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Effects of Enzyme Supplement on Nutrient Digestibility, Metabolizable Energy, Egg Production, Egg Quality and Intestinal Morphology of the Broiler Chicks and Layer Hens Fed Hull-Less Barley Based Diets

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Abstract: The effects of β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) supplementation on the nutrient digestibility and metabolizable energy of egg production, egg quality intestinal morphology of the broiler chicks and layer hens fed hull-less barley-based diets were examined in three similar experiments. The results of this study showed that the inclusion of β-glucanase and xylanase in the hull-less barley based diets had no significant improvement on the growth performance of broiler, feed conversion ratio. The results of this experiment showed that β-glucanase and xylanase had negative effects on egg shell quality as reduced egg shell weight (4.6%) and egg shell thickness (5.32%). The addition of β-glucanase and xylanase had also no effects on yolk color and Hugh units of eggs either. The results also demonstrated that β-glucanase and xylanase supplementation did not improve the metabolizable energy, organic matter, protein and starch digestibility of the diet contained hull-less barley. The addition of glucanase and xylanase to the diets significantly reduced villus height, villus width, crypt depth, villus height: crypt depth ratio and goblet cell numbers of the duodenum and jejunum of small intestine compared with the control group. But, the numbers of goblet cells were more in the jejunum than in duodenum of small intestine. On the other hand, these enzymes reduced villus width and crypt depth of the ileum while increased villus length of the ileum receptivity. The goblet cells numbers in the villi of the ileum of birds fed the hull-less based diet; with exogenous enzyme were significantly higher than those in the jejunum and duodenum section of small intestine of layer hens. Goblet cells are responsible for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium. Further studies are needed to evaluate the effects of exogenous enzyme on the nutrient digestibility, metabolizable energy, intestinal morphology and microflora activity of intestine of the broiler chickens and layer hen diet.

Key words: β-glucan, hull-less barley, nutrient digestibility, metabolizable energy, intestinal morphology, microflora activity, intestinal broiler chickens, layer hen

INTRODUCTION

The soluble non starch polysaccharides (NSP), pentosans and mixed-linked β-glucans, cannot be hydrolyzed by endogenous enzyme and result in poor feed conversion, reduced body weight gain and wet litter conditions (Ravindran, 2003). The enzyme widely used in the poultry feed industry, are xylanase and β-glucanases. These enzymes cleave the non-starch polysaccharides (NSP) in cereal grains such as barley or hull-less barley. Several mechanisms have been proposed to explain the beneficial effects of exogenous enzymes in improving energy and nutrient utilization of hull-less barley-based diets (Bedford and Schulze, 1998; Simon, 1993; Brufau *et al*., 1993). Enzymes were able, at low cost, to increase the metabolizable energy value of barley to virtually that of wheat and to improve litter quality (Brufau and Francesch, 1991). Al Bustany and Elwinger (1988) reported that amulti-enzyme product said to contain cellulose, β-glucanase and protease was found significantly to improve egg production and feed conversion, late in the laying period (Nasi, 1988). A modest but significant increase in egg production after the application of another multi-enzyme product to layers maintained on barley-based diets in a number of commercially sponsored trials were similarly reported by Graham (1991). The beneficial response to supplementary β-glucanase in barley-based diet occurs largely in the starter phase (0-3 weeks) with little or no response with older birds (Cambell *et al*., 1984). Yu *et al*. (1998) reported that the supplementing β-glucanase did not improve total weight gain (0-6 weeks) with a diet of 500 mg kg^{-1} barley

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substitution. Supplementation β-glucanase in diet up to 250 mg kg^{-1} of barley not only enhanced body weight gains of growing broiler, but also improved the live-weight of six-week broiler.

