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# The Effect of Triticale and Enzyme in Finisher Diet on Performance, Gut Morphology and Blood Chemistry of Broiler Chickens

Heydar Zarghi, Abolghasem Golian, Hasan Kermanshahi, Ahmad Reza Raji and Ali Reza Heravi
Department of Animal Science, College of Agriculture,
Ferdowsi University of Mashhad, Mashhad, Iran

**Abstract:** A completely randomized design experiment with 5×2 factorial arrangement was used to study the performance of broiler chickens fed diets with five different levels of triticale (0, 19, 36, 57 and 75%), with/without exogenous enzyme during finishing period. Each diet fed to five groups of ten male birds each. All birds fed similar commercial starter and grower diets up to 25 days of age. The experimental diets were isocalric and isonitrogenous and fed *ad-libitum* from 25-42 days of age. Similar weight gain, feed intake and feed conversion observed in birds fed control or diets contained up to 75% triticale. The exogenous enzyme supplementation did not significantly affect broiler performance. Relative weight of small intestine, large intestine and pancreas and gut chyme viscosity significantly increased with increase in level of triticale in diet when measured at 42 days of age whereas the highest values observed in birds fed highest level of triticale in soy based diet and the lowest values observed in birds fed with corn-soy based diet. Enzyme supplementation of diets caused a significant reduction in the intestinal and pancreas relative weights and gut viscosity. The jejunum villus of birds fed diet contained 75% triticale was significantly ($p<0.05$) shortened and thickened as compared to those fed control diet. Triticale seems to lower serum HDL in chickens.

**Key words:** Broilers, triticale, enzyme supplementation, performance, gut viscosity, gut morphology

## INTRODUCTION

Triticale, a hybrid of wheat and rye is an alternative cereal grain to use in poultry diets. Triticale has an excellent productivity potential and a greater flexibility to adapt to difficult agronomic conditions than wheat (Korver *et al*., 2004). Triticale has superior adaptation to stress conditions such as drought, excess moisture, acidic soils and low fertility situations where other crops are poorly adapted. Triticale has equal or higher yield than other crops (Chapman *et al*., 2005) and may be suitable grain as energy source in poultry diets. The use of triticale in broiler feed is limited by the presence of soluble non-starch polysaccharides components (Pourreza *et al*., 2007). Research findings have consistently demonstrated negative effects of water soluble Non Starch Polysaccharides ($NSP_S$) on bird performance. These compounds reduce the nutritive value of triticale by increasing gut chyme viscosity and thus reducing the availability of nutrients for digestion and absorption (Choct and Annison, 1992).

Digestion of Non-Starch Polysaccharide (NSP) fractions tends to be more variable due to lack of digestive enzymes and their tendency to create a viscous environment within the intestinal lumen (Jozefiak *et al*., 2004). An increase in gut chyme viscosity may reduce nutrient digestion in the small intestine because dietary components require mixing with pancreatic and other intestinal secretions for proper digestion; the gel-like environment caused by increased viscosity, reduces this mixing. Pentosans may also inhibit the activity of digestive enzymes by physically complexing with them and thereby reducing enzyme substrate reaction (Jaroni *et al*., 1999). Hasselman and Aman (1986) showed that cell walls containing arabinoxylans acted as a physical barrier to endogenous enzymes, reducing the utilization of starch and protein encapsulated within the endosperm wall.

Enlargement of digestive organs is also one of the effects observed with feeding soluble NSP. Partridge and Wyatt (1995) suggested that pancreatic relative weight as a proportion of body weight is increased in the presence of soluble fibers, implying that feedback mechanisms in the bird's gut stimulate hypertrophy of this organ. This could have implications for the protein economy of the bird, i.e., more protein synthesis directed toward organ growth and enzyme secretion, leaving less available for lean tissue growth.

**Corresponding Author:** Heydar Zarghi, Department of Animal Science, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Enzyme supplementations are used mainly in poultry diets in order to neutralise the effects of viscous NSPs in cereals such as barley wheat, rye and triticale (Joaquim *et al.*, 2006). Studies have demonstrated that the negative effects of the soluble arabinoxylan can be eliminated by partial hydrolysis of the fiber in the presence of crude enzyme preparations having high xylanase activity (Jaroni *et al.*, 1999). Dietary supplementation of enzymes plays a major role in reducing the viscosity, thereby increasing the rate of nutrient absorption and consequently improving digestibility (Burnett, 1996). Pettersson and Aman (1988) reported significant improvement in growth and feed conversion of broilers when triticale diets were supplemented with an enzyme cocktail containing a high level of β-glucanase and pentosanase activity. Other studies have also shown improved digestibility with enzyme supplementation in cereal-based diets (Friesen *et al.*, 1992).

Information about the use of triticale in broiler chick finisher diet and the effect of supplemental enzymes on its nutritional value is limited as compared to other cereal grains. The present investigation was conducted to study the effect of triticale and enzyme in finisher diet on performance, digestive tract size, gut viscosity, gut morphology and blood chemical characteristics of broiler chickens.

## MATERIALS AND METHODS

**Birds, housing and care:** About 500 days old male broiler chicks of commercial strain (Ross 308) were obtained from a commercial hatchery and fed a commercial starter and grower diets until 24 days of age. Chicks on day 25 were individually weighed and were randomly assigned to 10 dietary treatments with 5 replicate pens of 10 birds each. Each pen was one square meter and covered with wood shaving. The house temperature was initially maintained at 32°C and gradually decreased (2.5°C every week) to reach a constant temperature of 20-22°C at 28 days of age. From 0-6 days of age, the lighting program was constant and the 23:1 h light: dark cycle was applied for the rest of the experimental period. Birds in each pen were allowed free access to feed from a hanging feeder and fresh water from a drinker throughout the experiment.

