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Response of Broiler Chickens to Triticale-Based Diets Supplemented with Microbial Enzymes (1. Growth and Intestinal Function)

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Abstract

A total of 384 day-old male Ross 308 broiler chicks were allocated to 8 dietary treatments to examine the influence of supplementation with xylanase and phytase, individually or in combination, in diets based on two new cultivars of triticale (Bogong and Canobolas). The inclusion of phytase alone in either Bogong or Canobolas diets increased ($P < 0.01$) feed intake and body weight; however, the effect of grain was not significant. The ileal digestibility of crude protein, gross energy, starch, calcium, and phosphorus was increased by inclusion of phytase and xylanase ($P < 0.05$). The interaction between xylanase and phytase positively influenced ($P < 0.01$) the digestibility of crude protein, gross energy, calcium, and phosphorus. Ileal viscosity was decreased ($P < 0.05$) by the inclusion of xylanase and phytase individually or in combination. The inclusion of phytase and xylanase increased ($P < 0.001$) the phytate-P degradation. Birds on Bogong-based diet had a higher ($P < 0.05$) degradation of phytate than those on the Canobolas-based diet. The weight of various visceral organs on day 7 was not affected by the inclusion of enzymes, nevertheless the weight of proventriculus plus gizzard was higher ($P < 0.01$) for chickens offered Canobolas-based than chicks on the Bogong-based diets. On day 21, the liver weight was reduced ($P < 0.001$) by the inclusion of phytase. An interaction ($P < 0.01$) between grain and phytase inclusion led to an increased weight of proventriculus plus gizzard on the Bogong diets with phytase. The inclusion of xylanase increased ($P < 0.01$) maltase activity at the jejunum on day 7, while it decreased the pancreatic protein content on day 21. The activity of chymotrypsin amidase was reduced ($P < 0.01$) by the inclusion of phytase. These results show that supplementation of phytase and xylanase to triticale-based diets can improve broiler performance by increasing the activities of some digestive enzymes and nutrient utilization.

Introduction

In our previous study, broiler chickens that were given diets in which triticale completely replaced maize and wheat, without enzyme supplementation, performed better than birds on wheat-based diets as well as birds on a maize-based diet (Widodo *et al.*, 2015). In another study

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in which triticale partly substituted for maize in broiler chicken diets the performance of the birds was better than that of the birds on the maize-based diet (Zarghi *et al*., 2010). The potential of triticale to replace maize (Zarghi and Golian, 2009) and wheat (Osek *et al*., 2010), has been previously documented.

There have been some previous tests in which older cultivars of triticale constituted the only cereal grain in the poultry diet. The response to such diets was poor (Shimada *et al*., 1974), and similar results have been observed for later cultivars of triticale (Rundgren, 1988). The poor performance of the birds on such diets was attributed to the presence of non-starch polysaccharides (NSP), which are mainly arabinoxylans and β-glucans (Pourreza *et al*., 2007), in addition to phytic acid (Jondreville *et al*., 2007). Annison and Choct (1991), and Bedford (1995) suggested that supplementation with exogenous carbohydrase enzymes, such as xylanase, can reduce the viscosity of the intestinal contents and improve the digestibility of starch, protein and energy in broiler diets. Likewise, the inclusion of phytase in broiler chicken diets can improve feed utilisation and body weight, and also phosphorous content in excreta and mortality (Levic *et al*., 2006).

There has been a particular focus on dealing with the negative effects of phytic acid as well as the presence of xylans and arabinoxylans in chicken diets containing triticale (Çiftci *et al*., 2003; Jondreville *et al*., 2007; Pourreza *et al*., 2007; Zarghi *et al*., 2010). These researches show that the inclusion of enzyme preparations in the diet can improve chicken performance. Moreover, Vieira *et al*. (1995) reported that the inclusion of up to 40% triticale in a maize-soy diet did not have any negative effect on body weight of broiler chickens. In addition, Fayez *et al*. (1996) reported that even when the diet contained 100% of a Syrian cultivar of triticale for the grain portion without inclusion of any enzymes, the productivity of broiler chickens was unaffected. However, there is still dearth of information about the physiological response of broiler chickens fed the newer high-yielding cultivars of triticale when supplemented with microbial enzymes.

The objective of this trial was to examine the influence of supplementation with xylanase and phytase, individually or in combination, in diets based on two new cultivars of triticale (Bogong and Canobolas) on the gross response, visceral organ weight as well as some physiological responses in broiler chickens.

Materials and Methods

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No. AEC 10/098).

Nutrient content of Bogong and Canobolas

The grain samples were ground to pass through a one-mm sieve for laboratory analyses, while for the starch granule analysis, a sub-sample of whole grains was collected and stored in 20 mL container. Total starch and resistant starch in the samples were determined with the "Megazyme" total starch kit, using the enzyme procedure developed by McCleary *et al*. (1994). Soluble and insoluble NSP of the ground samples were measured as described by Englyst and Hudson (1993) and Theander and Westerlund (1993). Phytate-P content was measured using the method described by Haug and Lantzsch (1983). The nutrient contents of two grain cultivars are shown in Table 1.

Table 1. Analyzed nutrient and phytate-P content (g/kg DM) of Bogong and Canobolas

Items	Bogong	Canobolas
Crude protein	124.4	114.2
Total starch	653.7	612.3
Total free sugar	20.35	19.86
Total Soluble NSP	10.74	10.06
Total insoluble NSP	91.13	114.93
Arabinose	30.59	38.14
Xylose	38.43	48.34
Ca	0.30	0.31
P	3.70	3.50
Phytate-P	2.54	1.80

Dietary treatments and housing

The microbial enzymes used in this study were supplied by AB Vista® (Marlborough, UK). The xylanase preparation, Econase® XT, which contains thermostable endo-1,4-beta-xylanase, produced by *Trichoderma reesei*, was added to supply 160,000 BXU of xylanase activity. The microbial phytase, Quantum® 2500, which is a 6-phytase from *E. coli* was added to supply 500 FTU per kg diet.

