0.33	5.54^{c}	270.9
Maize	–	1045.2^{ab}	165.4^{ab}	0.32	6.41^{c}	247.3
	+	994.0^{bc}	158.6^{bc}	0.29	6.30^{c}	265.2
Sorghum	–	963.6^{bc}	155.0^{bc}	0.30	6.59^{bc}	230.7
	+	1037.6^{ab}	138.3^{d}	0.41	7.53^{ab}	216.0
Wheat	–	971.9^{bc}	151.2^{bcd}	0.37	6.44^{c}	236.9
	+	1119.0^{a}	143.8^{cd}	0.39	7.80^{a}	244.4
SEM		34.98	5.22	0.029	0.331	11.34
Source of variation						
Cereal		0.035	0.000	0.037	0.000	0.016
Enzyme		0.014	0.573	0.081	0.047	0.219
Cereal × enzyme		0.051	0.003	0.140	0.047	0.319

[ab]Means in the same row with different superscripts are significantly different at levels indicated for factors.

enzyme (Table 6). In general, villus height, surface area and villus height to crypt depth were higher ($p < 0.05$) in the birds fed the wheat-based diet than in the other cereal groups. Cereal-and-enzyme interaction increased villus height ($p = 0.051$) and villus:crypt ratio ($p < 0.05$) of birds on wheat diet, but had no effect ($p > 0.05$) on those receiving the other grains, resulting in an interaction ($p < 0.05$) between enzyme and cereal. The enzyme supplement reduced ($p < 0.01$) crypt depth in birds fed on the sorghum-based diet but not in birds fed on barley-based diets. The wheat-based diet increased ($p < 0.05$) the villus surface area compared with the barley- and maize-fed birds, but there was no cereal-and-enzyme interaction on this response. The thickness of the muscle layer was the least ($p < 0.05$) in birds on sorghum diet, with no significant difference from birds on the wheat-based diet.

Digestive enzyme activities

The results of digestive enzyme activities and tissue protein contents are summarized in Table 7. The jejunal protein content of the chickens receiving the exogenous enzyme was significantly ($p < 0.05$) decreased, but was not affected by cereal grains. In contrast, the activity of jejunal brush-border maltase and sucrase was influenced ($p < 0.05$) by cereal

Table 7 The effect of cereal grain type and microbial enzyme on tissues protein content and the activity of digestive enzymes in 21-day old chickens

Dietary treatments		Jejunum				Pancreas	
Cereal	Enzyme	Protein†	Maltase‡	Sucrase§	Alkaline phosphatase‡	Protein†	Chymotrypsin§
Barley	–	53.2	0.39	34.3	2.34	79.8	30.1
	+	36.9	0.33	28.9	1.57	96.7	32.2
Maize	–	55.7	0.42	38.9	1.83	81.4	29.3
	+	52.0	0.42	48.3	1.77	77.5	30.7
Sorghum	–	49.8	0.31	25.6	1.60	91.7	25.6
	+	39.8	0.39	22.7	1.68	85.2	32.0
Wheat	–	53.8	0.27	41.5	1.59	78.3	26.2
	+	38.0	0.25	40.0	1.42	81.2	23.8
SEM		6.34	0.045	5.37	0.223	6.12	2.63
Source of variation							
Cereal		0.436	0.008	0.017	0.225	0.264	0.119
Enzyme		0.015	0.483	0.985	0.149	0.593	0.317
Cereal × enzyme		0.729	0.742	0.659	0.257	0.237	0.434

†Concentration in tissue (mg/g tissue).
‡Specific activity (μmol/mg protein/min).
§Specific activity (*n*mol/mg protein/min).

grains. The highest ($p < 0.01$) activity of maltase was detected in birds fed on the maize-based diet, although the difference was not significant, compared with the birds on the sorghum-based diet. Feeding the sorghum-based diet to the birds decreased ($p < 0.05$) the activity of sucrase, in relation to the birds on maize and wheat diets. The activities of alkaline phosphatase, chymotrypsin and pancreatic protein content were not affected ($p > 0.05$) by dietary treatments.