**Experimental design and diets:** A completely randomized design experiment with a factorial arrangement of five diet triticale levels (0, 19, 38, 57 and 75% used in diet) and two levels of enzyme supplementation was used to study. The enzyme levels were 0 and 500 ppm of an enzyme cocktail (containing xylanase min.1200 unit $g^{-1}$ and β-glucanase 440 unit $g^{-1}$) in diet. Diets were formulated according to the recommended nutrients by the Ross 308, 2007 manual for broiler chicks and were offered in mash form. The composition of the experimental diets is shown in Table 1. The diets were provided isocalric with similar nutrients and fed *ad-libitum* from 25-42 days of age.

**Performance measurements:** Live body weight and feed weighed in and weighed back per pen were measured at day 24 and 42 of age. Weight gain, feed intake and feed conversion were calculated. Daily chick mortality was weighed, recorded and added to the total pen live body weight for the calculation of feed conversion during the experimental period.

**Slaughter and sampling:** At 42 days of age, one birds/pen, close to the average pen weight was selected and after 8 h of starvation with access to drinking water, weighed and slaughtered. Carcass was obtained by removing head, legs below the tibia-tarsal joint and gastro-intestinal tract. The digestive tract, from the proventriculus to the end of the intestine was excised. The crop and pro-ventriculus were emptied and weighed. The gizzard was emptied, trimmed of excess fat and weighed. The pancreas was excised and weighed. The small intestine was divided into three segments: duodenum (from gizzard to pancreo-biliary ducts), jejunum (from pancreo-biliary ducts to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileo-caecal junction). The jejunum and ileum digesta contents were removed for viscosity measurements. Then, the total segments of small intestine and large intestine were emptied and weighed. Weight of the edible carcass parts and digestive tract organs were expressed to body weight. After clearing the intestinal contents, a portion (about 1.5 cm in length) of intestine was taken from the midpoint between the bile duct entry and Meckel's diverticulum, flushed with 0.9% saline to remove the contents and then were fixed in 10% neutral buffered formalin solution for histological study.

**Gut viscosity measurements:** Intestinal contents, collected from the jejunum and ileum were divided into two subsamples for determination of viscosity. Approximately 1.5 g wet weight of the fresh digesta were immediately placed in a micro centrifuge tube and centrifuged at 12.700×g for 5 min. The supernatant (0.5 mL) was with drawn and viscosity (in centipoises, cps = 1/100 dyne second per square centimeter) was determined using a Brookfield digital viscometer (Model LVDVII+CP, Brookfield Engineering Labs, Inc., Stoughton, MA 02072)(Model DV-II). The average reading of two subsamples was taken for statistical analysis.

Table 1: Composition of the experimental diets

| | Percentage of triticale in diet[1] | | | | |
|---|---|---|---|---|---|
| Ingredients (%) | 0 | 19 | 38 | 57 | 75 |
| Corn | 63.10 | 47.10 | 31.11 | 15.10 | 0.00 |
| Triticale (11% CP) | 0.00 | 19.00 | 38.00 | 57.00 | 75.00 |
| Soybean meal (44% CP) | 30.73 | 27.55 | 24.40 | 21.22 | 18.21 |
| Soybean oil (8800 ME $kg^{-1}$) | 2.78 | 2.79 | 2.80 | 2.81 | 2.82 |
| Limestone | 1.05 | 1.05 | 1.05 | 1.05 | 1.03 |
| Di Ca-phosphate | 1.40 | 1.42 | 1.44 | 1.46 | 1.48 |
| Sodium chloride | 0.26 | 0.29 | 0.32 | 0.35 | 0.38 |
| Vit-min permix[2] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Hcl-lysine | 0.02 | 0.10 | 0.17 | 0.23 | 0.30 |
| DL-Methionine | 0.17 | 0.17 | 0.18 | 0.18 | 0.18 |
| Theronine | 0.00 | 0.03 | 0.06 | 0.10 | 0.13 |
| **Calculated nutrients** | | | | | |
| ME, (Kcal $kg^{-1}$) | 3050.00 | 3050.00 | 3050.00 | 3050.00 | 3050.00 |
| CP (%) | 19.00 | 19.00 | 19.00 | 19.00 | 19.00 |
| Ca (%) | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
| Av. P (%) | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Na (%) | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Lys (%) | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| Met (%) | 0.47 | 0.47 | 0.48 | 0.49 | 0.49 |
| Met+Cys (%) | 0.79 | 0.79 | 0.79 | 0.79 | 0.79 |
| Theronine | 0.71 | 0.71 | 0.71 | 0.71 | 0.71 |
| **Analyzed nutrients** | | | | | |
| Dry matter (%) | 90.50 | 90.90 | 89.50 | 89.40 | 90.00 |
| Crude protein (%) | 21.80 | 21.50 | 21.80 | 21.50 | 21.90 |
| Crude fiber (%) | 4.60 | 4.70 | 4.80 | 4.50 | 4.50 |
| Calcium (%) | 1.04 | 1.01 | 1.10 | 1.10 | 1.10 |
| Phosphorous (%) | 0.70 | 0.66 | 0.65 | 0.58 | 0.50 |