A 2 × 2 × 2 factorial arrangement was used to study 2 cultivars of high-yielding triticale (Bogong and Canobolas), with or without xylanase, and with or without phytase. Each diet was formulated to contain triticale (650 g/kg) as the sole cereal grain. The dietary treatments were as follows: a diet based on Bogong without any enzymes (B); Bogong with the inclusion of xylanase (BX); Bogong with the inclusion of phytase (BP); Bogong with the inclusion of xylanase and phytase (BXP); Canobolas without enzymes (C); Canobolas with the inclusion of xylanase (CX); Canobolas with the inclusion of phytase (CP), and Canobolas with the inclusion of xylanase and phytase (CXP). The diets were formulated to meet the minimum Aviagen recommendations (Aviagen, 2007). An indigestible marker, TiO_2, was incorporated in all diets to enable measurement of nutrient digestibility of the diets. Diets were pelleted and the ingredients and nutrient composition of them is shown in Table 2.

Table 2. Ingredients and nutrient composition (g/kg) of dietary treatments

Ingredients	B	BX	BP	BXP	C	CX	CP	CXP
Bogong	650.0	650.0	650.0	650.0	-	-	-	-
Canobolas	-	-	-	--	650.0	650.0	650.0	650.0
Soybean Meal	190.0	190.0	190.0	190.0	190.0	190.0	190.0	190.0
Soycomil K	69.4	69.4	69.4	69.4	61.3	61.2	61.2	61.2
L-Threonine	1.8	1.8	1.8	1.8	1.9	1.9	1.9	1.9
L-Lysine HCl	4.8	4.8	4.8	4.8	5.3	5.3	5.3	5.3
DL-Methionine	2.6	2.6	2.6	2.6	3.0	3.0	3.0	3.0
Sunflower oil	35.7	35.7	35.5	35.3	42.6	42.6	42.5	42.4
Limestone	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1
Dical. P	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8
Common Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline Cl-70%	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5
Xylanase	-	0.1	-	0.1	-	0.1	-	0.1
Phytase	-	-	0.2	0.2	-	-	0.2	0.2
Premix†	2.6	2.6	2.6	2.6	2.5	2.5	2.5	2.5
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient composition								
ME (Kcal/kg)	3033	3033	3033	3033	3081	3081	3081	3081
Crude protein	220.0	220.0	220.0	220.0	220.0	220.0	220.0	220.0
Crude fat	53.8	53.7	53.6	53.5	59.4	59.3	59.2	59.1
Crude fibre	25.5	25.5	25.5	25.5	25.2	25.2	25.2	25.2
Lysine	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Methionine	6.1	6.1	6.1	6.1	6.5	6.5	6.5	6.5
Met + Cys	10.5	10.8	10.8	10.8	11.3	11.3	11.3	11.3
Calcium	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Available P	5.2	5.2	5.2	5.2	5.6	5.6	5.6	5.6
Sodium	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chlorine	5.6	5.6	5.6	5.6	3.4	3.4	3.4	3.4

†Supplied per kg of diet (mg): vitamin A (as all-trans retinol): 3.6; cholecalciferol: 0.09; vitamin E (as d-α-tocopherol): 44.7, vitamin K_3: 2.0; thiamine: 2.0; riboflavin: 6.0; pyridoxine hydrochloride: 5.0, vitamin B_{12}: 0.2, biotin: 0.1, niacin: 50.1, D-calcium pantothenate: 12.0, folic acid: 2.0, Mn: 80.0, Fe: 60.0, Cu: 8.0, I: 1.0, Co: 0.3, and Mo: 1.0.

A total of 384 day-old male Ross 308 broiler chicks (Baiada Poultry Pty. Ltd, Tamworth, NSW, Australia), weighing 41.30 ± 0.35 g, were randomly allocated to 48 cages. The experimental chickens were raised in battery brooders, 60 × 42 × 23 cm, set up in a climate-controlled room. Each of the 8 treatments was randomly assigned to 6 cages, with 8 birds per cage. Water and feed were available *ad libitum*. The birds were initially brooded at a temperature of 34°C, and this was gradually reduced to 24 ± 1°C at 21 days of age when the

feeding trial ended. Light was provided for 18 h per day throughout the trial period. Birds were weighed at 1, 7, and 21 days of age, and feed intake was recorded during the experiment.

On days 7 and 21, one bird and three birds, respectively, from each cage, were randomly selected, weighed and killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and removed. The purpose of sampling was to weigh the visceral organs and obtain a section of jejunum (approximately 5 cm of anterior part of jejunum, immediately distal to the posterior end of the duodenal loop) and pancreas for analysis of enzyme activities. For the determination of the TiO_2 content as well as nutrient digestibility, the digesta from the ileum were collected on day 21 and pooled on a cage basis, homogenized and stored at –20°C. Later, the samples were freeze-dried, ground with a small grinding machine and stored in airtight containers at 4°C until they were analyzed to determine TiO_2, gross energy, starch and protein concentrations. The following is the details of the measurements and analysis.

Phytate-P content of ileal digesta

The phytate-P content of diets and ileal digesta was collected quantitatively and measured as described by Haug and Lantzsch (1983). In addition, the degradation of phytate (%), i.e. the percentage of dietary phytate-P apparently absorbed from the gut proximal to the ileum, was calculated by the following equation:

$$Phytate P degradation = \frac{(Phytate\ P_{(Diet)} - Phytate\ P_{(Digesta)})}{Phytate P_{(Diet)}} \times 100$$

Visceral organ weight

On days 7 and 21, the visceral organs (small intestine, proventriculus plus gizzard with contents, liver, pancreas, spleen, and bursa of Fabricius) of the randomly selected birds were obtained and weighed. The body weight of the birds was recorded.

Tissue protein content and digestive enzyme analysis

The pancreas and the anterior part of the jejunum were placed on crushed ice within one minute of death. The jejunal tissue was then opened longitudinally along one side of the section, using a pair of sharp scissors and the mucosal surface was cleaned with 1% (w/v) physiological saline. The jejunal tissue and pancreas samples were then wrapped in a small piece of labelled aluminium foil, and snap-frozen in liquid nitrogen. Samples were then stored at -20°C until preparation for analysis. The assessment of the digestive enzyme activities and protein concentration of the jejunal tissue was conducted as described by Shirazi-Beechey *et al.* (1991).