Rotter *et al.* (1990) reported that the available energy (AMEn) for young chicks significantly increased due to enzyme supplementation Friesen *et al.* (1992) showed that β-glucans content of hull-less barley had the most pronounced effect on apparent digestibilities. So, diets supplemented with increased AMEn, apparent protein digestibility and apparent lipid digestibility hull-less barley. Mannio (1981) reported that chicks fed diets supplemented at 4 week of age showed improve of body weight gain by 12-25% increasing feed consumption by 3-12% and metabolizable energy improve by 0.75-1.53 mJ kg^{-1} of dry matter, respectively. Kocher *et al.* (2000) showed the addition of enzymes to either canola meal or sunflower meal-based diets had no significant effects on growth performance and metabolizable energy. Also who reported that the commercial enzyme products had some effects in diets and these effects could only be seem after detail analysis of feed and digesta and did not result in significant improvement in growth performance of broiler. Salleh *et al.* (2005) reported that the commercial feed enzyme had no effect on body weight gain, but tended to improve the feed conversion of broiler chicks. The ileal crud protein and ash digestibility were improved by the commercial feed enzyme and increased ME. But the organic matter digestibility was not changed by the enzymes. Thus, Marsman *et al.* (1997) showed that ileal fat and starch digestibility are not affected by the feed enzyme, but the ileal CP digestibility increases. Danicke *et al.* (1999) showed that positive, xylanase effects were not significant for nitrogen and dry matter digestibility in jejunum and ileum (Which are the main nutrient absorptive sites)? Similar trends were reported by Salih *et al.* (1991) fed high viscosity hull-less barley diets with or without β-glucanse, to broiler or Single Comb White Leghorn (SCWL) cockerels, poor gain, feed conversion and lower fat digestibility with unsupplemented diets than chicks given the enzyme supplemented hull-less barley diets. Viveros *et al.* (1994), reported that the jejunum of birds fed a diet containing 60% barley showed shortening, thickening and atrophy of the villi and an increased number of goblet cells compared with those on a maize-soy diet. However, addition of β-glucanase counteracted these effects. In Contrast, Iji *et al.* (2001) found that the addition of xylanase to wheat-based diets had no effect on villus height, crypt depth or villus surface area in the duodenum, jejunum and ileum of broiler. Mathlouthi *et al.* (2002) observed that exogenous enzyme improve nutrient digestibility and broiler chicken performance, probably by improving the absorption capacity of the small intestine through increased villus surface and intestinal concentration of conjugated bile acid. Indigestible polysaccharides, which are known to be viscous, can act directly by increasing bile acid excretion (Garcia-Diez *et al.*, 1996), or indirectly through the intestinal microflora, which affect the morphology of the small intestine wall (Southon *et al.*, 1987). Therefore, the addition of exogenous enzymes is necessary to reduce the anti nutritive effects of viscous non starch polysaccharides (Choct and Annison, 1992). As reported by Yasar and Forbes (2000), the decrease in digesta viscosity after exogenous enzyme addition is most likely associated with an improvement in small intestine wall morphology. Wu *et al.* (2004a) found that the xylanase supplementation increased ileal villus height in the ileum. Xylanase supplementation had no effect on crypt depth in birds fed on diets containing ground wheat, but increased the crypt depth in cold-pelleting diets. A significantly interaction between diet form and xylanase supplementation was observed for ileal crypt depth. No significant effects of xylanase supplementation were observed for the villus height, crypt depth, goblet cell numbers or epithelial thickness in the ileum.

Wu *et al.* (2004b) demonstrated that the addition of xylanase supplementation to wheat-based diet had no effect on villus height and crypt in the duodenum, jejunum and ileum. Xylanase supplementation tended to increase goblet cell numbers in the duodenum and decreased crypt depth in the jejunum. The combination of phytase and xylanase increased villus height in the ileum and crypt depth in the jejunum and ileum. Mathlouthi *et al.* (2002) reported that small intestine wall decreased in villus length, width and surface area in broiler chickens fed the rye-based diet compared with those fed the corn-based diet. Van Leeuwen *et al.* (2004) showed that pectin affected the mucosal surface by decreasing the surface area with the zigzag pattern and increasing the surface area with convoluted, mainly ridge-shaped villi. When cell proliferation increases microbial activity, the mucin composition of goblet cell may also change. Goblet cells are responsible for the section of mucin (Schneeman, 1982). An increase in cell proliferation will reduce the age and maturity of the goblet cell, which might affect the quality of mucin, produced these cells. The maturity of enterocytes may also be reduced when cell proliferation increase, which consequently reduces absorption of fatty acid and nutrients and thus increase the energy requirement for maintenance of the digestive tract.