Discussion

The effect of both enzyme and cereal on weight gain of birds was found to be strongly related to feed intake. Although the diets were isocaloric and isonitrogenous, feeding the barley-based diet led to a low final body weight gain because of decreased feed intake and digestion, in relation to the other diets. The result was in accordance with previous studies (Hofshagen and Kaldhusdal, 1992; Almirall et al., 1995), reporting lower growth rate of birds on barley than those on maize. The addition of the enzyme supplement did not improve growth on the barley-based diet but did on the wheat-based diet. This improvement was associated with an increase in feed intake and a decrease in digesta viscosity. It is well established that wheat-soluble arabinoxylans can increase intestinal digesta viscosity and show antinutritional activity (Choct and Annison, 1992). Application of exogenous enzymes, which hydrolyze these components, can eliminate their deleterious effects. It seems that the wheat used in this experiment was a viscous cultivar, as evident from its effect on ileal digesta viscosity. It has been reported that high digesta viscosity can increase feed retention time in broiler GIT (van der Klis et al., 1993). Moreover, there is a relationship between the rate of feed passage through the GIT and feed consumption in young chickens (Almirall and Esteve-Garcia, 1994). Thus, the decreased digesta viscosity as a result of the supplemental enzyme in wheat-based diet may have resulted in the increased feed intake. It may be the reason for improved weight gain as well as the ileal digestibility of dry matter and energy by 5% and 5.3%, respectively, in relation to the non-supplemented group. Feed conversion ratio was unchanged because of an increase in both feed intake and weight gain. This may explain the increased feed intake of wheat-based diet but not of maize during the first 2 weeks. Maize has the lowest concentration of soluble NSPs among the commonly used cereal grains, at levels not sufficient to produce a detectable undesirable effect on performance (Chesson, 2001). The mechanism behind this effect of the NSP-degrading enzymes in maize remains unknown but may be associated with a reduction in cell wall integrity as structurally important arabinoxylans are hydrolysed (Cowieson, 2004). It must be stated that both barley and wheat are used at higher levels than would be recommended in practical diets.

The AID of starch improved in birds receiving the supplemental enzyme. This finding is in agreement with the previous reports (Annison, 1992; Choct et al., 1999) and may be associated with the hydrolysis of cell wall material, releasing encapsulated starch. With the exception of sorghum, the addition of enzyme numerically increased ileal starch digestibility of the diets as in relation to the control groups. This effect may not be explained by a change in digesta viscosity alone, but by the side activities of enzymes that are associated with the supplement.

Dietary treatments affected the bacterial populations in the different segments of GIT in this experiment. Diet composition can impact on gut microflora at least by two ways; by changing the physico-chemical properties (i.e. viscosity or pH) of chyme and by supplying the nutrients for the growth of specific groups of microflora. In this study, although ileal viscosity was reduced by enzyme supplementation in the wheat-based diet, the total number of bacteria did not change, compared with the control group. This is in contrast with previous reports (Choct et al., 1996, 1999), which indicated an increase in ileal microbial activity because of the presence of viscous NSPs in the diet.

The increase in the numbers of total anaerobic bacteria and lactobacilli in the gizzard because of supplemental enzyme indicates that substrates for microbial growth are released by the action of the enzyme supplement in the gizzard. Cereal type also resulted in a change of the pH of the gizzard contents and lactobacillus numbers, but only to a minimal extent. The change in the pH in the gizzard may be due to the effects of the diets on secretion of hydrochloric acid by the proventriculus. The reduction in pH of the gizzard is considered as an effective means of preventing potentially pathogenic bacteria such as *Salmonella* (Bjerrum et al., 2005) and *C. perfringens* (Engberg et al., 2004; Bjerrum et al., 2005) from entering the lower part of the GIT. However, in the present study, while the gizzard pH of birds on barley was significantly lower than those on sorghum-based diet, a higher number of *C. perfringens* was observed in the ileum and

caeca of birds fed on barley. Although the ileal viscosity of birds on barley decreased by 16% because of supplemental enzyme, the ileal and caecal counts of *C. perfringens* still showed a slight increase. *Clostridium perfringens* is the causative agent of necrotic enteritis; so this observation agrees with reports that the incidence of necrotic enteritis in chickens fed barley is usually higher (Riddell and Kong, 1992). Besides inducing necrotic enteritis and increasing mortality (Riddell and Kong, 1992), *C. perfringens* is considered as a very active bacterium in the hydrolysis of bile salts (Knarreborg et al., 2002), leading to depressed broiler performance (Stutz and Lawton, 1984; Engberg et al., 2000). It is likely that a reason for the decreased growth performance of birds on barley-based diet is related to high numbers of *C. perfringens* in the lower parts of GIT, in relation to those on the other grains.