Diets with or without the enzyme complex; 0 or 500 ppm of an enzyme cocktail containing xylanase min 1200 unit $g^{-1}$ and β-glucanase 440 units $g^{-1}$. Supplied per kilogram of diet: vitamin A, 11000 IU; vitamin D3, 1800 IU; vitamin E, 36 mg; vitamin K3, 5 mg; vitamin B12, 1.6 mg; thiamine, 1.53 mg; riboflavin, 7.5 mg; niacin, 30 mg; pyridoxine, 1.53 mg; biotin, 0.03 mg; folic acid, 1 mg; panthotenic acid, 12.24 mg; choline chloride, 1100 mg; etoxycoin, 0.125 mg; Zn-sulfate, 84 mg; Mn-sulfate, 160 mg; Cu-sulfate, 20 mg; Se, 0.2 mg; I, 1.6 mg; Fe, 250 mg

**Gut histological measurements:** Intestinal samples were transferred from formaldehyde, after dehydration by passing tissue through a series of alcohol solutions, cleared in xylene were embedded in paraffin. Intestinal samples were sectioned at 5 μm thickness using an auto microton (Model Lica RM 2145) placed on glass slides, prepared and processed for staining with Hematoxylin and Eosin (H and E). All chemical was purchased from sigma chemical company. Micrographs were taken with an Olympus microscopic, BX41 (Olympus, Tokyo, Japan). Morphometric measurements were performed on 9 villi chosen from each sample; magnification was 5 for villi and 10 for crypts. Morphometric indices included were Villus Height (VH) from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, Villus Width (VW; average of VW at one-third and two-third of the villus) and muscularis thickness from the submucosa to the external layer of the intestine (Geyra *et al*., 2001). Apparent Villus Surface Area (AVSA) was calculated by the formula: [(VW at one-third + VW at two-thirds of the height of the villus)×$(2)^{-1}$xvillus height] according to Iji *et al*. (2001).

**Blood chemistry measurements:** One bird from each replicate was randomly selected and after 8 h of starvation 3 mL of blood from wing vein was withdrawn into a syringe at 42 days of age. The blood serum samples were used to determine Triglyceride (TG), Cholesterol (Chol), high-density Lipoproteins (HDL) and low-density Lipoproteins (LDL) using Selectra E auto analysis.

**Diet analysis:** Diet samples were analyzed for Dry Mater (DM) at 100°C for 24 h, Crude Protein (CP) by the Kjeldahl method, Crude Fiber (CF), calcium by the dry ash method and phosphorus by the photometric method.

**Statistical analysis:** The data were tested for main effects of triticale levels and enzyme supplementation and for interaction of triticale and enzyme. Analysis of variance was performed using a randomized complete design experiment with a factorial arrangement of treatments. All percentage data were transformed to arc-sin before statistical analysis. All data were analyzed by ANOVA using the GLM procedure of the SAS software. Means were compared for significant differences using Tukey Kramer's multiple range test ($p<0.05$).

## RESULTS AND DISCUSSION

**Performance:** The average body weight of 25 days old chicks was 664 g and chicks pen weight were similar

Table 2: Effect of dietary triticale level and enzyme supplementation on body weight, weight gain, feed intake and feed conversion of broiler chicks during 25-42 days of age

| Items | Body weight | Weight gain | Feed intake | Feed conversion |
|---|---|---|---|---|
| Main effects | (g) | --------(g/b/d) ------- | | (g:g) |
| **Triticale** | | | | |
| 0 | 1913.0 | 74.14 | 134.07 | 1.81 |
| 19 | 1873.0 | 71.03 | 134.59 | 1.91 |
| 36 | 1916.0 | 72.91 | 138.17 | 1.89 |
| 57 | 1933.0 | 74.24 | 136.11 | 1.84 |
| 75 | 1870.0 | 71.67 | 136.03 | 1.90 |
| SE | 35.6 | 1.77 | 1.70 | 0.03 |
| **Enzyme** | | | | |
| 0 | 1875.0 | 71.81 | 135.11 | 1.89 |
| 500 | 1927.0 | 73.78 | 136.48 | 1.85 |
| SE | 22.5 | 1.12 | 1.07 | 0.02 |
| **Interaction effects** | | | | |
| Triticale (enzyme) | | | | |
| 0 (-) | 1841.0 | 72.12 | 130.76 | 1.82 |
| + | 1985.0 | 76.16 | 137.38 | 1.80 |
| 19 (-) | 1887.0 | 70.71 | 136.29 | 1.94 |
| + | 1860.0 | 71.35 | 132.89 | 1.86 |
| 36 (-) | 1891.0 | 71.35 | 138.48 | 1.95 |
| + | 1942.0 | 71.01 | 137.86 | 1.84 |
| 57 (-) | 1892.0 | 73.53 | 132.85 | 1.82 |
| + | 1973.0 | 74.94 | 139.36 | 1.86 |
| 75 (-) | 1866.0 | 71.68 | 137.14 | 1.91 |
| + | 1875.0 | 71.66 | 134.91 | 1.88 |
| SE | 50.3 | 2.50 | 2.41 | 0.05 |

Means with no common superscripts in each column and each effect are significantly different (p<0.05)