The main objectives of the present study carried out were to investigate the effects of dietary enzyme supplementation on the nutrients digestibility, Metabolizabe Energy (ME), performance, intestinal morphology in broilers and layer hens fed hull-less barley-based diets. The different responses of enzyme supplementation might be related to the non starch polysaccharide content of hull-less barley dietary. Therefore, this trial attempted to study the effects of enzyme supplementation on growth performance or egg production and egg quality, nutrients digestibility, Metabolizable Energy (ME), intestinal morphology in broiler and layer hen. We used these experimental models to ensure the greatest beneficial effects of exogenous enzyme and/or pronounced in non starch polysaccharide diets supplemented with hull-less barley.

MATERIALS AND METHODS

Three experiments were designed as follow: **First experiment:** Nine hundred and sixty one-day-old male broiler chickens (Arbor Acres) were housed and received hull-less barley-based diet. Room temperature was set at 32±3°C on day of placement and gradually decreased 32°C at week 1 to 22°C at day 42. Continuous fluorescent lighting was provided throughout the trial. Feed and water were available *ad libtum*. The experiment was designed Completely Randomized Design (CRD) with a 4*3 factorial arrangement of treatments, which were included 4 levels of hull-less barley (0, 10, 20 and 30%) and three levels of enzyme supplementation (0, 30 and 60 g/100 kg of diet). Each treatment replicated 4 times. The chickens were randomizely distributed to 48 pens (20 chickens per pen). The diets were given on the starter (0-21 days), grower (21-35 days) and finisher (35-49 days) (Table 1). All mortalities were weighed, recorded and their weights were included in the calculation of feed conversion. Group feed consumption (g) and body weights (g) were recorded weekly.

Second experiment: A digestion trial was performed from 21 days of age. To determine the effect of exogenous enzyme on the digestibility of nutrients and the metabolizable energy of the hull-less barley-based diets using, 252 birds (Arbor Acres) and fecal and ileal content were collection for calculating digestibility of diets fed. The birds were housed in the 36 battery cages (7 birds per cage) in a completely randomized design with a 4×3 factorial arrangement for hull-less barley (0, 10, 20 and 30%) and for enzyme addition β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) (0, 30 and 60 g kg^{-1}). Three replicates were used for each treatment. In all test diets, 3 g Cr_2O_3/kg of diet were used as a marker to measuring the digestibility of nutrient and metabolizable energy. In both periods, 21 and 42 days the chickens were adapted to diets for 3 days and then, excreta were collected twice per days. Excreta collected per cage during the 3 days were pooled together and represented one replicate. Contaminants such as feathers and scales were carefully removed and the excreta stored in container at -20°C. Four birds from each replicate were killed by cervical dislocation. Immediately after death, the intestinal tracts were removed. The total intestinal content was collected from 2 cm posterior to merckel's diverticulum to 2 cm anterior to the ileocaecal junction. Ileal digesta collected from the chickens in each pen was pooled, representing on replicate and then stored at -20°C in small plastic containers.

Table 1: Diet composition for broiler chicks

	21 days				42 days			
	Hull-less barley (%)				Hull-less barley (%)			
Diet	0	10	20	30	0	10	20	30
Maize	69.03	61.02	52.96	44.91	72.21	64.42	55.70	48.30
Soy bean meal	26.11	22.90	19.72	16.53	23.75	22.50	21.21	18.70
Fish meal	1.69	3.32	4.89	6.46	0.00	0.00	0.00	0.00
Other nutrients	3.17	2.76	2.43	2.10	4.04	3.08	3.09	3.00
Nutrient composition (%)								
MEn (kcal/kg)	2950.00	2950.00	2950.00	2950.00	2950.00	2950.00	2950.00	2950.00
CP (%)	18.50	18.50	18.50	16.60	16.60	16.60	16.60	16.60
SNSP (%)	3.70	3.89	4.09	4.29	3.27	3.84	4.30	4.60
INSP (%)	9.80	9.31	8.81	8.31	9.67	9.51	9.27	8.94
Nutrition determination (%)								
CP	19.50	19.00	18.80	19.20	16.30	16.10	15.70	15.60
SNSP	3.66	3.92	4.00	4.30	3.23	3.66	4.26	4.58
INSP	9.60	8.80	9.90	8.80	10.00	10.50	10.10	9.20
CF	5.80	5.60	4.80	5.00	4.60	4.20	3.80	4.20
Ash	6.30	6.30	6.80	6.70	6.70	5.60	8.00	6.70
EE	4.60	3.80	6.50	3.30	2.70	3.60	2.70	2.80
Sugar	6.17	5.51	4.88	5.25	36.26	35.68	41.04	46.37