The pancreas was processed in a similar manner to the jejunum except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used instead of buffer and the entire tissue was homogenized (Nitsan *et al*., 1974). The homogenised tissue was then centrifuged at high speed (30,000 × g) for 20 min to obtain a crude homogenate supernatant.

The specific activities of jejunal and pancreatic enzymes were evaluated by incubation with fixed substrate concentrations as standardized for poultry (Iji *et al*., 2001). Assays on the jejunal homogenate were conducted for mucosal protein content and activities of alkaline phosphatase (EC 3.1.3.1), maltase (EC 3.2.1.20), and sucrase (EC 3.2.1.10); whereas assays on the pancreas were conducted for protein and chymotrypsin amidase (EC 3.4.21.1). The specific activities of enzymes were measured according to the methods described for other species (Holdsworth, 1970; Serviere-Zaragoza *et al*., 1997) after standardization for poultry. The measurement of alkaline phosphatase activity was conducted according to Forstner *et al*. (1968). The protein content of both jejunal mucosa and pancreatic tissue was measured according to Bradford (1976), using the Coomassie dye-binding procedure. The protein absorbance data obtained by colorimetry (using Varian Cary 50 Bio UV-Visible Spectrophotometer) were converted into absolute values using Lowry Software (McPherson, 1985).

Ileal digestibility of nutrients

The TiO_2 contents of the ileal digesta and diet samples were measured by the method developed by Short *et al.* (1996). The TiO2 marker concentrations in the feed, and ileal digesta were used to calculate the digestibility coefficients for protein, gross energy, starch and minerals. Diets and ileal digesta were analyzed for gross energy, which was determined for individual samples using IKA® WERKE bomb calorimeter (C 7000, GMBH & Co., Staufen, Germany) as well as starch, which were determined with the "Megazyme" total starch kit, using the enzyme procedure developed by McCleary *et al*. (1994). The apparent digestibility

coefficient (ADC) of nutrients was calculated using the following equation:

$$ADC\% = 100 \times \left(\frac{\%\ TiO_2\ in\ feed}{\%\ TiO_2\ in\ ileal\ digesta} \times \frac{\%\ nutrient\ in\ ileal\ digesta}{\%\ nutrient\ in\ feed}\right)$$

Samples of diet and digesta were also analyzed for mineral and nitrogen contents. The nitrogen content was then converted to crude protein by multiplying with a factor of 6.25.

Statistical analyses

All data were analyzed by ANOVA using the general linear model (GLM) procedure of Minitab® Version 16 (Minitab, 2010) for the main factors (cultivar, xylanase, and phytase) and the interactions between these three factors. The significance of difference between means was determined by Fisher's least significant difference (LSD) test, for which the significant level was set at $P < 0.05$.

Results

Gross response

The gross response of the birds fed Bogong- and Canobolas-based diets with and without xylanase and phytase is shown in Table 3.The feed intake to day 7 was increased ($P < 0.01$) by the inclusion of phytase to both diets. Feed intake to 21d was also slightly ($P = 0.063$) affected by the xylanase inclusion. Body weight gain was increased ($P < 0.01$) by the inclusion of phytase, in addition to the interaction ($P = 0.081$) between xylanase and phytase at d 7 ($P = 0.081$) and 24 ($P< 0.01$).

The FCR to days 7 and 21 was not significantly affected by the treatments, but the FCR of birds on Bogongs-based was slightly ($P = 0.056$) better than on Canobolas-based diets. From hatch to d 7, the best FCR was found in chicks on the diet containing only xylanase for both Bogong and Canobolas (1.04), which is to some degree better than the FCR on the Canobolas diet without enzyme and with both xylanase and phytase; which was 1.08 (or 3.9% different). There was no significant interaction between grain and xylanase for all parameters measured, except for a significant interaction ($P < 0.05$) between grain and phytase as well as between xylanase and phytase ($P < 0.01$) on the feed intake on day 21.

Table 3. Feed intake (FI), body weight (BW) and feed conversion ratio (FCR) of chickens on triticale-based diet with or without enzymes between hatch and 7 or 21 d of age[1]

Treatments			1-7 days			1-21 days		
Grain	Xyl[2]	Phy[3]	FI (g/b)	BW (g/b)	FCR	FI (g/b)	BW (g/b)	FCR
Bogong	-	-	146.3^{c}	180.8^{cd}	1.05	1008.7^{de}	813.7^{b}	1.31
Bogong	+	-	147.2^{c}	182.8^{cd}	1.04	1043.0^{d}	826.6^{b}	1.33
Bogong	-	+	167.5^{a}	201.7^{a}	1.04	1385.5^{a}	1071.9^{a}	1.35
Bogong	+	+	164.6^{ab}	198.3^{ab}	1.05	1275.6^{c}	1045.4^{a}	1.27
Canobolas	-	-	147.4^{c}	178.1^{d}	1.08	954.9^{e}	775.2^{b}	1.31
Canobolas	+	-	154.1^{bc}	189.1^{bc}	1.04	961.1^{e}	788.0^{b}	1.29
Canobolas	-	+	168.3^{a}	201.2^{a}	1.05	1373.6^{ab}	1066.0^{a}	1.34
Canobolas	+	+	170.8^{a}	199.4^{a}	1.08	1305.1^{bc}	1048.4^{a}	1.30
Pooled SEM[4]			1.94	1.79	0.005	27.00	20.20	0.010
Source of variation			Significance of treatment effect					
Grain			ns	ns	0.056	ns	ns	ns
Xylanase			ns	ns	ns	0.063	ns	ns
Phytase			**	**	ns	**	**	ns
Grain x Xylanase			ns	ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	*	ns	ns
Xylanase x Phytase			ns	0.081	0.067	**	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates; [2]Xylanase; [3]Phytase; [4]*SEM* = Standard error of mean.
[a-d]Values with unlike superscripts within each column are significantly different at *P< 0.05; **P< 0.01; ns = not significant.

Nutrient digestibility

The ileal digestibility of CP did not differ ($P >$ 0.05) between the cultivars, but was increased by the inclusion of xylanase and the interaction between the grain and xylanase ($P < 0.05$), the inclusion of phytase, and the interaction between xylanase and phytase ($P < 0.01$). The digestibility of CP increased by 9.4% with the inclusion of phytase in the Bogong diet, while

the inclusion of the combination of supplemental xylanase and phytase increased CP digestibility by 11.5% in the Canobolas diet (Table 4).