Table 3: Effect of dietary triticale level and enzyme supplementation on carcass, portion weight of broiler chicks measured at 42 days of age

| Items | Eviscerated carcass | Femur | Pectoral | Abdominal fat |
|---|---|---|---|---|
| Main effects | ---------------------- (% LBW) -------------------- | | | |
| **Triticale** | | | | |
| 0 | 69.50[a] | 22.4 | 26.1 | 1.70 |
| 19 | 70.00[a] | 22.0 | 26.9 | 1.30 |
| 36 | 70.50[a] | 23.0 | 25.6 | 1.50 |
| 57 | 68.70[ab] | 22.5 | 25.7 | 1.20 |
| 75 | 66.50[b] | 22.7 | 25.8 | 1.40 |
| SE | 0.80 | 0.4 | 0.5 | 0.14 |
| **Enzyme** | | | | |
| 0 | 68.60 | 22.5 | 25.8 | 1.48 |
| 500 | 69.40 | 22.6 | 26.2 | 1.37 |
| SE | 0.50 | 0.3 | 0.3 | 0.09 |
| **Interaction effects** | | | | |
| Triticale (enzyme) | | | | |
| 0 (-) | 69.00 | 21.7 | 26.3 | 1.81 |
| + | 69.40 | 23.0 | 26.0 | 1.59 |
| 19 (-) | 68.20 | 21.9 | 25.7 | 1.41 |
| + | 71.80 | 22.3 | 28.1 | 1.30 |
| 36 (-) | 70.60 | 22.7 | 26.1 | 1.68 |
| + | 70.40 | 23.4 | 25.1 | 1.23 |
| 57 (-) | 69.00 | 23.2 | 25.6 | 1.08 |
| + | 68.50 | 21.8 | 25.7 | 1.31 |
| 75 (-) | 66.20 | 23.2 | 25.5 | 1.44 |
| + | 66.90 | 22.4 | 26.1 | 1.42 |
| SE | 1.19 | 0.6 | 0.8 | 0.19 |

[a, b]Means with no common superscripts in each column and each effect are significantly different (p<0.05)

before they were allocated to the dietary treatment. The average Body Weight (BW), Weight Gain (WG), Feed Intake (FI) and Feed Conversion Ratio (FCR) of chickens fed finisher diets with different levels of triticale with/without enzyme supplementation is shown in Table 2. There were not significant differences (p>0.05) in BW, WG, FI and FCR between birds fed diets contained different levels of triticale during experiment (25-42 days). Other studies reported similar results on performance, when fed diets contained up to 100% of grain replaced for triticale (Chapman *et al*., 2005). Boros (1998) reported octo and hexaploid triticale may not negatively affect the performance of broiler chickens and may successfully be replaced for a part of wheat in broiler diets. Similarly, Vieira *et al*. (1995) found that the inclusion of triticale up to 40% (substituted for corn) had no negative effect on weight gain or final weight of broilers. But other investigators reported a poor FCR in birds fed triticale-based diet as compared to those fed corn-based diet (Hermes and Johnson, 2004). These investigators concluded that the poor FCR of birds fed triticale based diet may be related to lower nutrients, lower nutrient digestibility or higher anti-nutrient factors in triticale as compared to corn. Exogenous enzyme supplementation did not significantly influenced body weight, weight gain, feed intake and/or FCR (Table 2). The addition of enzyme in other experiments significantly improved the performance of broiler chickens fed triticale- soy diet as compared to corn-soy diet (Al-Athari and Guenter, 1988; Pourreza *et al*., 2007; Jozefiak *et al*., 2007). Pettersson and Aman (1988) added enzyme with high level of β-glucanase and pentosanase activity to the triticale based diets and observed an improvement in growth and FCR of birds as compared to those fed basal diet without enzyme supplementation. The different results obtained in the experiment and other reports may be mainly due to the age of birds because the triticale was replaced for corn in the starter, grower and finisher diets in other researches as well as, type of enzyme cocktail and/or variety of triticale as compared to the experiment. Specially, the new varieties of triticale used in the experiment may contain fewer anti-nutrients such as pentosans and other non-starch polysaccharides than pervious varieties.

The average relative weight (weight as a percentage of LBW) of eviscerated carcass, femur, pectoral and abdominal fat of birds fed diet contained different levels of triticale with/without enzyme supplementation is shown in Table 3. There were not significant differences in femur, pectoral and abdominal fat as percentage of LBW of birds fed with different dietary treatments measured at 42 days of age. Eviscerated carcass as percentage of LBW was significantly (p<0.05) higher in birds fed control diet or diet contained 19 and 38% of triticale than birds fed 75% triticale diet. The significant decreased in carcass of bird fed diets with higher triticale may be due to increase relative weight of gastrointestinal tract which may be due to enhanced function of gut with subsequent increase in digesta viscosity.