Table 2: Diet composition of layer hens

	Diets					
Diet	1	2	3	4	5	6
Hull-less Barley	0	20	30	40	50	60
Barley	37	27	22	16	12	0
Maize	38	28	23	18	13	15
Other Nutrients	25	25	25	25	25	25
Nutrient composition (%)						
MEn (kcal/g)	2.65	2.63	2.63	2.60	2.60	2.63
CP	15.3	15.02	15.02	15.01	15.01	15.03
CF	3.45	3.87	3.55	2.96	2.96	2.45
Sugar	1.35	1.904	2.36	2.55	2.735	2.745
Starch	16.272	25.013	31.553	34.84	38.124	39.18
β-Glucan	1.05	1.93	2.51	2.88	3.25	3.48
Nutrition determination (%)						
CP	17.1	17.7	16.2	15.6	17.9	19
CF	4	3.6	3.2	3.8	3.6	3.8
NDF	10	808	804	9.4	9.4	8.6
Sugar	2.5	4.5	3	5.03	5.1	5.93
Starch	38.5	33.7	34.8	36.3	41.4	31.29
β-Glucan	1.34	1.89	2.66	2.70	2.64	2.22

Third experiment: This experiment was performed with 220 laying hen; Hy-line w36. Birds were housed in 20*20 cm cages (2 birds per cage). Birds were reared for 8 months (58 weeks) then 6 birds per treatment were killed for intestinal bacterial population or gut morphology. Experiment of diets were designed to used hull-less barley in layer ration 0, to 60%. Experiment was conducted with randomize complete design by factorial 6*3 arrangement for hull-less barley dietary inclusion rate of 0, 20, 30, 40, 50 and 60% of the diet and for addition enzyme supplementation β-glucanase(550 U g^{-1}) and xylanase (800 U g^{-1}) of 0, 50 and 100 g/100 kg diet with 6 replicates. Treatment means of comparisons were made by Duncan's multiple range tests.

Gut morphology: The whole parts of the small intestine comprising deudenum, jejonom and ileum were removed from the body immediately after death and transverse sections, were successively gut with 2 cm interval and fixed with 10% buffered formalin. Routine histological laboratory methods such as dehydration, Clearing and paraffin embedding were used and paraffin blocks were made. Sections with 6 μm thick were made by rotary microtome and stained with heamatoxylin-eosin (1) and PAS (1) and studied under light microscope. The length and width of the intestinal villi and the dept of the intestinal crypts of lieberkohn glands were measured with linear scaled graticule. The number of goblet cells/mm area of the vilus and crypts were measured by 25 squared graticule.

Diet composition for broiler chicks and layer hens are shown in Table 1 and 2. Nutrient values, non-starch polysaccharides and β-glucan were also determined.

RESULTS

Effect of exogenous enzyme supplement on the performance of 0-21 days, 22-45 days broiler chickens fed hull-less barley based diets are shown in Table 3. Chickens fed enzyme-supplemented hull-less barley diets did not significantly ($p>0.05$) improve weight gains during rearing periods. Feed intake and feed conversion ratio (FCR) were not different significantly ($p>0.05$) for chicks fed enzyme-supplemented β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) hull-less barley diets at 0-21 and 22-45 days. When data were calculated for metabolizable energy per kilogram body weight (MEn/GE) and crud protein intake per kilogram body weight (CP/BW) no significant differences were found for the broilers fed hull less barley based diets supplemented with enzyme β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) (Table 3). Analysis of data also showed that during the two stage of growing, the interaction between chicks fed diets without enzyme and chicks fed diet with enzyme were not different. So no significant ($p>0.05$) performance differences were found between the same two groups, either (Table 3).