Table 4. The ileal digestibility (%) of CP, gross energy, starch, Ca and P of chickens on triticale-based diets with or without enzymes at 21 days of age[1]

Treatments			Crude protein	Gross energy	Starch	Ca	P
Grain	Xyl[2]	Phy[3]					
Bogong	-	-	77.7^{c}	78.9^{d}	83.5^{c}	41.3^{b}	43.8^{bc}
Bogong	+	-	81.0^{b}	81.6^{c}	86.3^{ab}	46.4^{ab}	49.6^{ab}
Bogong	-	+	85.0^{a}	84.0^{ab}	86.8^{ab}	45.8^{ab}	56.4^{a}
Bogong	+	+	82.2^{ab}	82.9^{abc}	85.6^{b}	44.1^{ab}	55.0^{a}
Canobolas	-	-	75.8^{c}	77.0^{e}	83.0^{c}	32.2^{c}	39.4^{c}
Canobolas	+	-	81.2^{b}	82.1^{c}	85.8^{ab}	50.6^{a}	53.0^{a}
Canobolas	-	+	81.3^{b}	84.6^{ab}	87.2^{a}	41.2^{b}	51.1^{a}
Canobolas	+	+	84.5^{a}	82.6^{bc}	85.9^{ab}	48.0^{ab}	51.2^{a}
Pooled SEM[4]			0.55	0.41	0.26	7.03	1.10
Source of variation			Significance of treatment effect				
Grain			ns	ns	ns	ns	ns
Xylanase			*	*	*	*	*
Phytase			**	**	**	ns	***
Grain x Xylanase			*	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns
Xylanase x Phytase			**	**	ns	**	**
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of means.

[a-c] Values with unlike superscripts within each column are significantly different at *P< 0.05; **P< 0.01; **P< 0.001; ns = not significant.

The digestibility of gross energy was increased by the inclusion of xylanase (P < 0.05) and the inclusion of phytase (P < 0.01) and the interaction between xylanase and phytase (P < 0.01). Gross energy digestibility was increased by the inclusion of phytase by 9.4% and 9.9% in the Bogong and Canobolas diets, respectively. Likewise, the digestibility of starch was increased by the inclusion of xylanase (P < 0.05) and phytase (P < 0.01).

The ileal digestibility of Ca was increased by the inclusion of xylanase (P < 0.05) and the interaction (P < 0.01) between xylanase and phytase. Similarly, the ileal digestibility of P was significantly increased by the inclusion of xylanase (P < 0.05) and phytase (P < 0.001), as well as the interaction (P < 0.01) between xylanase and phytase. The inclusion of enzymes increased P digestibility by about 13.4% to 29.0%, and 30.0% to 35.0%, in the Bogong and Canobolas diets, respectively.

Ileal viscosity and phytate-P content

The results in Table 5 show the effect of dietary microbial enzyme supplementation on viscosity of ilealdigesta, the content of phytate-P in ileal digesta and phytate degradation in the ileum. In general, the inclusion of enzymes reduced the viscosity and phytate-P content of ileal digesta, as well as increasing the degradation of phytate in the diet.

The inclusion of xylanase and phytase, and the interaction between these two factors reduced (P < 0.05) the viscosity of ileal digesta. In addition, the concentration of phytate-P in the ileal digesta was also decreased (P < 0.001) by the inclusion of phytase in the diet, the interaction between grain and phytase, as well as the interaction between the three main factors. Furthermore, the interaction between xylanase and phytase simultaneously in the Bogong and Canobolas diets, tended (P = 0.092) to reduce the phytate-P content, compared to not only the control diets (no enzymes) but also when the diets were supplemented with xylanase only. The degradation of phytate-P at the ileum was numerically higher on the Canobolas than Bogong diet, while it was increased (P < 0.05) by the interaction of grain and xylanase, by the inclusion (P < 0.001) of xylanase and phytase, and the interaction between xylanase and phytase. The degradation of phytate-P by microbial enzymes was more than two times higher than on the diets without any enzyme supplementation.

Table 5. Ileal digesta viscosity, phytate-P content and degradation of phytate of broiler chickens on triticale-based diets with or without enzymes at 21 days of age[1]

Treatments			Viscosity	Phytate-P	Degradation of
Grain	Xyl[2]	Phy[3]	(cP)	(g/kg DMI)	phytate (%)
Bogong	-	-	3.8[a]	2.5[a]	15.2[d]
Bogong	+	-	2.6[b]	2.4[ab]	32.8[ab]
Bogong	-	+	2.7[b]	2.2[c]	30.7[bc]
Bogong	+	+	2.3[b]	2.0[d]	34.5[a]
Canobolas	-	-	3.9[a]	2.5[ab]	13.1[d]
Canobolas	+	-	2.4[b]	2.4[b]	28.4[c]
Canobolas	-	+	2.7[b]	2.0[d]	31.9[abc]
Canobolas	+	+	2.4[b]	2.2[c]	30.5[bc]
Pooled SEM[4]			0.15	0.03	1.20
Source of variation			Significance of treatment effect		
Grain			ns	ns	*
Xylanase			*	ns	***
Phytase			*	***	***
Grain x Xylanase			ns	***	*
Grain x Phytase			ns	ns	ns
Xylanase x Phytase			*	0.092	***
Grain x Xylanase x Phytase			ns	***	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of mean.
[a-d] Values with unlike superscripts within each column are significantly different at *P< 0.05; **P <0.01; ***P <0.001; ns = not significant.

Relative visceral organ weight

On day 7, there was no statistically significant effect of xylanase and phytase inclusion on the relative weight of any of the organs examined (Table 6); however, the relative weight of the proventriculus plus gizzard of birds on the Bogong diets was less (P < 0.01) than that on the Canobolas diets.

