

## Evaluation of Hull-Less Barley with or without Enzyme Cocktail in the Finisher Diets of Broiler Chickens

H. Teymouri<sup>1</sup>, H. Zarghi<sup>1\*</sup>, and A. Golian<sup>1</sup>

### ABSTRACT

An experiment was carried out to study the effect of Hull-Less Barley (HLB) replaced for dietary corn at the rate of zero, 25, 50, 75, and 100% with two levels of Enzyme Cocktail (EC) supplementation (0 and 0.5 g kg<sup>-1</sup> of diet) on performance of broiler chickens during the finisher period. Four hundred and fifty male broiler chickens aged 24-days were randomly assigned to 50 pens in a Complete Randomized Design (CRD) experiment, in a 5×2 factorial arrangement, with five replicates of 9 birds each. There were no significant differences in Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), and Feed Conversion Ratio (FCR) of birds fed diets with zero, 25, 50, and 75% HLB replacement for corn, whereas the complete replacement of HLB for corn in diet significantly decreased ADG and ADFI and increased FCR. The GastroIntestinal Tract (GIT) organs relative weights and ileal chyme viscosity were significantly increased, and serum lipid metabolites concentrations significantly decreased by the increase in dietary HLB levels. A significantly shorter and thicker villi and thicker muscular layer in jejunum of chickens were observed when diet HLB level increased. The dietary EC supplementation significantly reduced the adverse effects of high dietary level of HLB on performance and GIT characteristics. It is concluded that HLB is a good alternative for broiler finisher diet, if substituted for up to 75% of corn. In addition, supplementation of EC in the finisher diet can decrease the adverse effects of high level of HLB on performance of broiler chickens.

**Keywords:** Blood metabolites, Carcass yield, Cereal grains, Growth Performance, Intestinal histology and viscosity.

### INTRODUCTION

Hull-Less Barley (HLB) differs from conventional barley in that the hull is not firmly attached to the kernel and, as a result, is detached after thrashing, leading to a higher nutritional value and increased volume density than conventional barley (Thacker, 1999). HLB contains considerably higher levels of anti-nutritional factors consisting mainly of soluble Non-Starch Polysaccharides (NSPs), especially  $\beta$ -glucans, as compared to corn (Leeson and Summers, 2008). The levels of  $\beta$ -glucans in HLB range from 40 to 70 g kg<sup>-1</sup> (Baidoo and

Liu, 1998). High levels of NSPs in HLB can cause serious digestive problem (Classen *et al.*, 1985). It has been shown that the addition of extracted NSPs from cereal grains to broiler diets can increase intestinal chyme viscosity, decrease nutrient digestibility, modify intestinal micro-flora, and reduce physiological and morphological changes (White *et al.*, 1981; Choct *et al.*, 1996) and, as a result, depress growth performance (Preston *et al.*, 2001; Basmacioglu Malayoglu *et al.*, 2010). Many studies have shown that the use of supplemental NSP-degrading enzymes to viscous cereal based diets as triticale, wheat, barley, rye and oats positively affected

<sup>1</sup> Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.

\*Corresponding author; e-mail: h.zarghi@um.ac.ir



poultry health and productivity (Choct *et al.*, 1995; Wang *et al.*, 2005; Basmacioglu Malayoglu *et al.*, 2010; Zarghi *et al.*, 2010).

This study was designed to evaluation the effect of different levels of HLB with/without a blend enzyme of cellulases, xylanases and  $\beta$ -glucanases in finisher diet on performance, carcass characteristics, serum lipids, GIT organs relative weight, intestine chyme viscosity, and jejunal morphology of broiler chickens.

## MATERIALS AND METHODS

### Birds Housing and Care

The experimental procedure of this study was approved by the Animal Care Committee, Ferdowsi University of Mashhad, Mashhad, Iran. Five hundred day-old male broiler chicks of a commercial strain "Ross 308" were obtained from a commercial hatchery and fed with standard starter and grower commercial mash diets up to 24 days of age. Chickens were individually weighed and 450 of them randomly assigned to 10 dietary treatments with 5 replicates of 9 birds each at 24 days of age. Each pen had one square meter space and floors were 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 24 days of age. During the experimental period (25-39 days) room temperature and relative humidity were kept in the range of 20-22°C and 60-50 percent, respectively, and light: darkness program was 23:1 hours. In the course of the experiment, the chickens had continuous access to water and feed.

### Experimental Design and Diets

A Complete Randomized Design (CRD) experiment with a factorial arrangement (5×2) of five levels (0, 25, 50, 75, and 100%) of HLB replaced for dietary corn

with two levels of Enzyme Cocktail (EC) supplementation (0 and 0.5 g kg<sup>-1</sup> of diet). HLB that was used in this experiment (Lout variety) was obtained from the Khorasan Razavi Agricultural and Natural Resource Rresearch Centre (Northeast of Iran). The chemical compositions of HLB, corn and soybean meal were determined by NIR through Evonik-Degussa office in Tehran, Iran, and data were used to formulate the experimental diets. The enzyme cocktail (Safyzym, Lozafr, France) that was used in this experiment, was a blend of 3,500 U g<sup>-1</sup>  $\beta$ -glucanase, 1,600 U g<sup>-1</sup> xylanase, and 25 U g<sup>-1</sup> cellulases activity. The experimental diets, Five levels of HLB replaced for corn, were formulated by using the least-cost linear programming to meet or exceed nutrient requirements of Ross-308 rearing guideline (Aviagen, 2015). The experimental diets were adjusted to have equal energy, protein, and other nutrients and were prepared in mash form. Each diet was divided into two equal portions and enzyme cocktail was added to each part at rate of zero and 0.5 g kg<sup>-1</sup> and mixed to provide the 10 experimental diets and fed *ad-libitum* from 25 to 39 days of age. The ingredient and nutrient contents of experimental diets is presented in Table 1. Chemical composition of the experimental diets (dry matter, crude protein, crude fat, and crude fibre) were determined in the laboratory analysis after the samples were ground through 20  $\mu$ m mesh screen and were dried at 70°C for 48 hours. The proximate feed analysis were performed according to (AOAC, 2002).