Mean daily feed consumption of layers fed exogenous supplemented enzyme hull-less barley based diet were not significantly ($p>0.05$) different compared with control. There were no significant in percentage of egg production, egg weight and egg mass feed conversion ratio of layers fed diets supplemented with enzyme (Table 4). When data of the experiment analyzed for height of albumen, the results obtained were not different although control diet showed 0.40 and 0.26 (mm) higher than experimental diets. Hens fed control diet

Table 3: Effects of exogenous enzyme supplement on performance of broiler fed on hull-less based diets

Enzyme (g/100 kg)	Feed intake (g)	Body weight (g)	FCR	ME/WG (kg)	Pro/WG (kg)
Age 0-21 days					
0	805.1	506.1	1.60	4732.7	342.7
30	808.1	506.8	1.59	4748.2	343.8
60	820.5	521.7	1.57	4667.7	338.2
SEM	34.1	24.2	105	241.6	17.3
Significantly					
Enzyme	0.41	0.13	0.19	0.61	0.63
En.*Ration	0.73	0.72	0.12	0.67	0.67
Age 22-42 days					
0	2137.4	818	2.62	7739.6	417.5
30	2150.9	851.9	2.55	7531.26	406
60	2108.6	848.7	2.49	7353.6	396.5
SEM	236.5	93.2	0.27	805.3	43.4
Significantly					
Enzyme	0.87	0.53	0.41	0.41	0.40
En.*Ration	0.98	0.70	0.39	0.39	0.39

Table 4: Effect of exogenous supplemented enzyme on performance and egg quality of layer hens (58 weeks)

Enzyme (g/100 g)	Feed intake (g/HD)	Hen production (%)	Egg mass (g)	Egg weight (g)	FCR
0	141.69	80.51	45.10	55.24	3.30
50	138.84	80.26	45.9	56.02	3.44
100	138.89	78.91	45.36	56.11	3.42

Egg quality Enzyme (g/100 kg)	High albumen (mm)	Shell weight (g)	Shell thickness (mm)	Strength shell (kg cm^{-1})	Yolk Colour (Roach Fan)	Hugh unit
0	8.26	7.27a	0.297a	1.71	8.39	88.24
50	7.86	6.95b	0.282b	1.68	8.46	86.56
100	7.99	7.06b	0.284b	1.60	8.40	87.86

Mean values within columns with different letter(s) differ significantly ($p<0.05$)

Table 5: Influence of exogenous supplemented enzyme β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) on metabolizable energy, fecal and ileum nutrient digestibility of the broiler chicks (21 days)

Enzyme (g/100 kg^{-1})	ME (kg)	ME/GE (%)	MD (%)	DCP (%)	D fat (%)	Starch D.
Fecal collection						
0	13.12	74.3	76.04	62.5	8601	96.1
30	13.22	74.8	77.2	65.1	87.5	96.4
60	13.30	75.3	77.6	65.3	88.5	96.5
SEM	5.19	2.9	2.5	4.1	3.2	0.61
Probability						
Enzyme	0.1	0.71	0.30	0.21	0.18	0.29
Ration * Enzyme	0.62	0.63	0.36	0.4	0.11	0.88
Ileum collection						
0	12.82	72.6	71.8b	75.2	83.4	
30	12.74	72.1	72.5ab	75	85.4	
60	12.86	72.8	73a	74.2	85.6	
SEM	1.35	0.76	0.87	1.09	2.7	
Probability						
Enzyme	0.12	0.11	0.01	0.12	0.1	
Ration * Enzyme	0.12	0.11	0.20	0.003	0.8	

Mean values within columns with different letter(s) differ significantly ($p<0.05$)

showed significant ($p<0.05$) different shell weight compared with supplemented enzyme diet. But no significant differences were found between diets with enzyme (Table 4). The same trend was found with shell thickness. The variations were in the order of 0.29 (mm) for control diet and significantly ($p<0.05$) different from those obtained for diets contained enzyme. Strength of the shell, colour of the yolk and Hugh Unit calculated in this experiment, almost followed the same trend as other parameters mentioned before. But control diet with no enzyme showed numerically higher values from those contained enzyme (Table 4).

The main effect of exogenous enzyme were no significant ($p>0.05$) improve ME, organic matter, protein, fat and starch digestibility by fecal collection bioassay (21 days), respectively (Table 5). Enzyme supplementation also had a different effect on the ME, ME/GE, digestibility of organic matter, protein, fat and starch values but was not significantly. Therefore, exogenous supplemented enzyme did not significantly improve digestibility of CP, Fat and starch and ME or ME/GE values content of dietary hull-less barley from ileum collection assay (21 days) for broiler chickens (Table 5). But, data obtained of ileum collection shown that organic matter digestibility significantly, were improved for broiler chicks on the 21 days of age. By comparing data obtained from two bioassay for digestibility of protein, organic matter, fat, starch and ME or ME/GE values of fecal collection, were higher than those for ileum collection on the 21 days of broiler chicks.