On the other hand, the relative weight of liver of birds on the Bogong diets was higher (P < 0.01) than that of birds on the Canobolas diets. The heaviest weight of proventriculus plus gizzard was in birds on the Canobolas diet with phytase inclusion (5.90 g/100 g of body weight), and the lowest weight were observed on birds on the Bogong diet containing phytase (4.30 g/100 g body weight). In addition, there is an interaction (P < 0.05) between grain and phytase inclusion to reduce the relative weight of proventriculus and gizzard of birds on the Bogong diet, on the other hand, increase the relative weight of proventriculus and gizzard of birds on the Canobolas diet. The relative weight of lymphoid tissues (spleen and bursa of Fabricius) as well as yolk sac was not significantly different (P > 0.05).

On day 21 (Table 7) the only significant effect of the inclusion of enzymes in the diets was on the relative weight of liver, which was decreased (P < 0.001) by the inclusion of phytase in the diets. The relative weight of the proventriculus and gizzard tended (P =0.086) to be lower in the diets containing xylanase. The effect of inclusion of enzymes on the relative weight of small intestine was not significant; however, the values on the diet with enzymes were less than those on diets without enzymes. In addition, the inclusion of enzymes did not statistically affect the relative weight of pancreas and immune organs, the spleen and bursa.

Tissue protein content and digestive enzyme activities

In early life (at 7 d), there were no significant effects of grain cultivar, supplementary xylanase and phytase on the pancreatic and jejunal tissue protein content and enzyme activities, except for the effect of xylanase inclusion (P < 0.001) on maltase activity in jejunal tissue(Table 8).

The tissue protein content and enzyme activities at 21 d of age are presented in Table 9. There was no significant effect of grain variety, while the inclusion of xylanase significantly decreased (P < 0.05) the pancreatic tissue protein content and the inclusion of phytase decreased (P < 0.01) the activity of chymotrypsin amidase. The activities of jejunal tissue protein, alkaline phosphatase, maltase and sucrase were not significantly (P > 0.05) affected by the treatments.

Table 6. Relative weight of visceral organs (g/100g of body weight) of broiler chickens on triticale-based diets with or without enzymes at 7 day of age[1]

Treatments			Proventriculus and Gizzard	Small Intestine	Pancreas	Liver	Spleen	Bursa of Fabricius	Yolk sac
Grain	Xyl[2]	Phy[3]							
Bogong	-	-	4.5^{bc}	9.1	0.46	5.0^{ab}	0.07	0.17	0.12
Bogong	+	-	4.8^{bc}	9.2	0.52	5.0^{ab}	0.08	0.18	0.09
Bogong	-	+	4.3^{c}	9.2	0.49	5.2^{a}	0.08	0.18	0.09
Bogong	+	+	4.4^{bc}	10.0	0.46	4.9^{ab}	0.05	0.21	0.09
Canobolas	-	-	5.2^{ab}	9.6	0.50	4.5^{bc}	0.06	0.18	0.03
Canobolas	+	-	4.7^{bc}	10.1	0.49	4.6^{bc}	0.09	0.18	0.02
Canobolas	-	+	5.9^{a}	10.1	0.50	4.3^{c}	0.08	0.20	0.10
Canobolas	+	+	5.2^{ab}	8.6	0.45	4.5^{bc}	0.08	0.17	0.06
Pooled *SEM*[4]			0.12	0.16	0.010	0.08	0.003	0.006	0.015
Source of variation			Significance of treatment effect						
Grain			**	ns	ns	**	ns	ns	ns
Xylanase			ns	ns	ns	ns	ns	ns	ns
Phytase			ns	ns	ns	ns	ns	ns	ns
Grain x Xylanase			0.08	ns	ns	ns	ns	ns	ns
Grain x Phytase			**	ns	ns	ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of mean.

[a-c] Values with unlike superscripts within each column are significantly different at **P*< 0.05; ***P*< 0.01; ****P*< 0.001; ns = not significant.

Table 7. Relative weight of visceral organs (g/100g of body weight) of broiler chickens on triticale-based diets with or without enzymes at 21 day of age[1]

Treatments			Proventriculus and Gizzard	Small Intestine	Pancreas	Liver	Spleen	Bursa of Fabricius
Grain	Xyl[2]	Phy[3]						
Bogong	-	-	2.0	6.9	0.27	3.5^{ab}	0.08	0.20
Bogong	+	-	1.9	6.4	0.27	3.8^{a}	0.07	0.17
Bogong	-	+	2.3	6.5	0.26	2.9^{c}	0.06	0.25
Bogong	+	+	1.9	6.2	0.26	3.3^{bc}	0.08	0.21
Canobolas	-	-	2.2	6.9	0.28	3.8^{a}	0.12	0.25
Canobolas	+	-	2.2	6.4	0.29	3.7^{a}	0.09	0.21
Canobolas	-	+	2.2	6.8	0.29	3.1^{bc}	0.08	0.19
Canobolas	+	+	1.9	6.3	0.30	3.2^{bc}	0.08	0.19
Pooled *SEM*[4]			0.05	0.09	0.006	0.06	0.005	0.008
Source of variation			Significance of treatment effect					
Grain			ns	ns	ns	ns	ns	ns
Xylanase			0.086	ns	ns	ns	ns	ns
Phytase			ns	ns	ns	***	ns	ns
Grain x Xylanase			ns	ns	ns	0.081	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of mean.

[a-c] Values with unlike superscripts within each column are significantly different at *P< 0.05; **P< 0.01; ***P< 0.001; ns = not significant.

Table 8. Tissue protein content and enzyme activities of broiler chickens at day 7, on triticale-based diets with or without microbial enzyme supplementation[1]

Treatments			Pancreas		Jejunum			
Grain	Xyl[2]	Phy[3]	Protein (mg/g tissue)	Chymotrypsin Amidase (ηmol/mg protein/min)	Protein (mg/g tissue)	Alkaline Phosphatase (µmol/mg protein/min)	Maltase	Sucrase
							(ηmol/mg protein/min)	
Bogong	-	-	175.6	7.0	195.2	5.4	147.1^{c}	27.1
Bogong	+	-	163.8	5.7	183.5	5.4	197.7^{a}	29.7
Bogong	-	+	166.1	6.9	187.3	4.4	153.1^{bc}	30.0
Bogong	+	+	165.1	6.5	185.1	5.2	187.5^{ab}	28.0
Canobolas	-	-	171.1	6.9	207.2	5.4	156.5^{bc}	27.3
Canobolas	+	-	167.4	5.3	182.4	5.7	194.9^{a}	30.2
Canobolas	-	+	169.5	5.4	193.7	4.8	157.6^{bc}	29.5
Canobolas	+	+	164.4	6.1	190.9	5.1	185.6^{ab}	33.3
Pooled *SEM*[4]			2.180	0.198	4.100	0.212	4.920	0.804
Source of variation			Significance of treatment effect					
Grain			ns	ns	ns	ns	ns	ns
Xylanase			ns	ns	ns	ns	**	ns
Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase			ns	ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of mean.