Table 4: Effect of dietary triticale level and enzyme supplementation on relative weights of different sections of the gastrointestinal tract and gut viscosity of broiler chicks measured at 42 days of age

| | | | | | | | Gut viscosity | |
|---|---|---|---|---|---|---|---|---|
| Item | Crop | Pro-ventriculus | Gizzard | Pancreas | Small intestine | Large intestine | Jejunum | Ileum |
| Main effects | (%LBW) | | | | | | cps[1] | |
| **Triticale** | | | | | | | | |
| 0 | 0.35 | 0.40 | 1.63 | 0.21$^{c}$ | 2.91$^{b}$ | 0.52$^{b}$ | 1.59$^{c}$ | 1.89$^{c}$ |
| 19 | 0.40 | 0.44 | 1.67 | 0.26$^{b}$ | 3.33$^{ab}$ | 0.59$^{ab}$ | 2.16$^{bc}$ | 3.31$^{bc}$ |
| 36 | 0.29 | 0.45 | 1.64 | 0.29$^{ab}$ | 3.35$^{ab}$ | 0.62$^{ab}$ | 2.98$^{ab}$ | 5.05$^{bc}$ |
| 57 | 0.31 | 0.43 | 1.72 | 0.28$^{ab}$ | 3.43$^{a}$ | 0.63$^{a}$ | 3.12$^{ab}$ | 6.02$^{ab}$ |
| 75 | 0.35 | 0.46 | 1.80 | 0.33$^{a}$ | 3.73$^{a}$ | 0.63$^{a}$ | 3.85$^{a}$ | 8.79$^{a}$ |
| SE | 0.04 | 0.02 | 0.06 | 0.06 | 0.11 | 0.03 | 0.27 | 0.91 |
| **Enzyme** | | | | | | | | |
| 0 | 0.32 | 0.45 | 1.70 | 0.30$^{a}$ | 3.43 | 0.59 | 3.12$^{a}$ | 6.22$^{a}$ |
| 500 | 0.37 | 0.42 | 1.69 | 0.25$^{b}$ | 3.27 | 0.61 | 2.36$^{b}$ | 3.81$^{b}$ |
| SE | 0.02 | 0.01 | 0.04 | 0.01 | 0.07 | 0.02 | 0.17 | 0.57 |
| **Interaction effects** | | | | | | | | |
| Triticale (enzyme) | | | | | | | | |
| 0 (-) | 0.34 | 0.42 | 1.60 | 0.22$^{d}$ | 2.92$^{b}$ | 0.51 | 1.58$^{d}$ | 1.76$^{c}$ |
| + | 0.35 | 0.38 | 1.67 | 0.21$^{e}$ | 2.91$^{b}$ | 0.53 | 1.61$^{d}$ | 1.99$^{c}$ |
| 19 (-) | 0.34 | 0.47 | 1.64 | 0.28$^{bcd}$ | 3.40$^{ab}$ | 0.55 | 1.92$^{cd}$ | 2.59$^{bc}$ |
| + | 0.47 | 0.41 | 1.70 | 0.24$^{cd}$ | 3.26$^{b}$ | 0.63 | 2.40$^{bcd}$ | 4.03$^{bc}$ |
| 36 (-) | 0.30 | 0.45 | 1.66 | 0.33$^{ab}$ | 3.31$^{ab}$ | 0.61 | 3.59$^{abc}$ | 6.52$^{abc}$ |
| + | 0.29 | 0.46 | 1.62 | 0.26$^{bcd}$ | 3.38$^{ab}$ | 0.64 | 2.37$^{bcd}$ | 3.59$^{bc}$ |
| 57 (-) | 0.29 | 0.44 | 1.78 | 0.30$^{abc}$ | 3.48$^{ab}$ | 0.66 | 3.68$^{ab}$ | 7.93$^{ab}$ |
| + | 0.34 | 0.41 | 1.66 | 0.27$^{bcd}$ | 3.38$^{ab}$ | 0.60 | 2.56$^{bcd}$ | 4.11$^{bc}$ |
| 75 (-) | 0.31 | 0.47 | 1.76 | 0.38$^{a}$ | 4.03$^{a}$ | 0.63 | 4.83$^{a}$ | 12.26$^{a}$ |
| + | 0.39 | 0.45 | 1.81 | 0.29$^{abc}$ | 3.43$^{ab}$ | 0.63 | 2.88$^{bcd}$ | 5.33$^{bc}$ |
| SE | 0.06 | 0.03 | 0.09 | 0.02 | 0.16 | 0.04 | 0.38 | 1.30 |

$^{a-e}$Means with no common superscripts in each column and each effect are significantly different (p<0.05)

**Digestive organ weights:** The relative weights (weight as a percentage of LBW) of different sections of the gastrointestinal tract of birds fed diet with different levels of triticale with/without enzyme supplementation at 42 days of age is shown in Table 4. There were not significant (p>0.05) differences in relative weights of digestive organs such as crop, pro-ventriculus and gizzard of birds fed different dietary treatments. The relative weights of pancreas, small intestine and large intestine numerically increased when birds fed diets with increased level of triticale, the highest weight observed in birds fed triticale-soy based diet and lowest weights observed in birds fed corn-soy based diet. The relative weight of pancreas was significantly increased (p<0.001) in birds fed diet contained 75% triticale than those fed control and/or 19% triticale diet. Pancreas weight was significantly higher in birds fed 57, 36 and/or 19% triticale diet than those fed with corn-soy based diet. The small intestine relative weight was significantly increased (p<0.004) in birds fed 57 and/or 75% triticale diet than those fed with control diet. The large intestine relative weight was significantly higher (p<0.04) in birds fed 57 and/or 75% triticale diet than those fed control diet.The significant increase in empty small and large intestine relative weights in birds had high level of triticale in their diet may be due to enhanced function of these parts, because of an increase in water soluble NSP and subsequent increase in digesta viscosity which implying a feedback mechanism in gut motility and thus size of this organ. The higher water soluble non starch polysaccharides in triticale as compared to corn can increase gut chyme viscosity and reduce enzyme-nutrient reactions and subsequent substrate digestions, leading to significant modifications of the structure and function of intestine (Wang *et al*. 2005) and to adapt to these changes, the activities of the intestinal secretary mechanisms may be enhanced. Thus this may lead to an increase in the size of the gastrointestinal tract and pancreas. Brenes *et al*. (1993a, b) indicated that this increased size of intestine and gastrointestinal tract could be an adaptive response to an increased need for enzymes. Partridge and Wyatt (1995) suggested that pancreatic weight as a proportion of body weight is increased in the presence of soluble fibers, implying that feedback mechanisms in the bird's gut stimulate hypertrophy of this organ. Enzyme supplementation did not have a significant effect (p>0.05) on relative weight of crop, pro-ventriculus, gizzard, small intestine and/ or large intestine of birds. The pancreas relative weight was significantly decreased in birds fed diet supplied with enzyme than those fed diet without enzyme supplementation. The small intestine relative weight was significantly (p<0.05) greater in birds fed 75% triticale diet without enzyme supplementation than those fed corn-soy based diet with/without enzyme supplementation or birds fed 19% triticale diet with enzyme supplementation.