### Growth Performance Traits

All birds (pen groups) were weighed at 24 (commence of experiment) and 39 days of ages. The birds were fasted for 4 hours prior to being weighed. Mortality in each pen was weighed and recorded to correct the growth performance traits. The growth performance as mean 39 days live body weight, Average Daily weight Gain (ADG) and Average

**Table 1.** Ingredients and composition of experimental diets <sup>a</sup>.

Item	Hull-less barley levels replaced for dietary corn (%)				
	0.0	25	50	75	100
<u>Ingredient, g/kg as-fed</u>					
Corn (ME= 3340 kcal kg <sup>-1</sup> and CP= 74.1 g kg <sup>-1</sup> )	573.8	445.9	310.6	163.4	0.0
Hull-less barley (ME= 3253 kcal kg <sup>-1</sup> and CP= 124.6 g kg <sup>-1</sup> )	0.0	150.0	310.6	485.8	675.5
Soybean meal (ME= 2270 kcal kg <sup>-1</sup> and CP= 446.9 g kg <sup>-1</sup> )	334.0	312.3	289.0	264.3	237.0
Soybean oil, (ME= 8850 kcal kg <sup>-1</sup> )	58.5	57.4	55.0	52.8	51.4
DL-Methionine (Methionine= 980 g kg <sup>-1</sup> )	2.6	2.7	2.7	2.7	2.7
L-lysine-HCl (Lysine= 780 g kg <sup>-1</sup> )	1.2	1.5	1.8	2.1	2.5
L- Threonine (Threonine= 999 g kg <sup>-1</sup> )	0.6	0.7	0.8	0.9	1.1
Limestone	10.9	10.9	11.2	11.4	11.7
Di-calcium phosphate	10.3	10.3	10.0	9.9	9.6
Salt (Na Cl)	2.4	2.3	2.3	2.3	2.5
Na HCo <sub>3</sub>	0.7	1.0	1.0	1.0	1.0
Vitamin-premix <sup>b</sup>	2.5	2.5	2.5	2.5	2.5
Mineral premix <sup>c</sup>	2.5	2.5	2.5	2.5	2.5
Total	1000	1000	1000	1000	1000
<u>Calculated composition (g kg<sup>-1</sup> as-fed, except for ME)</u>					
ME (kcal kg <sup>-1</sup> )	3200	3200	3200	3200	3200
Crude protein	195.0	195.0	195.0	195.0	195.0
Crude fibre					
Calcium	7.9	7.9	7.9	7.9	7.9
Available phosphorus	3.9	3.9	3.9	3.9	3.9
Sodium	1.6	1.6	1.6	1.6	1.6
Digestible Lysine	10.3	10.3	10.3	10.3	10.3
Digestible Methionine	5.3	5.3	5.3	5.3	5.3
Digestible Methionine+Cysteine	8	8	8	8	8
Digestible Threonine	6.9	6.9	6.9	6.9	6.9
<u>Analyzed nutrient contents [% (as fed basis)]</u>					
Dray mater	93.30	93.28	92.93	92.85	92.85
Crude protein	19.40	18.50	18.75	19.80	19.80
Crude fat	7.97	7.40	6.91	5.93	5.21
Crude fibre	3.50	3.55	3.60	3.65	3.70

<sup>a</sup> Each diet was divided into two equal portions and enzyme cocktail (Safyzym: containing 1,600 U g<sup>-1</sup> xylanases, 3,500 U g<sup>-1</sup>  $\beta$ -glucanases and 25 U g<sup>-1</sup> cellulases) was added to each part at rate of zero and 0.5 g kg<sup>-1</sup> and mixed to provide the 10 experimental diets. <sup>b</sup> Vitamin premix Supplied the following per kilogram of diet: Vitamin A (all-trans-retinol), 11,000 IU; vitamin D<sub>3</sub>, 1,800 IU; vitamin E ( $\alpha$ -tocopherol), 36 mg; vitamin K<sub>3</sub> (Menaquinone), 5 mg; cyanocobalamin, 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, 1,100 mg; etoxycoin, 0.125 mg. <sup>c</sup> Mineral premix Supplied the following per kilogram of diet: Zn-sulfate, 84 mg; Mn- sulfate, 160 mg; Cu-sulfate, 20 mg; Se, 0.2 mg; I, 1.6 mg; Fe, 250 mg.

Daily Feed Intake (ADFI) were calculated during experimental period. The Feed Conversion Ratio (FCR) was calculated as the amount of feed consumed divided by pen weight gain including the weight gain of the dead chickens.

### Blood Metabolites

One bird per replicate (pen) was randomly selected and blood samples were collected from the wing vein by a syringe at day 39 after 4 hours fasting. Blood samples were collected in labelled sterile test tubes and



were centrifuged at 3,000×g for 5 minutes to isolate serum. After centrifugation, gained serum was stored at -20°C for later analysis. Serum lipid metabolites including Triacylglycerol (TG), Cholesterol (Cho), High-Density Lipoproteins (HDL) and Low-Density Lipoproteins (LDL) were determined enzymatically in an auto-analyzer (Vitalab Selectra E, Vital Scientific, Argentina).