Table 6: Influence of enzyme supplement β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) on the metabolizable energy, fecal and ileum nutrient digestibility of the broiler chicks (42 days)

Enzyme (g/100 kg)	ME (kJ/g)	ME/GE (%)	%OMD	DCP%	D Fat%	Starch D.
Fecal Collection						
0	14.66	83.7	82.6	69.9	79.6a	97.2
30	14.71	83.9	84.0	69.8	82.1ab	97.6
60	14.75	84.2	84.5	71.02	84.8a	97.8
SEM	0.499	2.8	2.9	4.5	2.9	0.62
Probability						
Enzyme	0.9	0.91	0.73	0.70	0.01	0.05
Ration * Enzyme	0.2	0.19	0.16	0.12	0.47	0.05
Ileum collection						
0	14.12	80.6	81.5	76.1	84.5	
30	13.91	79.4	80.7	77.3	84.4	
60	14.02	80.0	81.4	77.0	85.1	
SEM	5.23	2.5	2.2	3.13	3.1	
Probability						
Enzyme	0.52	0.52	0.61	0.60	0.84	
Ration * Enzyme	0.01	0.04	0.02	0.001	0.38	

Table 7: Effects of exogenous supplemented enzyme β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) on the morphology and shapes villus of the small intestinal of broiler chickens (42 days)

Enzyme (g/100 kg^{-1})	Villus height (mm)	Villus width (mm)	Crypt depth (mm)	Villus height/width (mm)	Villus height/Cr Dep (mm)
0	0.96	0.69	0.19	1.37	5.15
30	0.92	0.72	0.19	1.30	5.05
60	0.97	0.72	0.19	1.35	5.32
Probability					
Ration	0.003	0.45	0.001	0.04	0.001
Enzyme	0.31	0.23	0.89	0.15	0.35

Enzyme (g/Ton)	Leaf-shaped	Tongue -shaped villi	Finger-position shaped villi	Ridges partly	Convoluted ridges villi
0	19.2	72.7	5.2	1.1	1.4
30	18.9	73.5	5.6	0.99	1.0
60	18.6	72.7	6.1	0.96	1.6
Probability					
Enzyme	0.75	0.65	0.37	0.81	0.09

The effect of exogenous supplementation enzyme on the digestibility of organic matter, protein, fat and starch ileal and excreta (fecal) digestibility and metabolizable energy in broiler for 42 days presented in Table 6. The exogenous enzyme supplemented to hull-less barley was significantly improved fat digestibility of excreta (fecal) bioassay compared to ileal assay for broiler chicks on the 42 days age ($p<0.05$). Enzyme supplementation did not improve digestibility of organic matter, protein, starch and metabolizable energy or ME/GE of dietary hull-less barley from using excreta and ileal collection in broiler chicks ($p>0.05$). However, data obtained from fecal collection bioassay shown that digestibility of organic matter, fat and metabolizable energy values were higher than ileal collection for broiler chicks respectively. The results of experiment also shown that protein digestibility obtained from ileal collection bioassay was higher than those excreta collection bioassay (Table 6).

Addition of exogenous enzyme did not affect the shape of villi in the small intestine of broiler chickens (Table 7). No exogenous supplemented enzyme affected the villus height, villus width, crypt depth and villus height/width and villus height/Crypt depth ratio from the small intestine of broiler chickens.

The result of experiment showed that supplemented β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) effects, on villus height, villus width, crypt depth, villus height/Crypt depth ratio and number of goblet cell in different sections of the small intestine of birds fed on hull-less barley (Table 8). Inclusion 100 g kg^{-1} exogenous supplemented enzyme in the diet significantly reduced villus height in duodenum, therefore, 50 and 100 g kg^{-1} exogenous enzyme was similar in reducing villus width, crypt depth and, villus height: crypt depth ratio in the duodenum of small intestine of layer hen ($p<0.05$). However, the addition exogenous supplemented enzyme significantly reduced villus height, villus width, crypt depth and goblet cell number at the jejunum of small intestine compared with the control group. Thus, goblet cell number increased more in the jejunum camper to duodenum of small intestine, respectively (Table 8). Exogenous supplemented enzyme had no effect ($p>0.05$)

Table 8: Effects of exogenous supplemented enzyme β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) on the morphology of the intestinal mucosa at different sites in the small intestinal of layer hens (58 weeks).