[a-c] Values with unlike superscripts within each column are significantly different at $^{**}P < 0.01$; ns = not significant.

Table 9. Tissue protein content and enzyme activities of broiler chickens at day 21 following rearing on triticale-based diets with or without microbial enzyme supplementation[1]

Treatments			Pancreas		Jejunum			
Grain	Xyl[2]	Phy[3]	Protein (mg/g tissue)	Chymotrypsin Amidase (ηmol/mg protein/min)	Protein (mg/g tissue)	Alkaline Phosphatase (µmol/mg protein/min)	Maltase	Sucrase
							(ηmol/mg protein/min)	
Bogong	-	-	228.9^{ab}	5.8^{a}	250.2	8.9	133.6	18.9
Bogong	+	-	171.5^{c}	4.7^{ab}	199.5	9.9	159.5	23.8
Bogong	-	+	212.1^{abc}	5.6^{a}	232.8	8.1	144.8	21.3
Bogong	+	+	179.3^{c}	4.1^{b}	219.1	8.4	208.8	26.7
Canobolas	-	-	234.6^{a}	5.7^{a}	249.8	10.3	149.9	18.4
Canobolas	+	-	172.4^{c}	5.1^{ab}	155.8	9.8	184.6	26.1
Canobolas	-	+	189.7^{abc}	5.5^{a}	194.4	7.8	140.2	22.0
Canobolas	+	+	184.4b^{c}	3.9^{b}	193.9	8.6	175.1	27.2
Pooled *SEM*[4]			6.420	0.186	8.35	0.288	6.52	0.920
Source of variation			Significance of treatment effect					
Grain			ns	ns	ns	ns	ns	ns
Xylanase			*	ns	ns	ns	ns	ns
Phytase			ns	**	ns	ns	ns	ns
Grain x Xylanase			ns	ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns	ns
Xylanase x Phytase			0.091	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of mean.

[a-c] Values with unlike superscripts within each column are significantly different at *P< 0.05; **P< 0.01; ns = not significant.

Discussion

Gross response

The results of this study demonstrate that supplementation with microbial enzymes improve the performance of broiler chickens in terms of FI, BW, and FCR up to 21 days of age. This finding is consistent with previous study (Zarghi *et al.,* 2016) who showed that supplementation of triticale-based diets with xylanase and β-glucanase increased broiler performance. The feed intake of chickens on a diet based on an 'old' triticale cultivar (Pettersson and Aman, 1988) was improved by enzyme supplementation than response on a 'new' triticale cultivar diet (Pourreza *et al*., 2007). One of the reasons for the differences may be the lower content of NSP in the new cultivars of triticale (Elangovan *et al.,* 2011) compared to the cultivars used in previous trials. The enzyme (xylanase) appeared to have a bigger impact under higher fibre content. Nevertheless, Oettler (2005) argued that there are many other factors that can affect the nutritional value of triticale used in feeding, such as genotype, growing environment, animal species, feed formula, and methodologies implemented in the experiments.

The highest FI and BW were found in the diets with only phytase inclusion. These diets also exhibited the highest CP, GE, starch and P digestibility. This finding, however, was unexpected, because the ileal viscosity of birds on the diet with only phytase supplementation was significantly higher than that of birds on the diets containing only xylanase or those containing a combination of supplemental xylanase and phytase. This response may be due to the fact that the microbial phytase used in the present study was produced by solid state fermentation and contains significant activities of beta-glucanase and xylanase (Wu *et al*., 2004). It might be as effective as xylanase, or more than xylanase, in improving the performance of broiler chickens fed on triticale diets containing adequate levels of phosphorus. Improved performance with enzyme supplementation was generally associated with reduced digesta viscosity, increased AME, and reduced relative weight of the small intestine.

The improvement in body weight and feed intake owing to the supplementation of phytase and the combination of supplemental xylanase and phytase is consistent with the findings reported by Widodo *et al.* (2015). Except for FCR, these findings are in agreement with previous studies (Olukosi *et al.,* 2008; Selle *et al*., 2009) i.e. xylanase and phytase had a synergistic effect with respect to increasing the digestibility of energy and nutrients, which contributed to the higher FI, BW and FCR.

Numerically, the diets with only phytase or a combination of supplemental xylanase and phytase had higher nutrient digestibility than diets containing xylanase alone. These results are consistent with the results of phytate degradation, where there is an increase in phytate degradation in the diets with the microbial enzyme preparation compared to the control diet. Phytic acid (phytate-P) is a critical antinutrient present in grains that can bind minerals, protein, lipids and starch (Thompson and Yoon, 1984), thereby reducing nutrient digestibility in poultry (Sebastian *et al*., 1997). Other workers have also reported that phytase in broiler chicken diets improved the total amino acid digestibility and ME (Ravindran *et al*., 2006; Truong *et al.,* 2015); however, some of these responses were not assessed in this study. Bedford (1996) described the capacity of xylanase in poultry diet to potentially improve the nutritive value of the diet by hydrolysing polysaccharides which encapsulate the starch or protein. Another advantage in using the various exogenous enzymes is that they can improve the nutritional value of diets by reducing the loss of endogenous material (Cowieson and Ravindran, 2007).

As has been noted above, the results indicate that Bogong and Canobolas have relatively similar nutritive values for broiler chickens, and it can also be said that in triticale-based diet, the activity of one type of enzyme is facilitated by the other, possibly in a complementary way.