### Small Intestine Chyme Viscosity

One bird per replicate was euthanized to determine small intestine chyme viscosity at day 39. The intestinal tract was immediately removed to obtain small intestine chyme by gentle finger stripping of the intestinal segments. For viscosity measurement, the chyme taken from jejunum and or ileum were divided into two sub samples, homogenized thoroughly, and approximately 1.5 g wet weight centrifuged at 12,700×g for 5 minutes to obtain the supernatants. The supernatant (0.5 mL) was withdrawn and its viscosity in centipoises (1/100 dyne second per cm<sup>2</sup>) was measured in a Brookfield digital viscometer (Model LVDVII+CP, Brookfield Engineering Labs, Inc., Stoughton, MA 02072) at 37°C. The average value obtained from two subsamples was used for the statistical analysis.

### Jejunal Morphology

The middle part of jejunum was excised for morphological study. Tissue samples (0.5 cm<sup>2</sup>) were taken from jejunum midpoint and then were immersed in a 10% buffered formalin solution for 72 hours. Then, samples were excised and were washed with physiological saline solution. The tissue samples were treated in tissue processor apparatus and embedded in paraffin wax. Transverse sections were cut (6 µm) using a rotary microtome (Leica RM 2145), placed on a glass slide and stained with Hematoxylin and Eosin (H&E), then, they

were analyzed under a light microscope to determine morphological indices (Bancroft and Gamble, 2002). Morphological parameters were measured using the Image Pro Plus v 4.5 software package on 9 villi chosen from each slide and only vertically oriented villus were selected for measuring (Saki *et al.*, 2012). The morphological traits were: (1) Villus height, (2) Villus width, (3) Crypt depth, and (4) Intestinal muscular thickness (Ganjali *et al.*, 2015). The villus surface area was calculated according to the Equation (1) (Solis de los Santos *et al.*, 2007).

$$AVSA = \frac{1}{2} \times VW \times VH \times 2\pi \quad (1)$$

Where, VSA= Villus Surface Area, VW= Villus Width, VH= Villus Height, and  $\pi=3.14$

### Carcass Yield and Gastrointestinal Parameters

At 39 d of age, one birds/pen, close to the average pen weight was selected and, after 4 hours of feed withdrawal but with free access to drinking water, was weighed, slaughtered, plucked and the gastrointestinal tract, giblets, and other inner organs were excised to determine the carcass and gastrointestinal parameters. Carcass was obtained by removing head, feathers, feet, and gastro-intestinal tract. After chilling for 24 hours at 4°C, carcasses were weighed to determine the slaughter yield (%) and were cut according to a standardized procedure (Uijttenboogaart and Gerrits, 1982) to determine carcass, breast, thigh, and abdominal fat weights using weighing scale (0.001-g, model GF 400, A&D Weighing, San Jose, CA, USA).

### Statistical Analysis

All data were analyzed by ANOVA using GLM procedure of SAS (SAS, 2003). Analysis of variance was performed using a complete randomized design with a factorial

arrangement of treatments. Before statistical analysis, the percentage data was transformed by Equation (2) for normalization. Data statistically tested for main effects of HLB levels and enzyme supplementation. Means were compared for significant differences using Duncan multiple range test ( $P < 0.05$ ). Statistical plan model is shown in Equation (3). Linear and quadratic models were fitted to data to describe the relationships between treatment and variables.

$$X = \text{Degrees} \left( \arcsin \sqrt{\frac{x}{100}} \right); \quad (2)$$

Where,  $X$  = transformed data, and  $x$  = basic data

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}; \quad (3)$$

Where,  $Y_{ijk}$  = Value that view,  $\mu$  = Mean population,  $\alpha_i$  = Effect of HLB levels,  $\beta_j$  = Effect of enzyme cocktail addition,  $(\alpha\beta)_{ij}$  = Interaction between HLB level  $\times$  Enzyme cocktail addition, and  $\varepsilon_{ijk}$  = Effect of experimental error.

## RESULTS AND DISCUSSION

### Growth Performance

Increasing the diet HLB levels up to 75% of corn in diet did not have a significant effect on LBW, ADG, ADFI and FCR of birds, whereas replacing diet corn with feeding diet containing 100% HLB significantly decreased 39 d LBW, ADG and ADFI ( $P < 0.01$ ) and significantly increased FCR ( $P < 0.04$ ) during finisher period (Table 2). The growth performance factors differed quadratically with increasing dietary HLB levels. The LBW, ADG, and ADFI numerically increased as HLB level increased up to 75% HLB for diet corn, but over that, with increasing HLB level to 100% of corn in diet, the above growth performance factors decreased. The greatest LBW, ADG and ADFI response to HLB

levels were observed at 75% HLB for diet corn. Body weight gain and FCR were influenced by exogenous enzyme supplementation (Table 2). Addition of EC to the diet significantly improved growth ( $P < 0.03$ ) and feed efficiency ( $P < 0.01$ ) of birds as compared to those fed diet without enzyme supplementation.

A poor performance in the birds fed HLB soybean-meal based diet as compared to those fed corn soybean-meal based diet related to lower nutrient digestibility and higher ant nutrient factors in HLB as compared to corn. This result is in agreement with (Scott *et al.*, 1998), who reported that barley significantly reduced FI, LBW, and increased FCR during 1-17 days of age. The results indicated that supplementing the finisher diet with EC improved the ADG and feed efficiency of broiler chickens. The cause of positive performance effects achieved by the addition of enzymes in feed are proposed as follows: (1) It has been shown that the anti-nutritive effects of 'viscous cereals' are associated with raised intestinal viscosity caused by soluble  $\beta$ -glucans and arabinoxylans present in those cereals (Choct and Annison, 1992; Bedford and Morgan, 1996). These hold significant amounts of water and, due to the resulting high viscosity; the absorption of nutrients becomes limited. These problems can be overcome by the addition of  $\beta$ -glucanases and xylanases, resulting in improved poultry performance. (2) As a consequence, it is widely assumed that the ability of  $\beta$ -glucanases and xylanases to degrade plant cell walls leads to release the nutrients from grain endosperm and aleurone layer cells. Therefore, this mechanism played an important role in improving the feed energy value. (3) A third proposed mechanism having a positive influence on the nutritive value of feed is the prebiotic effect achieved via the release of oligosaccharides (Choct and Cadogan, 2001).