The number of lactose-negative enterobacteria in the caeca was affected by both cereal type and enzyme supplementation. The reduction in the population of lactose-negative enterobacteria, through the effect of the enzyme supplement, can be regarded as a significant effect. *Salmonella* is a key potential pathogen in broiler chickens, and is of major concern to the human consumer. The birds on barley and wheat diets had a lower number of lactose-negative enterobacteria than those on maize and sorghum in their caeca. It was convenient with higher number of lactobacilli in the former birds' caeca too. This may suggest the involvement of competitive exclusion (Nurmi and Rantala, 1973) of lactose-negative enterobacteria by lactobacilli.

Significant differences were observed between the treatments in morphometric indices, as a result of the main factors and their interaction effects. Thomas et al. (2005) noted no significant differences in the jejunal morphology of chickens on diets based on maize, sorghum or wheat. However, other workers have found some significant differences between the studied morphometric parameters by feeding of diets based on different cereals (Viveros et al., 1994; Yasar and Forbes, 1999; Mathlouthi et al., 2002a). Our finding was in agreement with that of Viveros et al. (1994), who reported shorter jejunal villi in birds fed on barley compared with those fed on a maize-based diet. Intestinal cell proliferation occurs mainly in the crypts (Geyra et al., 2001; Iji et al., 2001a). Thus, the large crypt suggests a high nutrient requirement for intestinal maintenance and reduced efficiency of the bird. In this view, the large crypt in birds fed on the barley-based diet may partly explain the poor growth performance. It has been reported that enzyme supplementation can lower the rate of cell proliferation in the crypt (Silva and Smithard, 2002). In this respect, the lower crypt depth as a result of enzyme addition can be considered as a beneficial way to decrease the cost of intestinal maintenance in birds. The observed increase in villus height as a result of supplemental enzyme agrees with the results obtained by Mathlouthi et al. (2002a) and Wu et al. (2004), who also observed similar results on rye- and wheat-based diets respectively. The increase in jejunal villus height and surface area in birds fed the enzyme-supplemented wheat-based diet may explain the improved performance of chickens on this diet, compared with the non-supplemented group.

Although the pancreatic protease (chymotrypsin) activity and protein concentration were not influenced by dietary treatments in this study, the activities of jejunal mucosal disaccharidases were affected by cereal type, but not by supplemental enzyme. The increase in intestinal enzymes is stimulated mechanically by chyme passing through the digestive tract (Duke, 1986). The bulk of digesta in the intestines of birds fed on maize or sorghum diets, resulting from a higher feed intake than those on barley and wheat may stimulate maltase activity. The activities of the other enzymes was, however, not increased, suggesting that different mechanisms may be involved in the priming of the enzymes. Based on dry matter, maize and sorghum-based diets contained the highest amount of starch. The high activity of maltase in the birds receiving these diets is probably related to substrate (maltose) availability in the jejunum. Alkaline phosphatase is known as a marker of mucosal enterocyte maturation because it is expressed by the enterocytes on the top of the villi (Weiser, 1973; Traber et al., 1991). Therefore, the lack of significant differences between different treatments in respect to alkaline phosphatase indicated that all sampled jejunal villi were at the same level of maturation. The relation between different cereal grains and the activity of digestive enzymes, especially the mucosa membrane-bound enzymes, apparently has not been previously investigated. A reduction in jejunal tissue protein content was observed in this study because of the microbial enzyme application. The same observations have been reported by other workers (Danicke et al., 2000; Iji et al., 2004). This can be interpreted as a positive effect of the microbial enzyme supplementation to save on the cost of mucosal renewal and re-direction of nutrients to body growth.