Visceral organ weight

In early life of the birds, the relative weight of the proventriculus plus gizzard, duodenum and liver was affected by the cultivars and phytase inclusion. The weight of proventriculus plus gizzard on the Canobolas diet was higher than on the Bogong diet which may be associated with increased feed intake of birds on Canobolas diet. The result is partly consistent with previous study by Zarghi *et al.* (2016) who reported that increasing triticale level in diet significantly increased the whole gastrointestinal tract, gizzard, small intestine, large intestine, and pancreas. The effect of microbial enzymes could be found on the weight of small intestine

(reduced by xylanase inclusion), liver (reduced by phytase inclusion) and bursa of Fabricius (interaction of grain and phytase). In addition, in the current study, xylanase inclusion reduced the weight of the small intestine. This may be due to diminished physical function of the intestine because of a decrease in concentration of water-soluble NSP and subsequent reduction in digesta viscosity could reduce the muscular activity needed to propel the digesta through the tract (Wang *et al*., 2005).

Tissue protein and digestive enzyme activities

The protein content and the activities of pancreatic protease (chymotrypsin amidase) were higher in birds on the Bogong and Canobolas diets without enzyme inclusion. This may be the result of the need for greater endogenous secretions in order to accomplish digestion. This finding is in agreement with that reported by Mahagna *et al*. (1995) who indicated that a reduction in the secretion of pancreatic chymotrypsin was caused by enzyme supplementation. The authors added that the reduction in secretion of pancreatic enzymes was most probably the result of the presence of exogenous enzymes in the intestine; however, this proposal does not correspond with the results reported by Engberg *et al*. (2004) who found an increased activity of pancreatic chymotrypsin by the inclusion of xylanase. Xylanase may reduce viscosity and enhance the activity of enzymes that target nutrients other than carbohydrates.

The activities of jejunal mucosal disaccharidases, as reflected by maltase and sucrase, were significantly affected by the inclusion of xylanase. This result is in agreement with Pinheiro *et al*. (2004) who proposed that supplementation with carbohydrase and protease could increase the activities of sucrase and maltase compared with the response on unsupplemented diets. This may be caused by release of substrates targeted by these enzymes.

Conclusions

The response of birds on the diets based solely on triticale was close to or better than breed standard. Supplementation with phytase alone or combination of phytase and xylanase further improved productivity. The beneficial effect of exogenous enzymes may be due to improvement in the digestibility of CP, gross energy, starch, Ca, and P. The relative weight of visceral organs especially that of the small intestine, was lower on the diets containing enzymes; however, the weight of the proventriculus plus gizzard differed between the Bogong and Canobolas groups possibly because of intrinsic differences in the coarseness of these two cultivars. The nutritive values of Bogong and Canobolas are similar, and diets based on these cultivars can be improved by the concurrent inclusion of xylanase and phytase.

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References

Annison G & Choct M. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World's Poultry Science Journal, 47: 232-242. DOI: 10.1079/WPS19910019

Aviagen. 2007. ROSS 308 BROILER: Nutrition Spesification. Newbridge, Midlothian EH28 8SZ, Scotland, UK.

Bedford MR. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Animal Feed Science and Technology, 53: 145-155. DOI: 10.1016/0377-8401(95)02018-U

Bedford MR. 1996. The effect of enzymes on digestion. Journal of Applied Poultry Research, 5: 370-378. DOI: 10.1093/japr/5.4.370

Bradford MM. 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. DOI: 10.1016/0003-2697(76)90527-3

Çiftci I, Yenice E & Eleroglu H. 2003. Use of triticale alone and in combination with wheat or maize: effects of diet type and enzyme supplementation on hen performance, egg quality, organ weights, intestinal viscosity and digestive system characteristics. Animal Feed Science and Technology, 105: 149-161. DOI: 10.1016/S0377-8401(03)00010-5

Cowieson AJ & Ravindran V. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous

protein at the terminal ileum of growing broiler chickens. British Journal of Nutrition, 98: 745-752. DOI: 10.1017/S0007114507750894

Engberg RM, Hedemann MS, Steenfeldt S & Jensen BB. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poultry Science, 83: 925-938. DOI: 10.1080/00071660220000044480

Englyst HN & Hudson G J. 1993. Dietary fiber and starch: Classification and measurement. In: CRC Hanbook of Dietary Fiber and Human Nutrition (2nd ed.), Ed. Spiller, G., Pages. 53-71. CRC Press Boca Raton, Florida, US.

Elangovan AV, Bhuiyan M, Jessop R & Iji, PA. 2011. The potential of high-yielding triticale varieties in the diet of broiler chickens. Asian Journal of Poultry Science, 5: 68-76. DOI: 10.3923/ajpsaj.2011.68.76

Fayez EY, Nedal HO & Mousa A. 1996. Nutritive value and feed efficiency of broiler diets containing different levels of triticale. In: Triticale: Today and Tomorrow, Eds. Guedes-Pinto H, Darvey N & Carnide VP. Vol. 5, Pages, 819-826. Kluwer Academic Publishers, Dordrecht/Boston/London.

Forstner GG, Sabesin SM & Isselbacher KJ. 1968. Rat intestinal microvillus membranes. Purification and biochemical characterization. Biochemical Journal, 106: 381-390. DOI: 10.1042/bj1060381

Haug W & Lantzsch HJ. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of the Science of Food and Agriculture, 34: 1423-1426. DOI: 10.1002/jsfa.2740341217

Holdsworth ES. 1970. The effect of vitamin D on enzyme activities in the mucosal cells of the chick small intestine. Journal of Membrane Biology, 3: 43-53. DOI: 10.1007/BF01868005

Iji PA, Saki A & Tivey DR. 2001. 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. DOI: 10.1080/00071660120073142

Jondreville C, Genthon C, Bouguennec A, Carre B & Nys Y. 2007. Characterisation of European varieties of triticale with special emphasis on the ability of plant phytase to improve phytate phosphorus availability to chickens. British Poultry Science, 48: 678-689. DOI: 10.1080/00071660701691292

Levic J, Djuragic O & Sredanovic S. 2006. Phytase as a factor of improving broilers growth performance and environmental protection. Archiva Zootechnica, 9: 95-100.