### Carcass Yield

Different levels of HLB and EC in the finisher diet did not have a significant effect

**Table 2.** Effect of dietary Hull-Less Barley (HLB) levels and Enzyme Cocktail (EC) supplementation in finisher diet on average Live Body Weight (LBW), Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) in broiler chickens (25-39 days of age) <sup>a</sup>.

Effects	25 d LBW	39 d LBW	ADG	ADFI	FCR
	(g)		(g b <sup>-1</sup> d <sup>-1</sup> )		
<u>Hull-less barley levels replaced for dietary corn (%)</u>					
0	1039	2093 <sup>a</sup>	75.25 <sup>a</sup>	140.96 <sup>ab</sup>	1.88 <sup>b</sup>
25	1039	2095 <sup>a</sup>	75.42 <sup>a</sup>	135.52 <sup>b</sup>	1.85 <sup>b</sup>
50	1039	2104 <sup>a</sup>	76.05 <sup>a</sup>	143.10 <sup>a</sup>	1.90 <sup>ab</sup>
75	1046	2123 <sup>a</sup>	76.87 <sup>a</sup>	146.63 <sup>a</sup>	1.93 <sup>ab</sup>
100	1040	2007 <sup>b</sup>	69.05 <sup>b</sup>	134.59 <sup>b</sup>	1.96 <sup>a</sup>
<i>SEM</i> <sup>b</sup>	7.06	22.20	1.46	2.38	0.02
<u>Enzyme cocktail levels (g kg<sup>-1</sup>)</u>					
0	1046	2070	73.13 <sup>b</sup>	141.15	1.95 <sup>a</sup>
0.5	1035	2098	75.93 <sup>a</sup>	139.18	1.86 <sup>b</sup>
<i>SEM</i> <sup>b</sup>	4.46	14.04	0.92	1.51	0.02
<u>Source of variation, <i>P</i>-value</u>					
HLB	0.94	0.01	0.01	0.01	0.04
EC	0.08	0.17	0.03	0.36	0.01
HLB×EC	0.10	0.99	0.79	0.35	0.17
<u>Hull-less barley levels response, <i>P</i>-value</u>					
Liner	0.73	0.08	0.06	0.07	0.99
Quadratic	0.78	0.02	0.01	0.05	0.44

<sup>a</sup> Growth performance data are means of 10 pens with 10 birds per each for HLB levels and 25 pens with 10 birds per each for EC levels effects. <sup>b</sup> SEM indicates Standard Error of Mean. <sup>a, b</sup> Values with different superscripts within a column for each effect are significantly different (P< 0.05).

on relative weights of carcass, breast, and thigh of broiler chickens. Relative weights of abdominal cavity fat decreased (P< 0.05) with addition of graded levels of HLB. A lower abdominal fat deposition to HLB levels were observed at higher HLB levels, and the abdominal fat decreased linearly (P< 0.01) with increasing HLB levels in finisher diet (Table 3).

The results of this experiment showed that abdominal fat in broiler chickens decreases with the addition of graded levels of HLB in finisher diet. This result is in agreement with the finding of Esteve-Garcia *et al.* (1997), who reported that broiler chickens fed diets based on barley reduced abdominal fat to 2.5 g 100 g<sup>-1</sup> of carcass weight. Deposition of fat

in the abdominal region in broiler chickens is considered a waste by the poultry industry. Abdominal fat is not only a loss but also it represents an added expense for the processing effluent treatment. In further processing, it appears that the larger the quantity of abdominal fat, the lower is the processing yields (Yusrizal and Chen, 2003).

#### Gastrointestinal Tract Organs Relative Weight

The results of the current study demonstrated that in broiler chickens fed diet with HLB levels more than 50% HLB replacing corn in diet significantly increased

**Table 3.** Effect of dietary Hull-Less Barley (HLB) levels and Enzyme Cocktail (EC) supplementation in finisher diet on carcass yield (g 100 g<sup>-1</sup> of live body weight) and abdominal fat of broiler chickens slaughtered at 39 days of age <sup>a</sup>.

Effects	Relative weight (g 100 g <sup>-1</sup> of live body weight)			
	Carcass	Breast	Thigh	Abdominal fat
<u>Hull-less barley levels replaced for dietary corn (%)</u>				
0	71.66	26.83	19.83	2.02 <sup>a</sup>
25	71.55	27.39	20.43	1.57 <sup>bc</sup>
50	72.37	27.05	19.78	1.71 <sup>ab</sup>
75	71.64	26.87	19.58	1.28 <sup>c</sup>
100	70.69	25.94	19.76	1.51 <sup>bc</sup>
SEM <sup>b</sup>	0.60	0.43	0.35	0.11
<u>Enzyme cocktail (g kg<sup>-1</sup>)</u>				
0	71.61	26.86	19.85	1.63
0.5	71.56	26.77	19.90	1.60
SEM <sup>b</sup>	0.17	0.27	0.22	0.07
<u>Source of variation, P-value</u>				
HLB	0.42	0.21	0.50	0.01
EC	0.93	0.82	0.84	0.80
HLB×EC	0.12	0.06	0.21	0.32
<u>Hull-less barley levels response, P-value</u>				
Linear	0.34	0.30	0.90	0.01
Quadratic	0.21	0.12	0.91	0.08