Conclusion

It can be stated that the inimical effect of barley on the growth performance of broiler chickens compared with other cereal grains is associated with its ability to alter intestinal morphology. The effects of barley on microbial populations, especially *C. perfringens*, may also lead to reduced efficiency in nutrient utilization by the birds. The positive effect of the supplemental enzyme on the performance of chickens fed on the wheat-based diet may be related to improved intestinal morphometric parameters and decreased digesta viscosity. Further studies into the absorptive function and microflora of the gut may be useful to explain the difference in growth performance of broiler chickens on different cereal grains and the exogenous enzymes investigated in this study.

Acknowledgements

The first author wishes to express thanks to Professor Mingan Choct, Australian Poultry CRC, for inviting him to Australia. We gratefully acknowledge the technical assistance of the Poultry group technicians, University of New England. We further wish to thank Danisco Animal Nutrition, Marlborough, UK for providing us with Gridazym™ GPL 5000.

References

Almirall, M.; Esteve-Garcia, E., 1994: Rate of passage of barley diets with chromium oxide: Influence of age and poultry strain and effect of β-glucanase supplementation. *Poultry Science* **73**, 1433–1440.

Almirall, M.; Francesch, M.; Perez-Vendrell, A. M.; Brufau, J.; Esteve-Garcia, E., 1995: The differences in intestinal viscosity produced by barley and beta-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *The Journal of Nutrition* **125**, 947–955.

Annison, G., 1992: Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improved apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science and Technology* **38**, 105–121.

Apajalahti, J.; Kettunen, A.; Graham, H., 2004: Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal* **60**, 223–232.

Bedford, M. R.; Schulze, H., 1998: Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews* **11**, 91–114.

Bjerrum, L.; Pedersen, K.; Engberg, R. M., 2005: The influence of whole wheat feeding on salmonella infection and gut flora composition in broiler. *Avian Diseases* **49**, 9–15.

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

Branton, S. L.; Lott, B. D.; Deaton, J. W.; Maslin, W. R.; Austin, F. W.; Pote, L. M.; Keirs, R. W.; Latour, M. A.; Day, E. J., 1997: The effect of added complex carbohydrates or added dietary fiber on necrotic enteritis lesions in broiler chickens. *Poultry Science* **76**, 24–28.

Burnett, G. S., 1966: Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. *The British Journal of Nutrition* **7**, 55–75.

Chesson, A., 2001: Non-starch polysaccharide degrading enzymes in poultry diets: influence of ingredients on the selection of activities. *World's Poultry Science Journal* **57**, 251–263.

Choct, M.; Annison, G., 1990: Anti-nutritive activity of wheat pentosans in broilers diets. *The British Journal of Nutrition* **30**, 811–821.

Choct, M.; Annison, G., 1992: Antinutritive effect of wheat pentosans in broiler chicken: role of viscosity and gut microflora. *The British Journal of Nutrition* **33**, 821–834.

Choct, M.; Hughes, R. J.; Wang, J.; Bedford, M. R.; Morgan, A. J.; Annison, G., 1996: Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *The British Journal of Nutrition* **37**, 609–621.

Choct, M.; Hughes, R. J.; Bedford, M. R., 1999: Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. *The British Journal of Nutrition* **40**, 419–422.

Cowieson, A. J.; Acamovic, T.; Bedford, M. R., 2004: The effects of supplementation of maize-based diets with exogenous phytase on amino acid digestibility and nitrogen retention by young broiler chicks. *British Poultry Science* **45**, S5–S6.

Dahlqvist, D., 1964: Method for assay of intestinal disaccharidases. *Analytical Biochemistry* **7**, 18–25.

Danicke, S.; Bottcher, W.; Jeroch, H.; Thielebein, J.; Simon, O., 2000: Replacement of soybean oil with tallow in rye-based diets without xylanase increases protein synthesis in small intestine of broiler. *The Journal of Nutrition* **130**, 827–834.

Duke, G. E., 1986: Alimentary canal: secretion and digestion, special digestive functions, and absorption. In: P. D. Sturkie (ed), *Avian Physiology*. Springer-Verlag, New York, pp. 289–302.