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. DOI: 10.1016/0926-5287(96)80192-4

McCleary BV, Gibson TS, Solah V & Mugford DC. 1994. Total starch measurement in cereal products: Interlaboratory evaluation of a rapid enzymic test procedure. Cereal Chemistry, 71: 501-505.

McPherson GA. 1985. Lowry Program: Elsevier-BIOSOFT. 68 Hills Rd, Cambridge, CB2, 1LA, UK.

Minitab. 2010. Minitab 16 Statistical Software. Minitab Inc. State College. PA. USA.

Nitsan Z, Dror Y, Nir I & Shapira N. 1974. The effects of force-feeding on enzymes of the liver, kidney, pancreas and digestive tract of chicks. British Jorunal of Nutrition, 32: 241-247. DOI: 10.1079/BJN19740077

Oettler G. 2005. The fortune of a botanical curiosity – Triticale: past, present and future. Journal of Agricultural Science, 143: 329-346. DOI: 10.1017/S0021859605005290

Olukosi OA, Cowieson AJ & Adeola O. 2008. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. British Journal of Nutrition, 99: 682-690. DOI: 10.1017/S0007114507815807

Osek M, Milczarek A, Janocha A & Swinarska R. 2010. Effect of triticale as a partial or complete wheat and maize substitute in broiler chicken diets on growth performance, slaughter value and meat quality. Annals of Animal Science, 10: 275-283.

Pettersson D & Åman P. 1988. Effects of enzyme supplementation of diets based on wheat, rye or triticale on their productive value for broiler chickens. Animal Feed Science and Technology, 20: 313-324. DOI: 10.1016/0377-8401(88)90005-3

Pinheiro DF, Cruz VC, Sartori JR and Vicentini Paulino ML. 2004. Effect of early feed restriction and enzyme supplementation on

digestive enzyme activities in broilers. Poultry Science, 83: 1544-1550. DOI: 10.1093/ps/83.9.1544

Pourreza J, Samie AH & Rowghani E. 2007. Effect of supplemental enzyme on nutrient digestibility and performance of broiler chicks fed on diets containing triticale. International Journal of Poultry Science, 6: 115-117.

Ravindran V, Morel, PCH, Partridge GG, Hruby M & Sands JS. 2006. Influence of an escherichia coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poultry Science, 85: 82-89. DOI: 10.1093/ps/85.1.82

Rundgren M. 1988. Evaluation of triticale given to pigs, poultry and rats. Animal Feed Science & Technology, 19: 359-375. DOI: 10.1016/0377-8401(88)90026-0

Sebastian S, Touchburn SP, Chavez ER & Laguë PC. 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poultry Science, 76: 1760-1769. DOI: 10.1093/ps/76.12.1760

Selle PH, Ravindran V & Partridge GG. 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilization, mineral retention and growth performance in wheat-based broiler diets. Animal Feed Science and Technology, 153: 303-313. DOI: 10.1016/j.anifeedsci.2009.06.011

Serviere-Zaragoza E, del Toro MAN & García-Carreño FL. 1997. Protein-hydrolyzing enzymes in the digestive systems of the adult Mexican blue abalone, Haliotis fulgens (Gastropoda). Aquaculture, 157: 325-336. DOI: 10.1016/S0044-8486(97)00169-5

Shimada A, Cline TR & Rogler JC. 1974. Nutritive value of triticale for the nonruminant. Journal of Animal Science, 38: 935-940. DOI: 10.2527/jas1974.385935x

Shirazi-Beechey SP, Smith MW, Wang Y & James PS. 1991. Postnatal development of lamb intestinal digestive enzymes is not regulated by diet. Journal of Physiology, 437: 691–698. DOI: 10.1113/jphysiol.1991.sp018619

Short FJ, Gorton P, Wiseman J & Boorman KN. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology, 59: 215-221. DOI: 10.1016/0377-8401(95)00916-7

Theander O & Westerlund E. 1993. Determination of individual component of dietary fibre. In: CRC Handbook of Dietary Fiber and Human Nutrition (2nd ed.), Ed. Spiller, G., Pages, 77-98. CRC Press Boca Raton, Florida, US.

Thompson LU & Yoon JH. 1984. Starch digestibility as affected by polyphenols and phytic acid. Journal of Food Science, 49: 1228-1229. DOI: 10.1111/j.1365-2621.1984.tb10443.x

Truong HH, Bold RM, Liu SY & Selle PH. 2015. Standard phytase inclusion in maize-based broiler diets enhances digestibility coefficients of starch, amino acids and sodium in four small intestinal segments and digestive dynamics of starch and protein. Animal Feed Science and Technology, 209: 240-248. DOI: 10.1016/j.anifeedsci.2015.08.012

Vieira SL, Penz AM Jr, Kessler AM & Catellan EV Jr. 1995. A nutritional evaluation of triticale in broiler diets. Journal of Applied Poultry Research, 4: 352-355. DOI: 10.1093/japr/4.4.352

Wang Z, Qiao S, Lu W & Li D. 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poultry Science, 84: 875-881. DOI: 10.1093/ps/84.6.875

Widodo AE, Nolan JV & Iji PA. 2015. The nutritional value of new varieties of high-yielding triticale: Feeding value of triticale for broiler chickens. South African Journal of Animal Science, 45: 74-81. DOI: 10.4314/sajas.v45i1.9

Wu YB, Ravindran V, Thomas DG, Birtles MJ & Hendriks WH. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. British Poultry Science, 45: 76-84. DOI: 10.1080/00071660410001668897

Zarghi H & Golian A. 2009. Effect of triticale replacement and enzyme supplementation on performance and blood chemistry of broiler chickens. Journal of Animal and Veterinary Advances, 8: 1316-1321.

Zarghi H, Golian A, Kermanshahi H, Raji AR & Heravi AR. 2010. The effect of triticale and enzyme in finisher diet on performance, gut morphology and blood chemistry of broiler

chickens. Journal of Animal and Veterinary Advances, 9: 2305-2314.

Zarghi H, Golian A & Kermanshahi H. 2016. The effect of triticale and enzyme cocktail (xylanase & beta-glucanase) replacement in grower diet on performance, digestive organ relative weight, gut viscosity and gut morphology of broiler chickens. Iranian Journal of Animal Science Research, 8: 298-312. (In Persian with English abstract)