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## Effect of Diets Containing Different Qualities of Barley on Growth Performance and Serum Amylase and Intestinal Villus Morphology

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**Abstract:** A study was conducted to investigate protein metabolism and differences in length of intestinal villi in broiler chickens which consumed one of the barley based diets. The treatments were corn diet (1) as a control, barley diet with (4) or without (2) a commercial  $\beta$ -glucanase enzyme, barley treated with rumen fluid without protozoa (3) and hullless barley (5). The effect of treatments were investigated in a 42 d trial using 360 sexed, broiler chickens. In a digestibility trial, 15 male broiler chicks were used at 45 days old. In this regard, five treatments offered to chicken in three replicates individually. The experimental design for performance investigation was a completely randomized one with a  $5 \times 2$  factorial arrangement of treatments. Each of five treatments was replicated three times per sex ( $n = 3$ ). The levels of barley in treatments of (2) to (5) were 35% during growing (14 to 42 d) and finishing (42 to 52 d) periods. At the end of trial, two birds from each pen were selected and slaughtered. For histological studies the length, width and surface area of intestinal villi has been determined on male chickens. Blood sample has been taken just before slaughter of birds. No significant difference ( $P > 0.05$ ) was observed between (3) to (5) treatments with corn diet for weight gain, feed intake and feed conversion, but barley with no treatment (2) has shown less weight gain to compare enzyme treatment and hullless barley diet ( $P < 0.05$ ). Digestibility of dry matter (DM) and crude protein (CP) were less in barley diet with no treatment, to compare other treatments ( $P < 0.05$ ).

**Key words:** Intestinal villi, hulled barley, hullless barley, uric acid

### Introduction

Mixed linkage  $\beta$ -(1-3)(1-4)-D-glucan ( $\beta$ -glucan) is the major constituent of barley endosperm cell walls. The amount of this non-starch polysaccharide (NSP) in barley can vary considerably and is affected by growing conditions as well as genotype (Molina-Cano and Conde, 1982). The  $\beta$ -glucans have been identified as a major cause of poor growth rate and low nutrient digestibility (Ward and Marquardt, 1987) in broiler chicken. These antinutritive effects of NSP are attributed to an increase in intestinal digesta viscosity (Choct and Annison, 1992b). Mathlouthi *et al.* (2002) demonstrated that intestinal mucosa was modified in chickens receiving rye alone, but not in those receiving rye plus enzymes. This damage to the small intestinal mucosa may be caused indirectly by the viscous characteristics of NSPs (Stanogias and Pearce, 1985). In this regard, increasing endogenous losses of amino acid and 20% of nitrogen are well documented by NSPs (Angkanaporn and Coct, 1994). On the other hands, Sakata (1987) demonstrated that an increase in bacterial activity in the gastrointestinal tract was associated with a change in the morphology of the gut wall. Therefore, the addition of exogenous enzymes is necessary to reduce the antinutritive effects of viscous NSP (Choct and Annison, 1992a). The NSP-degrading enzymes markedly increase the nutritive value of wheat (Choct *et al.*, 1995) and rye (Bedford and Classen, 1993) in broiler chicken. This

improved performance of birds fed NSP-rich cereal diets by exogenous enzyme such as  $\beta$ -glucanase supplementation is not due to release of simple sugars, but rather to the ability of the enzymes to prevent the formation of viscous digesta (Choct and Annison, 1992a). 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.

The present study was undertaken to elucidate whether the barley treatments or use of hullless barley was associated with an effect on protein metabolism and/or with a change in the gut morphology of broiler chickens fed a barley based diet.

### Materials and Methods

**Animals and diets:** Three hundred and sixty 1-d-old sexed commercial broiler chickens (Ross, Iranian agency) were housed separately for each sex in floor pens containing litter composed of wood shaving and received a corn-based starter diet (Table 1). At 14 days of age, all the chickens from each sex were divided into 15 groups, 12 chicken per group. Each one of the 5 experimental diets was fed to 3 groups of chickens (in each sex) for 42 days. Dietary treatments as grower and finisher diets (Table 1) consist of:

1. A corn-based diet as a control,
2. A hulled barley-based diet without any treatment or enzyme addition on it,

3. A same diet as a mention for diet (2) but the barley treated with rumen fluid without protozoa. The rumen fluid collected from rumen content in slaughterhouse and strained through four layers of cheesecloth and centrifuged once at  $1000 \times g$  for 10 min. The supernatant fluid decanted and diluted with water (50:50). This fluid was mixed with ground barley thoroughly and transferred to large plastic barrel and kept anaerobically for 48 hours in about  $20^{\circ}C$ . Then the barrel evacuated on plastic sheet for drying on sunlight. This type barley which is named as processed barley included to grower and finisher diet as same amount of other type of barley for treatment no 3.
4. The exogenous enzyme used in this treatment was the commercial powdered preparation Endofeed, Reg No. 280003 (GNC Bioferm INC. Saskatchewan, Canada S7H 3A6) with  $\beta$ -glucanase activities. The enzyme preparation was added to the barley-based diet (same percent of treatment 2 and 3) at the level of 0.5g/kg of diet.
5. A normal hullless barley-based diet (same percent the treatments 2 to 4) without any treatment or enzyme addition on barley. The CP content in this grain was about 2% higher than hulled barley but, the removal of the hull is expected to result in modest increases in metabolizable energy, which can adjusted energy/protein levels as same as regular barley. This is why same amount of hullless barley used for comparing to hulled barley.

Birds were fed a mash feed mixture twice daily and weighed once weekly. Feed was fed in a uniform mash mixture and there was no selection of particles by the *ad libitum* fed birds. Fresh feed was weighed and offered to all the birds twice daily to prevent wastage. At the conclusion of the experiment, all birds and the remaining feed were weighed. Water also was supplied *ad libitum* throughout the entire experiment. Body weights were determined at 14, 42 and 56 d of age and feed intake over these periods was recorded. Feed conversion (g feed per body mass – FC) was calculated after adding the cumulated feed consumption for each pen to the total bird mass of that pen.

**Digestion trial:** A digestion trail experiment was performed using 15 male broiler chicks from 45 to 52 d of age. This consisted of 4 days of adaptation, followed by 72 h with access to 85 g feed from each treatment. Fifteen birds (3 replicate for each diet) were housed in individual layer cages with wire bottoms. Birds had free access to water throughout the experiment. The cages were kept in a room at  $22^{\circ}C$  and approximately  $58\% \pm 3$  relative humidity. Excreta were collected for each 24-hour period for days 50, 51 and 52. Contamination, such as

down and scales, was carefully removed, and the excreta stored in containers at  $-25^{\circ}C$ . Excreta samples were subsequently dried in oven at  $80^{\circ}C$ , weighed, ground through a 0.5-mm sieve, and stored in airtight plastic vessel at  $4^{\circ}C$  until analysis.

#### **Carcass Characteristics and Blood Samples**

**Collection:** At the end of the experiment (56 d of age), two birds of each pen were bled by cutting the carotid artery and blood has been taken from these artery. The blood samples were centrifuged for 15 min at  $2500 \times g$ , and serum was harvested and stored at  $-80^{\circ}C$ .

The carcass feather's removal was accomplished in a free-action picker after subscalding at approximately  $60^{\circ}C$ . The weights of the gizzard as well as the liver and intestine were recorded. The percentage yield of each part was calculated on the basis of carcass weight.

**Chemical measurements:** The determination of nitrogen in feed and excreta was performed with the macro-Kjeldahl method. Because a part of nitrogen in excreta is originated from uric acid, the faecal nitrogen should be corrected for uric acid nitrogen. In this regard, the excreta were calculated as total nitrogen minus nitrogen in the uric acid.

Serum samples were also analyzed for amylase (Moharrery and Das, 2001).

**Intestine morphometry:** Segments (1 cm) were removed from the duodenum, jejunum, and ileum as follows from: 1) the apex of the duodenum, 2) midway between the point of entry of the bile ducts and Meckel's diverticulum (jejunum), and 3) 10 cm proximal to the cecal junction (ileum). For morphometric analysis, segments were fixed in 10% neutral buffered formalin solution and routinely processed to 5-mm hematoxylin-eosin stained sections. Tissue slices were dehydrated manually and embedded in paraffin wax. Sections were cut from the waxed tissue on a Leitz 1512 microtome (Ernst Leitz Westlar GmbH, Austria) and were cleared of wrinkles by floating on warm water ( $45-50^{\circ}C$ ) prior to mounting on 10% poly-L-lysine coated slides.