<sup>a</sup> Data are mean of 10 samples for HLB levels and 25 samples for EC levels effects.<sup>b</sup> SEM indicates Standard Error of Mean.<sup>a, b</sup> Values with different superscripts within a column for each effect are significantly different (P < 0.05).

the GIT, proventriculus, pancreas, and small intestine relative weight. These relative organ weights at 39 d of age were significantly increased (P < 0.01) as more HLB replaced corn in diet. The GIT organs relative weight differed linearly with increasing dietary HLB levels. The GIT, proventriculus, small intestine, and jejunum relative weight numerically increased as HLB level increased. The greatest above GIT organs relative weight response to HLB levels were observed at 75 and 100% HLB replaced corn in diet. Enzyme supplementation did not have a significant effect (P > 0.05) on GIT, proventriculus, and small intestine relative weights measured at 39 d age, but birds fed diets supplemented with the enzyme cocktail had lower (P < 0.05) pancreas relative weight than those fed diets without enzyme addition. The dietary HLB levels and EC supplementation

interaction effects were significant for GIT and small intestine relative weight (P < 0.05). The birds that fed diet without EC as the dietary HLB levels increased the GIT (P < 0.05) and small intestine (P < 0.03) relative weight linearly increased, but, in the birds that fed diet supplemented with EC, the GIT and small intestine relative weight showed a straight line with non-significant regressions against different dietary HLB levels (Table 4 and Figure 1).

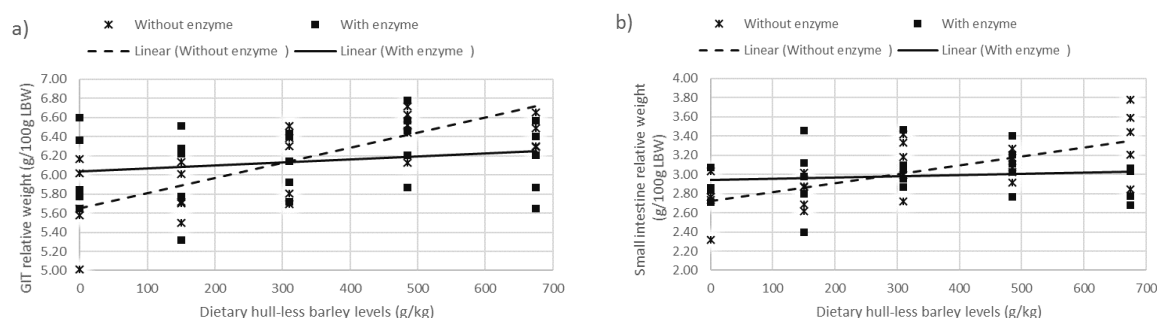
The significant increase in empty GIT organs relative weight in birds that had higher HLB in their finisher diet may be due to enhanced function of GIT to subsequent increase in HLB levels replacement for corn. This result agrees with the finding that reported feeding HLB to birds significantly increased relative weight (g 100 g<sup>-1</sup> of body weight) of GIT (Sharifi *et al.*, 2012). The increase in relative weight of GIT may be



**Table 4.** Effect of dietary Hull-Less Barley (HLB) levels and Enzyme Cocktail (EC) supplementation in finisher diet on GastroIntestinal Tract (GIT) organs relative weight ( $\text{g } 100 \text{ g}^{-1}$  of live body weight) and small intestine chyme viscosity (cPs) of broiler chickens at 39 days of age <sup>a</sup>.

Effects	GIT relative weight ( $\text{g } 100 \text{ g}^{-1}$ of live body weight)						Viscosity	
	GIT	Proventriculus	Small intestine	Jejunum	Ileum	Pancreas	Jejunum	Ileum
<u>Hull-less barley levels replaced for dietary corn (%)</u>								
0	5.86 <sup>b</sup>	0.34 <sup>b</sup>	2.79 <sup>b</sup>	1.19 <sup>b</sup>	0.92 <sup>b</sup>	0.25 <sup>b</sup>	1.39	1.68 <sup>b</sup>
25	5.92 <sup>b</sup>	0.33 <sup>b</sup>	2.88 <sup>b</sup>	1.27 <sup>ab</sup>	0.92 <sup>b</sup>	0.25 <sup>b</sup>	1.41	1.86 <sup>b</sup>
50	6.13 <sup>ab</sup>	0.36 <sup>ab</sup>	3.11 <sup>a</sup>	1.35 <sup>a</sup>	1.01 <sup>ab</sup>	0.29 <sup>ab</sup>	1.46	1.84 <sup>b</sup>
75	6.43 <sup>a</sup>	0.43 <sup>a</sup>	3.09 <sup>a</sup>	1.37 <sup>a</sup>	1.00 <sup>ab</sup>	0.29 <sup>a</sup>	1.45	1.96 <sup>a</sup>
100	6.41 <sup>a</sup>	0.37 <sup>ab</sup>	3.14 <sup>a</sup>	1.36 <sup>a</sup>	1.04 <sup>a</sup>	0.31 <sup>a</sup>	1.59	2.07 <sup>a</sup>
SEM <sup>b</sup>	0.11	0.01	0.08	0.04	0.03	0.01	0.11	0.13
<u>Enzyme cocktail (<math>\text{g kg}^{-1}</math>)</u>								
0	6.16	0.36	3.02	1.31	1.00	0.29 <sup>a</sup>	1.53	2.02 <sup>a</sup>
0.5	6.14	0.36	2.98	1.30	0.96	0.26 <sup>b</sup>	1.38	1.74 <sup>b</sup>
SEM <sup>b</sup>	0.01	0.01	0.21	0.02	0.02	0.01	0.07	0.08
<u>Source of variation, P-value</u>								
HLB	0.01	0.01	0.01	0.02	0.01	0.01	0.75	0.05
EC	0.87	0.61	0.58	0.78	0.12	0.04	0.15	0.03
HLB×EC	0.05	0.45	0.04	0.22	0.02	0.32	0.37	0.74
<u>Hull-less barley levels response, P-value</u>								
Liner	0.05	0.02	0.03	0.01	0.21	0.16	0.95	0.50
Quadratic	0.59	0.14	0.22	0.10	0.72	0.67	0.66	0.92