**Intestinal viscosity:** The average gut (jejunum and ileum) chyme viscosity of chickens at 42 days of age fed diet with different levels of triticale with/without enzyme supplementation is shown in Table 4. The gut chymes viscosity numerically increased with increased in the levels of triticale in diet. The jejunum and ileum chyme viscosity significantly ($p<0.001$) increased in birds fed diets with 36, 57 and 75% triticale than those fed control diet. Enzyme supplementation significantly reduced ($p<0.001$) intestine chyme viscosity measured at 42 days of age. The jejunum and ileum chime viscosity was significantly decreased in birds fed with diet contained enzyme supplementation than those fed diet without enzyme supplementation. The interaction between triticale level and enzyme supplementation was significant ($p<0.02$) whereas enzyme supplementation showed more effect in diet with high level of triticale.

The main problem associated with the feeding of NSPs to poultry is viscosity which is a high water holding capacity of these compounds. Research has shown that viscosity is due to soluble pectins or β-glucans that even in small amounts, markedly increase intestinal viscosity (Jozefiak *et al*., 2004). The reduction of the intestinal chyme viscosity is suggested to be one of the main functions of exogenous enzymes. Thus, many researchers (Steenfeldt *et al*., 1998; Bergh *et al*., 1999; Silva and Smithard, 2002; Lazaro *et al*., 2003) suggested that the beneficial role of NSP degrading enzymes is by their action in the ileum. In the presented experiment, the viscosity of the ileal digesta was highest in birds fed triticale-soy based diets which contained the highest amounts of NSPs. The higher NSPs in triticale as compared to corn can increase digestive chyme viscosity and reduce endogenous enzyme-nutrient actions and their subsequent substrates, leading to significant modifications of the structure and function of intestine (Wang *et al*., 2005). To adapt to these changes, the activities of the intestinal secretary mechanisms may be enhanced. Thus this may lead to an increase in the size of the gastro intestinal tract and pancreas (Table 4). Brenes *et al*. (1993a) indicated that this increased size of intestine and the gastro intestinal tract could be an adaptive response to an increased need for enzymes. When supplementing exogenous enzymes in the triticale based diet, a greater proportion of NSP may be hydrolyzed which might attenuate the secretary function of the responding organs and the tract segments and then the organ sizes may decrease. Brenes *et al*. (1993b) also implied that the reduction in relative organ weight had a direct economic benefit as the dressing yield of broilers should increase proportionally. According to Morgan *et al*. (1995), feed enzymes in wheat-based diets for poultry function by reducing viscosity at the intestinal level. Classen and Bedford (1991) showed a small but significant reduction in digesta viscosity with pentosanase supplementation of wheat-based diets.

**Intestinal histology:** The average Villus Height (VH), Villus Width (VW), crypt depth, Muscular Thickness (MT) and Apparent Villus Surface Area (AVSA) of small intestine (jejunum) of birds fed different levels of triticale with/without enzyme supplementation measured at 42 days of age is shown in Table 5. The VH and crypt depth significantly ($p<0.01$) decreased with increased levels of triticale in diet whereas the lowest VH and crypt depth observed in birds fed 75% triticale diet. The highest VH and crypt depth observed in birds fed control diet. The VW, MT and AVSA significantly ($p<0.01$) increased in birds fed 75% triticale diet than those fed control diet. Villus hight, crypt depth and AVSA significantly ($p<0.05$)

Table 5: Effect of dietary triticale level and enzyme supplementation on small intestinal (jejunum) morphology[1] of broiler chicks measured at 42 days of age