Enzyme (g/100 kg)	Villus length (μm)	Villus width (μm)	Crypt depth (μm)	Villus length/Crypt depth (μm)	Goblet cell No. in mucosal epithelial (mm^2)
Duodenum					
0	1005.52a	170.28a	178.68a	5803.0b	1464.54a
50	979.43a	150.86b	137.90b	7997.9a	1320.63ab
100	912.76b	153.42b	142.09b	6549.1b	1274.07b
Jejunum					
0	912.38a	132.19a	123.43a	1259.02a	1540.74b
50	899.43a	121.90b	119.24ab	1262.97a	1731.22a
100	915.05a	111.62c	112.76b	1401.50a	1583.07b
Ileum					
0	661.71b	131.05a	113.90a	968.84b	3208.5a
50	706.29a	104.00b	101.33b	1152.49a	3047.6a
100	674.34ab	113.17b	107.32ab	1026.60b	3276.2a

[a-d] Mean values within columns with different letter(s) differ significantly ($p<0.05$)

Table 9: Effect of exogenous supplemented enzyme β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) on the morphology in the small intestinal of layer hens (58 weeks)

Enzyme (g/100 kg)	Villus length (μm)	Villus width (μm)	Crypt depth (μm)	Villus length/Crypt depth (μm)	Goblet cell No. in mucosal epithelial (mm^2)
0	859.87a	144.51a	138.67a	2677.0c	2073.60a
50	861.71a	125.59b	119.49b	3471.1a	2011.99a
100	836.32b	126.47b	120.76b	2995.1b	2041.62a
Duodenum	965.90a	158.22a	152.89a	6783.3a	1353.08c
Jejunum	908.95b	121.90b	118.48b	1307.8b	1618.34b
Ileum	683.05c	116.44b	107.56c	1052.0b	3164.44a

[a-c]Mean values within columns with different letter(s) differ significantly ($p<0.05$)

on villus length and the ratio of villus length to crypt depth in the jejunum. The exogenous supplemented enzyme reduced villus width and crypt depth of the ileum and increased villus length in the ileum of intestine ($p>0.05$). However, the ileum goblet cell numbers were not significantly increased ($p>0.05$) by exogenous supplemented enzyme.

The results of the current study showed that villus height, villus width, crypt depth in the small intestine mucosa were affected by exogenous supplemented enzyme of hull-less based diet (Table 9). However, the addition of exogenous enzyme had no effect on goblet cell number in small intestine wall of birds ($p>0.05$). The addition of exogenous supplemented enzyme had significant differences effects on the villus height, villus width and crypt depth in the duodenum, jejunum and ileum of small intestine of bird. However, exogenous supplemented enzyme reduced villus height, villus width, crypt depth villus and length/crypt depth in the duodenum, jejunum and ileum of the small intestine of layer hens ($p<0.05$). Goblet cell numbers in the villi of the small intestine were not affected ($p>0.05$) by the exogenous supplemented enzyme. Across all groups, the villus height, villus width, crypt depth in the duodenum was significantly higher than those in the jejunum and ileum. The goblet cells number in the villi of the ileum of birds fed the hull-less based diet with exogenous enzyme were higher ($p<0.05$) than those in the jejunum and duodenum section of small intestine in layer hens.

DISCUSSIONS

The results of the present study showed that the inclusion of β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) to the hull-less barley based diets did not ($p>0.05$) improve body weight (BW), feed conversion ratio (FCR), metabolizable energy (MEn/BW) and crud protein intake per kilo gram of body weight (CP/BW). Also, the interaction between diets and the exogenous enzyme supplemented, on broiler performances was not significantly different. These results confirmed those reported by Yu *et al.* (1998) who showed that supplementing β-glucanase to diets did not improve body weight gain from 0-6 weeks of age. These negative responses could be due to non starch polysaccharides (NSP) that cannot be hydrolyzed by exogenous enzyme, therefore, no improvement in feed conversion and body weight gain. These results also, agree with those reported by Kocher *et al.* (2000). They reported that the addition of enzymes to either canola meal or sunflower meal-based diets had no significant improvement in growth performance of broilers. But in contrast, Mannio (1981) reported that the body weight gain was improved by 12 to 25% and food consumption increased by 3 to 21% when chicks at 4 weeks of age fed diets supplemented with enzyme.