<sup>a</sup> Data are mean of 10 samples for HLB levels, 25 samples for EC levels and 5 samples for interaction effects. <sup>b</sup> SEM indicates Standard Error of Mean. <sup>a, b</sup> Values with different superscripts within a column for each effect are significantly different ( $P < 0.05$ ).



**Figure 1.** Effect of diet Enzyme Cocktail (EC) supplementation at five dietary HLB levels in finisher diet on digestive organs relative weight ( $\text{g } 100 \text{ g}^{-1}$  of live body weight) of meal broiler chickens at 39 days of age: (a) GastroIntestinal Tract (GIT) relative weight plotted from the equations;  $Y = 0.0016X + 5.65$ ,  $R^2 = 0.53$  (Without Enzyme = WOE),  $Y = 0.0003X + 6.04$ ,  $R^2 = 0.04$  (With Enzyme = WE), and (b) Small intestine relative weight plotted from the equations;  $Y = 0.0009X + 2.72$ ,  $R^2 = 0.47$  (Without Enzyme = WOE)  $Y = 0.0001X + 2.94$ ,  $R^2 = 0.018$  (With Enzyme = WE).

related to an increase in the intestinal function, due to an increase in NSPs and digesta viscosity which led to increasing gut motility and digestive excretions and, therefore, to increasing the size of GIT and its organs. In agreement with the present

study, other researchers have also pointed out that fibre ingestion leads to increase in size and length of the digestive organs in chickens (Iji *et al.*, 2001), pigs (McDonald, 2001), and rats (Ikegami *et al.*, 1990). The reduction in weight of GIT of birds fed HLB



diets supplemented with EC in the present study may be attributed to the decrease of intestinal chyme viscosity as a consequence of exogenous enzyme. The presence of grains such as wheat, barley and rye in diets tends to increase the relative weight and relative length of the GIT and gastrointestinal organs and supplemental enzyme significantly decreases intestinal weight and length (Silva and Smithard, 2002).

### Intestinal Chyme Viscosity

Ileal chyme viscosity was significantly increased ( $P < 0.01$ ) by the supplemental graded levels of HLB to diet, whereas it was significantly decreased ( $P < 0.01$ ) by the addition of EC to the diet (Table 4). Increase in intestinal viscosity previously has been reported by other researchers using viscous cereal grains in their experiments. Non-Starch Polysaccharides (NSP) are polymeric carbohydrates, which differ in composition and structure from starch (Bedford and Morgan, 1996) and possess chemical cross-linking among them and, therefore, are not digested by poultry (Adams and Pough, 1993; Annison, 1993). A part of these NSPs is water-soluble which is notorious for forming a gel like viscous consistency in the intestinal tract (Ward, 1995), thus reducing gut performance. Predominantly water soluble, on the other hand,  $\beta$ -glucans adversely affect all nutrients, especially protein and starch utilization and are known to give rise to highly viscous conditions in the small intestine of the chicks (Hasselman and Aman, 1986). Baidoo and Liu, (1998) reported that the levels of  $\beta$ -glucans in HLB range from 40 to 70 g kg<sup>-1</sup>, which are consistently higher than the 3 to 45 g kg<sup>-1</sup> in hulled barley.

The reduction ( $P < 0.05$ ) of intestinal chyme viscosity has been reported in broiler chickens fed wheat or barley-based diets supplemented with NSP-degrading enzymes (Fuente *et al.*, 1998; Shirzadi *et al.*, 2010), which might be a consequence of the

breakdown of polysaccharides into smaller polymers, thereby reducing viscosity (Wang *et al.*, 2005). As a result of xylanases and  $\beta$ -glucanases supplementation, the long backbones of the arabinoxylans and  $\beta$ -glucans are cleaved into shorter fragments, thereby reducing their viscosity (Gruppen *et al.*, 1993).

### Blood Serum Lipid Metabolites

The blood serum Triacylglycerol (TG), total Cholesterol (Cho), Very Low Density Lipoprotein (VLDL) and High Density Lipoprotein (HDL) concentrations were significantly decreased ( $P < 0.05$ ) when birds were fed diets containing high level of HLB (100% HLB replacement for corn). The blood serum total Cho, VLDL and HDL concentration numerically decreased (Table 5). The effect of HLB to reduce TG and Cho concentrations is believed to be attributable to the  $\beta$ -glucans in the NSPs fraction of this cereal grain (Delaney *et al.*, 2003). This result is in agreement with the finding of other researchers who reported that an increase in NSP of intestinal content can reduce cholesterol absorption and plasma cholesterol concentration (Smits *et al.*, 1997). It has been indicated that barley-based diets reduced serum total cholesterol in broilers (Moharrery, 2006). A negative correlation has been found between dietary fibre and serum cholesterol level (Pettersson and Aman, 1992). Fibre has a binding property with bile acids and may directly increase bile acid excretion and reduce serum cholesterol levels (Adrizal and Ohtani, 2002). Hajati *et al.* (2009) reported that dietary enzyme addition increased ( $P < 0.05$ ) the concentration of blood total cholesterol, HDL-cholesterol, and triacylglycerol levels. The Hypercholesterolemia effects of EC can be related to: (1) Reducing restrictions in the function of bile salts and their emulsifying properties in intestinal chyme (Hajati *et al.*, 2009), and (2) Digestion of big molecules of carbohydrates by enzyme which can change

**Table 5.** Effect of dietary Hull-Less Barley (HLB) levels and Enzyme Cocktail (EC) supplementation in finisher diet on blood serum lipid metabolites (mg dl<sup>-1</sup>) of broiler chickens at 39 days of age<sup>A</sup>.