| Item | Villus height | Villus width | Crypt depth | Muscularis thickness | Villus apparent surface area |
|---|---|---|---|---|---|
| Main effects | ---------------- | ---------------- | μm | ---------------- | $\mu m^2$ |
| **Triticale** | | | | | |
| 0 | $613.63^{a}$ | $59.84^{b}$ | $139.86^{a}$ | $57.15^{b}$ | $35590^{b}$ |
| 36 | $572.25^{a}$ | $71.12^{b}$ | $126.82^{ab}$ | $81.28^{a}$ | $40547^{ab}$ |
| 75 | $491.24^{b}$ | $102.93^{a}$ | $119.66^{b}$ | $70.18^{ab}$ | $49695^{a}$ |
| SE | 13.32 | 5.31 | 4.06 | 4.83 | 2835 |
| **Enzyme** | | | | | |
| 0 | $522.11^{b}$ | 78.94 | $121.78^{b}$ | 67.41 | $38329^{b}$ |
| 500 | $595.98^{a}$ | 76.98 | $135.77^{a}$ | 71.66 | $45559^{a}$ |
| SE | 10.88 | 4.33 | 3.32 | 3.94 | 2315 |
| **Interaction effects** | | | | | |
| Triticale (enzyme) | | | | | |
| 0 (-) | $585.19^{ab}$ | $60.33^{c}$ | $132.09^{abc}$ | $53.70^{b}$ | $33279^{b}$ |
| + | $642.06^{a}$ | $59.35^{c}$ | $147.63^{a}$ | $60.59^{ab}$ | $37901^{b}$ |
| 36 (-) | $596.12^{ab}$ | $66.23^{bc}$ | $113.36^{c}$ | $77.30^{ab}$ | $39314^{ab}$ |
| + | $548.39^{b}$ | $76.00^{abc}$ | $140.29^{ab}$ | $85.27^{a}$ | $41780^{ab}$ |
| 75 (-) | $385.01^{c}$ | $110.27^{a}$ | $119.91^{bc}$ | $71.25^{ab}$ | $42393^{ab}$ |
| + | $597.48^{ab}$ | $95.58^{ab}$ | $119.40^{bc}$ | $69.12^{ab}$ | $56996^{a}$ |
| SE | 18.84 | 7.51 | 5.75 | 6.83 | 4010 |

[a-c]Means with no common superscripts in each column and each effect are significantly different ($p<0.05$); Measurement were done under 5× objective lens

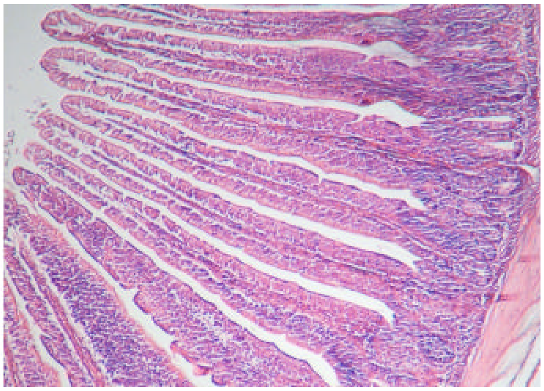

Fig. 1: Light micrograph of part of a section through the jejunum of a bird fed the corn-soy based diet showing normal length and distribution of the villi

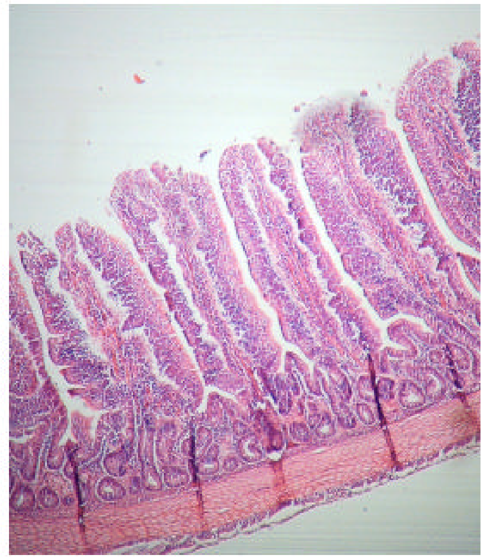

Fig. 2: Light micrograph of part of a section through the jejunum of a bird fed the 75% level of triticale in diet without enzyme supplementation, showing very shortening, thickening and atrophy of the villi

increased in birds fed diet with enzyme supplementation compared to those fed diet without enzyme supplementation. The interaction between triticale level in diet and enzyme supplementation on VH was significant ($p<0.01$) whereas the effect of enzyme supplementation on VH was more noticeable in birds fed triticale-soy diet. Histological observations of the small intestine indicated apparent morphological changes in the jejunum of birds fed triticale-soy based diet compared with those fed corn-soybean meal diet. The birds fed the control diet had elongated and distinct villi (Fig. 1) in contrast, the villi of the jejunum of bird fed triticale-soy based diets without enzyme supplementation were shorten and thickened (Fig. 2). Although most studies have been done with cereal diets other than triticale one can relate the effects of triticale NSPs to those shown by other cereal diets, such as wheat, barley, oats and rye. In a study by Viveros *et al*. (1994), histological observations of the small intestinal epithelium of birds fed barley-soy revealed morphological changes in the jejunum compared with birds fed corn-soy diet. The section through the jejunum of birds fed a diet containing barley showed shortening, thickening and atrophy of the villi compared with the elongated villi from birds fed the corn-soy diet. In this study histological observations were similar to those of Jaroni *et al* . (1999) who described normal characteristics of the villus high and crypts of Lieberkuhn in birds fed corn-soy diet and short, thick and damaged villi in birds fed wheat middlings diets. The birds examined in the study showed short, thick and damaged villi especially with the higher level (75%) of triticale in diet compared to those fed a corn-soy diet and these are in agreement with the visual observations of Jaroni *et al*. (1999) and Viveros *et al*. (1994) for barley-based diets. The higher water soluble non starch polysaccharides in triticale as compared to corn can increase digestive chyme viscosity. Increased viscosity is known to stimulate the growth of anaerobic microflora and their interaction with nutrients (Campbell and Bedford, 1992). Microbes can deconjugate bile salts, contributing to lower digestibility of nutrients especially fat (Jaroni *et al*., 1999).