The addition of β-glucanase (550 U g^{-1}) and xylanase (800 U g^{-1}) to hull-less barley based diets had no effect ($p>0.05$) on albumen, yolk color and Hugh unit of the

eggs. Data obtained in this experiment, demonstrated that exogenous β-glucanase and xylanase had no significant ($p>0.05$) effects on the metabolizable energy (ME), ME/BW, protein, fat and starch digestibility of hull-less barley diets. These results are in agreement with that report by Kocher *et al.* (2000). Our data demonstrated that addition of β-glucanase and xylanase to diets had no effects ($p>0.05$) on egg production. Similar results were obtained by Jaroni *et al.* (1999) and Brenes *et al.* (1993). They reported that enzyme addition to a laying diet containing wheat did not improve egg production. But recent studies by Al Bustany and Elwinger, (1988a); Aimonen and Uusi-Rawa, (1991), they also found that enzyme supplementation had no effects on egg production, feed consumption and feed conversion efficiency. But these results are in contrast with those reported by Pawlik *et al.* (1990) and Brenes *et al.* (1993). The results obtained in this experiment showed that egg weight and egg mass of hens fed enzyme supplemented diets were not significantly ($p>0.05$) different and are in contrast to other studies reported increased egg weight and egg mass (Jaroni *et al.*, 1999; Wyatt, 1992). Our experiment showed that the exogenous β-glucanase and xylanase significantly reduced ($p<0.05$) shell weight by 4.6% and shell thickness by 5.32%. Studies by Aimonen and Uusi Rawa, (1991) also reported that enzyme supplementation can have a negative effect on shell quality. The exogenous supplemented β-glucanase and xylanase to diets significantly improved fat digestibility when excreta compared to ileal bioassay for broiler chicks on the 42 days age ($p<0.05$). These results are in agreement with Friesen *et al.* (1992). Exogenous supplemented did not affect the villus height, villus width, crypt depth and villus height/width and villus height/Crypt depth ratio in small intestine of broiler chickens. These results are in consistent with those reported by Iji *et al.* (2001) and Wu *et al.* (2004b). They reported that addition of xylanase had no effect on villus height in the ileum of broiler given wheat-based diets.

Addition of glucanase and xylanase in layer hen diets, significantly reduced villus height villus width, crypt depth and, villus height: crypt depth ratio and goblet cell number in the duodenum and jejunum of small intestine compared with the control group. These results are in consistent with those reported by Jaroni *et al.* (1999) and Vivero *et al.* (1994). Viveros *et al.* (1994) found that the addition of β-glucanase to barley-based diets decreased the number of goblet cells compared with those birds fed the maize-soy diet. Jaroni *et al.* (1999) reported that the shortening, thickening and atrophy of the villi in the jejunum of laying hens fed diets based on wheat middling were reversed with xylanase addition.

The goblet cells number in the villi of the ileum intestine of birds fed the hull-less based diet contain exogenous enzyme were significantly higher than those in the jejunum and duodenum section of small intestine in layer hens. Wu *et al.* (2004) demonstrated that the xylanase supplementation tended to increase goblet cell numbers in the duodenum and decreased crypt depth in the jejunm. Goblet cells are responsible for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium (Schneeman, 1982). Thus, a higher density of goblet cells may result in an increase in the secretion of mucin. An increase in cell turnover associated with a reduction in the maturity of the goblet cell (Filipe, 1977). An increase in cell proliferation will reduce the age and maturity of the goblet cell, increases microbial activity, which might affect the quality of mucin composition, produced these cells. The maturity of enterocytes may also be reduced when cell proliferation increase, which consequently reduces absorption of fatty acid and nutrients and thus increase the energy requirement for maintenance of the digestive tract (Van Leeuwen *et al.*, 004). In conclusion, the addition of xylanase and β-glucanase to hull-less barley-based diets did not significantly improve the performance of broiler chickens and egg production of layer hens, as well as nutrients digestibility and metabolizable energy values. Further more research needed to evaluate the effect of exogenous enzyme on the nutrients digestibility and metabolizable energy and morphological or bacterial activity for broiler chickens and layer hen diet.

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