Effects <sup>B</sup>	TG	Cho	HDL	LDL	VLDL
<u>Hull-less barley levels replaced for dietary corn (%)</u>					
0	109 <sup>a</sup>	162 <sup>a</sup>	86 <sup>a</sup>	54	22 <sup>a</sup>
25	100 <sup>a</sup>	128 <sup>bc</sup>	68 <sup>ab</sup>	39	20 <sup>a</sup>
50	84 <sup>ab</sup>	142 <sup>ab</sup>	75 <sup>ab</sup>	50	17 <sup>ab</sup>
75	106 <sup>a</sup>	123 <sup>bc</sup>	62 <sup>b</sup>	40	21 <sup>a</sup>
100	76 <sup>b</sup>	114 <sup>c</sup>	41 <sup>c</sup>	58	15 <sup>b</sup>
SEM <sup>C</sup>	7.82	8.85	6.29	6.45	1.56
<u>Enzyme cocktail (g kg<sup>-1</sup>)</u>					
0	93	127	63	45	18
0.5	96	140	69	50	19
SEM <sup>C</sup>	4.94	5.60	3.97	4.08	0.98
<u>Source of variation, P-value</u>					
HLB	0.02	0.01	0.01	0.18	0.02
EC	0.71	0.12	0.24	0.36	0.72
HLB×EC	0.75	0.08	0.78	0.01	0.75
<u>Hull-less barley levels response, P-value</u>					
Liner	0.65	0.14	0.62	0.19	0.67
Quadratic	0.80	0.58	0.33	0.14	0.79

<sup>A</sup> Data are mean of 10 samples for HLB levels, 25 samples for EC levels and 5 samples for interaction effects.

<sup>B</sup> TG= Triacylglycerol, Cho= Cholesterol, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein, VLDL= Very Low Density Lipoprotein. <sup>C</sup>SEM indicates Standard Error of Mean. <sup>a, b</sup> Values with different superscripts within a column for each effect are significantly different ( $P < 0.05$ ).

the viscous nature of intestinal chyme and, therefore, improves fat digestibility (Van Der Klis *et al.*, 1995).

### Morphological Observations of Jejunum

The morphometric variables including Villus Height (VH), Villus Width (VW), Crypt Depth (CD), Villus Height to Crypt Depth ratio (VH/CD), Muscular Thickness (MT), and Apparent Villus Surface Area (AVSA) were not influenced by dietary HLB levels. Dietary EC supplementation showed significant effect on MT ( $P < 0.01$ ) and the interaction effect between HLB levels and EC supplementation on VH was significant ( $P < 0.02$ ), while the effect of enzyme supplementation on VH was more noticeable in birds fed HLB based finisher

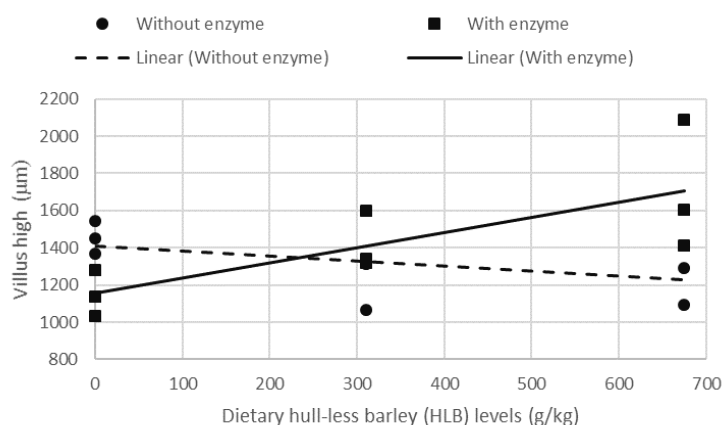
diet. In birds fed HLB based finisher diet compared with birds fed corn based finisher diet, VH significantly decreased, while birds fed HLB based finisher diet supplemented with enzyme, affected reversely on these morphological criteria (Table 6 and Figure 2). Histological observations of the small intestine indicated apparent morphological changes in the jejunum of birds fed HLB-based finisher diets compared with those fed corn-based finisher diets. The villi of the jejunum in birds fed HLB based diets without enzyme supplementation were shorter and thicker and had lower crypt depth (Figure 3-a). In contrast, the birds fed HLB-based diet supplemented with enzyme had elongated and distinct villus (Figure 3-b).

Similar to the results found in the current study, the birds that were fed with 80 and 160 g wheat middling kg<sup>-1</sup> of diet showed

**Table 6.** Effect of dietary Hull-Less Barley (HLB) levels and Enzyme Cocktail (EC) supplementation in finisher diet on jejunum morphological observations of broiler chickens at 39 days of age<sup>A</sup>.