The microorganisms that are generally found in large numbers in the caeca of the bird also tend to migrate to the small intestine where most nutrient absorption takes place (Campbell and Bedford, 1992). High bacterial counts can thus irritate the gut lining and result in a thicker lining with damaged microvilli reducing nutrient absorption further (Visek, 1978). Enzyme supplementation also appears to reduce the microbial population in the gastrointestinal tract (Choct *et al*., 1995; Dunn, 1996) and thus the negative effects associated with it such as atrophy of the intestinal villi, enlarged digestive organs and increased size of the gastrointestinal tract (Brenes *et al*., 1993a, b; Viveros *et al*., 1994).

**Blood biochemistry:** The average Triglyceride (TG), cholesterol, Low-Density Lipoproteins (LDL) and High Density Lipoprotein (HDL) in serum of chicks fed diet with different levels of triticale with/without enzyme supplementation measured at 42 days of age is shown in Table 6. There were not significant differences in TG,

Table 6: Effect of dietary triticale level and enzyme supplementation on Triglyceride (TG), Cholesterol (Chol), High-density Lipoproteins (HDL) and Low-Density Lipoproteins (LDL) of broiler chicks measured at 42 days of age

| Item | Chol | TG | HDL | LDL |
|---|---|---|---|---|
| Main effects | ---mg $dL^{-1}$--- | | | |
| **Triticale** | | | | |
| 0 | 135.0 | 146.0 | 96.0[a] | 19.0 |
| 19 | 125.0 | 121.0 | 86.0[ab] | 19.0 |
| 36 | 133.0 | 138.0 | 88.0[ab] | 23.0 |
| 57 | 130.0 | 147.0 | 82.0[ab] | 20.0 |
| 75 | 124.0 | 126.0 | 75.0[b] | 22.0 |
| SE | 5.5 | 10.0 | 4.0 | 2.5 |
| **Enzyme** | | | | |
| 0 | 126.0 | 127.0 | 84.0 | 21.0 |
| 500 | 133.0 | 144.0 | 86.0 | 20.0 |
| SE | 3.4 | 6.4 | 2.6 | 1.6 |
| **Interaction effects** | | | | |
| Triticale (enzyme) | | | | |
| 0 (-) | 130.0 | 155.0 | 91.0 | 20.0 |
| + | 140.0 | 136.0 | 100.0 | 17.0 |
| 19 (-) | 128.0 | 117.0 | 91.0 | 17.0 |
| + | 122.0 | 124.0 | 81.0 | 21.0 |
| 36 (-) | 130.0 | 123.0 | 88.0 | 22.0 |
| + | 136.0 | 153.0 | 89.0 | 23.0 |
| 57 (-) | 130.0 | 120.0 | 76.0 | 19.0 |
| + | 130.0 | 173.0 | 88.0 | 20.0 |
| 75 (-) | 111.0 | 119.0 | 74.0 | 25.0 |
| + | 137.0 | 134.0 | 75.0 | 19.0 |
| SE | 7.7 | 14.2 | 5.8 | 3.5 |

Means with no common superscripts in each column and each effect are significantly different ($p<0.05$)

cholesterol and LDL between birds fed diet contained different levels of triticale but the blood serum HDL significantly ($p<0.01$) decreased in birds fed diet with 75% triticale than those fed corn-soy diet. The chickens fed diet supplemented with 500 ppm of enzyme supplementation had higher cholesterol concentration but was not significantly differs from those fed diet without enzyme addition (126 Vs 133 mg $mL^{-1}$). The inclusion of highly viscous grain in diet induced bird plasma cholesterol and HDL levels as compared to control one. Soluble dietary fibers such as mixed linked β-Glucans, may reduce the absorption of fat and cholesterol and are known to have cholesterol lowering properties. These effects are all associated with the viscosity forming properties of soluble dietary fibers (Pettersson and Aman 1993). Aline *et al.* (2001) reported that plasma cholesterol was lower than control rats fed whole wheat and triticale flour diets. Several mechanisms that might explain the hypocholesterolemic effect of dietary fiber, whether functioning alone or in combination have been proposed as slowing down the rate of gastric emptying, modification of bile acid absorption and metabolism, interference with lipid absorption and metabolism, production of Short-Chain Fatty Acids (SCFA) from fermentation of fiber in the colon, up regulation of the hepatic LDL receptor and alterations in plasma concentration or tissue sensitivity to insulin or other hormones (Anderson *et al.*, 1990; Jackson *et al.*, 1994).

## CONCLUSION

Triticale may be used as an alternative source of grain in finishing broiler diets. The use of 75% triticale in finisher diet did not have an adverse effect on broiler chicken performance. Gut chymes viscosity and relative weight of pancreas and gastrointestinal tract increased in broiler chickens fed diet with high levels of triticale. Enzyme supplementation of triticale-soy finisher diet caused a reduction in the intestinal and pancreas relative weights and gut chyme viscosity of birds. Histological observations on the small intestine epithelium of birds fed triticale-soy diet showed morphological changes in the jejunum (shortening, thickening and atrophy of the villi). The addition of enzymes supplementation; to the triticale-soy based diet improved these histological alterations. Triticale seems to have a lowering property for serum HDL in chickens.

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