Engberg, R. M.; Hedemann, M. S.; Jensen, B. B.; Lesser, T. D., 2000: Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poultry Science* **79**, 1311–1319.

Engberg, R. M.; Hedemann, M. S.; Steenfeldt, S.; Jensen, B. B., 2004: Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poultry Science* **83**, 925–938.

Fengler, A. I.; Marquardt, R. R., 1988: Water-soluble pentosans from rye: II. Effect on rate of dialysis and on the retention of nutrients by the chick. *Cereal Chemistry* **65**, 298–302.

Gabriel, I.; Mallet, S.; Leconte, M., 2003: Differences in digestive tract characteristics of broiler chickens fed on complete pelleted diet or on whole wheat added to pelleted protein concentrate. *British Poultry Science* **44**, 283–290.

Geyra, A.; Uni, Z.; Sklan, D., 2001: Enterocyte dynamics and mucosal development in the posthatch chick. *Poultry Science* **80**, 776–782.

Hesselman, K.; Elwinger, K.; Nilsson, M.; Thomke, S., 1981: The effect of β-glucanase supplementation, stage of ripeness, and storage treatment of barley in diets fed to broiler chickens. *Poultry Science* **60**, 2664–2671.

Hofshagen, M.; Kaldhusdal, M., 1992: Barley inclusion and avoparcin supplementation in broiler diets. I. Effect on small intestinal bacterial flora and performance. *Poultry Science* **71**, 959–969.

Holdeman, L. V.; Cato, E. P.; Moore, E. C., 1977: *Anaerobic Laboratory Manual*. Virginia Polytechnic Institute and State University, Blacksburg.

Holdsworth, E. S., 1970: The effect of vitamin D on enzyme activities in the mucosal cells on the chick small intestine. *The Journal of Membrane Biology* **3**, 43–53.

Iji, P. A.; Saki, A.; Tivey, D. R., 2001a: Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. *British Poultry Science* **42**, 505–513.

Iji, P. A.; Saki, A.; Tivey, D. R., 2001b: Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. *British Poultry Science* **42**, 514–522.

Iji, P. A.; Khumalo, K.; Slippers, S.; Gous, R., 2004: Intestinal function and body growth of broiler chickens on maize-based diets supplemented with mimosa tannins and a microbial enzyme. *Journal of the Science of Food and Agriculture* **84**, 1451–1458.

van Immerseel, F.; de Buck, J.; Pasmans, F.; Huyghebaert, G.; Haesebrouck, F.; Ducatelle, R., 2004: *Clostridium perfringens* in poultry: an emerging threat for animal and public health. *Avian Pathology* **33**, 537–549.

Jozeflak, D.; Rutkowski, A.; Jensen, B. B.; Engberg, R. M., 2006: The effect of β-glucanase supplementation of barley- and oat-based diets on growth performance and fermentation in broiler chicken gastrointestinal tract. *British Poultry Science* **47**, 57–64.

van der Klis, J. D.; van Voorst, A.; van Cruyningen, C., 1993: Effect of a soluble polysaccharide, carboxymethyl cellulose: on the physico-chemical conditions in the gastrointestinal tract of broilers. *British Poultry Science* **34**, 971–983.

Knarreborg, A.; Engberg, R. M.; Jensen, S. K.; Jensen, B. B., 2002: Quantitative determination of bile salt hydrolase activity in bacteria isolated from the small intestine of chickens. *Applied and Environmental Microbiology* **68**, 6425–6428.

Mahagna, M.; Nir, I.; Larbier, M.; Nitsan, Z., 1995: Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks. *Reproduction, Nutrition, Development* **35**, 201–212.

Mathlouthi, N.; Lalles, J. P.; Lepercq, P.; Juste, C.; Larbier, M., 2002a: Xylanase and β-glucanase supplementation improve conjugated bile acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet. *Journal of Animal Science* **80**, 2773–2779.

Mathlouthi, N.; Mallet, S.; Saulnier, L.; Quemener, B.; Larbier, M., 2002b: Effects of xylanase and β-glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. *Animal Research* **51**, 395–406.