Effects <sup>B</sup>	VH	VW	CD	MT	AVSA	VH/CD
<u>Hull-less barley levels replaced for dietary corn (%)</u>						
	..... $\mu$ .....				1000 $\mu^2$	
0	1303	188	245	222	750	5.70
50	1329	156	232	223	634	6.48
100	1484	201	236	236	924	6.44
SEM <sup>C</sup>	77.34	23.77	25.30	14.73	111.65	0.98
<u>Enzyme cocktail (g kg<sup>-1</sup>)</u>						
0	1319	197	237	254 <sup>a</sup>	817	6.51
0.5	1424	166	238	200 <sup>b</sup>	722	6.27
SEM <sup>C</sup>	63.15	19.41	20.65	12.03	91.69	0.80
<u>Source of variation, P-value</u>						
HLB	0.24	0.41	0.93	0.74	0.29	0.81
EC	0.26	0.28	0.97	0.01	0.54	0.91
HLB×EC	0.02	0.07	0.65	0.42	0.64	0.28
<u>Hull-less barley levels response, P-value</u>						
Linear	0.88	0.31	0.71	0.92	0.24	0.63
Quadratic	0.60	0.26	0.76	0.79	0.15	0.73

<sup>A</sup> Data are mean of 10 tissues samples for HLB levels, 25 tissues samples for EC levels and 5 tissues samples for interaction effects. <sup>B</sup> VH = Villus High, VW= Villus Width, CD= Crypt Depth, MT= Muscular Thickness, AVSA= Apparent Villus Surface Area. <sup>C</sup> SEM indicates Standard Error of Mean. <sup>a, b</sup> Values with different superscripts within a column for each effect are significantly different ( $P < 0.05$ ).

**Figure 2.** Effect of diet Enzyme Cocktail (EC) supplementation at five dietary HLB levels in finisher diet on jejunum morphological observations of meal broiler chickens at 39 days of age: Villus High (VH) plotted from the equations;  $Y = -0.2691X + 1407.9$ ,  $R^2 = 0.2535$  (WithOut Enzyme= WOE),  $Y = 0.8145X + 1156.3$ ,  $R^2 = 0.5877$  (With Enzyme= WE).



**Figure 3.** Light micrograph of a section through the jejunum of broiler chicken at 39 days of age: (a) Fed HLB soymeal diet (showing very shortening, thickening, and atrophy of villous), and (b) Fed the HLB soymeal diet supplemented with exogenous enzyme (showing normal length and distribution of the villous) in finisher period.

shorter and thicker villus (Jaroni *et al.*, 1999). The NSP in HLB caused an increase in the viscosity of intestinal chyme which stimulates the growth of anaerobic microflora in caeca. Microorganisms migrate from caeca to small intestine where the absorption of most nutrients takes place (Campbell and Bedford, 1992), thereby this high bacterial concentration can irritate the gut lining and cause thickening and atrophy of villus (Vissek, 1978). Enzymes supplementation can reduce both microbial population (Choct *et al.*, 1995) and atrophy of villus (Brenes *et al.*, 1993). This is in agreement with the results of other researchers (Santos *et al.*, 2004; Wu *et al.*, 2004), who observed longer and narrower villus and deeper crypts in birds fed wheat or rye-based diets supplemented with enzyme.

### CONCLUSIONS

Hull-less barley can replace up to 75% of corn in finisher broiler chickens diets without any adverse effect on performance. Gut chymes viscosity and relative weight of gastrointestinal tract organ increased in broiler chickens fed diet with higher levels of HLB. Histological observations on the small intestine epithelium of birds fed HLB based diet showed morphological changes in the jejunum. Dietary exogenous enzyme

supplementation can decrease the adverse effects of high HLB level in finisher diets. Hull-less barley seems to have a lowering property in serum lipid metabolites in chickens. Finally, HLB may be used as an alternative source of grain in poultry diets.

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### بررسی جو بدون پوشینه با و بدون مکمل آنزیمی در جیره پایانی جوجه‌های گوشتی

ح. تیموری، ح. زرقی، و ا. گلیان

#### چکیده

به منظور بررسی اثر جایگزینی ذرت با سطوح مختلف "صفر، 25٪، 50٪، 75٪ و 100٪" جو بدون پوشینه با و بدون افزودن مکمل آنزیمی (صفر و 0/5 گرم در کیلو گرم جیره) در جیره پایانی جوجه‌های گوشتی این آزمایش انجام شد. 450 قطعه جوجه گوشتی در سن 24 روزگی بین 50 قفس در یک طرح کاملاً تصادفی، با ترتیب فاکتوریل 2\*5، با پنج تکرار و نه قطعه پرند در هر تکرار توزیع شدند. جایگزینی ذرت در سطوح صفر، 25٪، 50٪ و 75٪ با جو بدون پوشینه اختلاف معنی داری بر افزایش وزن و مصرف خوراک روزانه و ضریب تبدیل غذایی نداشتند ولی با جایگزینی کامل جو بدون پوشینه به جای ذرت به طور معنی داری رشد و مصرف خوراک کاهش و ضریب تبدیل غذایی افزایش یافت. افزایش مقدار جو بدون پوشینه در جیره باعث افزایش وزن نسبی اندام‌های گوارشی و چسبندگی محتویات ایلتوم و کاهش غلظت متابولیت‌های چربی سرم خون به طور معنی دار شد. با افزایش سطح جو بدون پوشینه در جیره به طور معنی داری ویلی‌های ژرنوم پهن تر و کوتاه تر و ضخامت لایه عضلانی ضخیم تر شد. افزودن آنزیم به جیره مصرفی باعث کاهش اثرات منفی سطوح بالای جو بدون پوشینه در جیره مصرفی شد. نتایج حاصل از این آزمایش نشان داد که جو بدون پوشینه یک غله مناسب برای جایگزینی به جای ذرت در سطح 75 درصد در جیره پایانی جوجه‌های گوشتی است. بعلاوه استفاده از آنزیم های برون زادی باعث تعدیل اثرات منفی مقادیر زیاد جو بدون پوشینه در جیره می شود.