Miller, T. L.; Wolin, M. J., 1974: A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. *Applied Microbiology* **27**, 985–987.

Miller, G. L.; Blum, R.; Glennon, W. E.; Burton, A. L., 1960: Measurement of carboxymethyl cellase activity. *Analytical Biochemistry* **2**, 127–132.

National Research Council, 1994: *Nutrient Requirements for Poultry*. National Academy Press, Washington, DC.

Nitsan, Z.; Dror, Y.; Nir, I.; Shapira, I., 1974: The effects of force-feeding on enzymes of the liver, kidney, pancreas and digestive tract of chicks. *The British Journal of Nutrition* **32**, 241–247.

Noy, Y.; Sklan, D., 1995: Digestion and absorption in the young chick. *Poultry Science* **74**, 366–373.

Nurmi, E.; Rantala, M., 1973: New aspects of *Salmonella* infection in broiler production. *Nature* **241**, 210–211.

Porter, R. E. J., 1998: Bacterial enteritides of poultry. *Poultry Science* **77**, 1159–1165.

Riddell, C.; Kong, X. M., 1992: The influence of diet on necrotic enteritis in broiler chickens. *Avian Diseases* **36**, 499–503.

SAS Institute, 2003: *SAS User's Guide*. SAS Institute, Cary, NC.

Serviere-Zaragoza, E.; Navarrete del Toro, M. A.; Garcia-Carreno, F. L., 1997: Protein-hydrolyzing enzymes in the digestive system of the adult Mexican blue abalone, *Haliotis fulgens*, gastropoda. *Aquaculture* **157**, 325–336.

Shirazi-Beechey, S. P.; Smith, M. W.; Wang, Y.; James, P. S., 1991: Postnatal development of lamb intestinal digestive enzymes is not regulated by diet. *Journal of Physiology* **437**, 691–698.

Silva, S. S.; Smithard, R. R., 2002: Effect of enzyme supplementation of a rye-based diet on xylanase activity in the small intestine of broilers, on intestinal crypt cell proliferation and on nutrient digestibility and growth performance of the birds. *British Poultry Science* **43**, 274–282.

Sklan, D.; Noy, Y., 2000: Hydrolysis and absorption in the small intestines of posthatch chicks. *Poultry Science* **79**, 1306–1310.

Stutz, M. W.; Lawton, G. C., 1984: Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens* and ileal weight of broiler chicks. *Poultry Science* **63**, 2036–2042.

Thomas, D. V.; Ravindran, V.; Thomas, D. G., 2005: Performance, digestive tract measurements and gut morphology in broiler chickens fed diets containing maize, wheat or sorghum. *Proceedings of the Australian Poultry Science Symposium* **17**, 61–62.

Traber, P.; Gumucio, D. L.; Wang, W., 1991: Isolation of intestinal cell for the study of differential gene expression along the crypt-villus axis. *American Journal of Physiology* **260**, G895–903.

Uni, Z.; Ganto, S.; Sklan, D., 1998: Posthatch development of mucosal function in the broiler small intestine. *Poultry Science* **77**, 75–82.

Uni, Z.; Noy, Y.; Sklan, D., 1999: Posthatch development of small intestinal function in the poult. *Poultry Science* **78**, 215–222.

Viveros, A.; Brenes, A.; Pizarro, M.; Castano, M., 1994: Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Animal Feed Science and Technology* **48**, 237–251.

Wanger, D. D.; Thomas, O. P., 1978: Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* **57**, 971–975.

Weiser, M. M., 1973: Intestinal epithelial cell surface membrane glycoprotein synthesis. i. an indicator of cellular differentiation. *The Journal of Biological Chemistry* **248**, 2536–2541.

Wu, Y. B.; Ravindran, V.; Thomas, D. G.; Birtles, M. J.; Hendriks, W. H., 2004: Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. *British Poultry Science* **45**, 385–394.

Yasar, S.; Forbes, J. M., 1999: Performance and gastrointestinal response of broiler chickens fed on cereal grain-based food soaked in water. *British Poultry Science* **40**, 65–76.