The morphometric variables analyzed included: villus length (from the tip of the villi to the villus crypt junction), villus width, and villus surface area was calculated from villus height and width at half height. Values are means from 10 adjacent villi and only vertically oriented villi were measured.

**Statistical analysis:** The complete randomized model was used to analyze digestibility data. In this regard, five treatments offered to chicken in three replicates individually. The experimental design for performance investigation was a completely randomized one with a  $5 \times 2$  factorial arrangement of treatments. Each of five treatments was replicated three times per sex ( $n=3$ ). The

Table 1: Composition of experimental diets

Ingredients and analysis	Experiment diet				
	14 to 42d			42 to 56 d	
	Starter	Corn-based	Barley-based <sup>1</sup> (g/kg)	Corn-based	Barley-based <sup>1</sup>
Ground yellow corn	618	485	150	615	300
Ground barley			350		350
Soybean meal (44% CP)	280	330	310	190	180
Fish meal	49.5	27	30	20	18
Plant oil	19	19	50	25	50
Wheat bran		84	55	95	50
Dicalcium phosphate	12	28	28	28	28
Oyster shell	13	10	10	10	10
Sodium chloride	1	4.5	1.5	4.5	1.5
DL- methionine	0.5	2.5	2.5	2.5	2.5
Vitamin/mineral premix <sup>2</sup>	7	10	10	10	10
Analyses (calculated) <sup>3</sup>					
AME Kcal/kg	3000	2720	2719	2891	2891
Crude protein (%)	20.78	21.89	21.85	16.62	16.64
Methionine (%)	0.44	0.61	0.59	0.53	0.51
Methionine + Cysteine (%)	0.75	0.94	0.92	0.79	0.77
Lysine (%)	1.21	1.26	1.26	0.86	0.86

<sup>1</sup>In diets of (2) to (5) same level of barley were used but with different type of treatment or variety.

<sup>2</sup>The premix supplied the following (mg/kg diet): retinol 3.6 (about 1.1 IU.KJ<sup>-1</sup>), cholecalciferol .075 (about .26 IU.KJ<sup>-1</sup>), biotin 1, dl- $\alpha$ -tocopheryllacetate 10, riboflavin 10, pantothenate 20, choline 2000, niacin 100, thiamine 10, pyridoxine 10, menadin sodium bisulphate 1.5, cyanocobalamin .1, folic acid 2, ethoxyquin 150, Mn 100, Fe 100, Cu 10, Co 1, I 1, Zn 100. <sup>3</sup>Estimated from NRC (1994) composition tables.

data were analyzed using general linear model procedure of SAS (1988). Duncan's multiple range test (SAS, 1988) ( $P < 0.05$ ) was used to test the significance of difference between means. The percentage values was transformed to arcsine and analyzed, but the values, which are reported, converted to the initial form. Values are given as means, and the homogeneity of variance was checked.

### Results and Discussion

Growth Performance Growth performance and feed intake and feed conversion are shown in Table 2. No differences ( $P > 0.05$ ) in feed intake or feed conversion were observed through day 42 or 56. Weight gain was significantly reduced ( $P < 0.05$ ) by the use of treatment (2) at 42 and 56 d. The barley diets which is treated by rumen fluid or commercial enzyme showed same result with corn based diet and superior performance than barley without any treatment (2), indicating that the efficiency of dietary utilization was increased in chicks fed these regimen. The significant reduction in weight gain that occurred in broiler fed (2) regimens to compare (5) regimens suggested that the insoluble fibre may be impacted, because the  $\beta$ -glucan concentration in hullless barley is more than whatever we can see in

hulled barley.

At present there are two major types of hullless barley, normal and waxy. Improvements in the performance of poultry fed on diets containing barley to which enzymes had been added were first reported more than 45 years ago (Jensen *et al.*, 1957). In present study it was shown that adding enzyme to the diet (treatment no 4) increased the weight attained by broiler at 8 weeks of age by just over 6% ( $P < 0.05$ ). Corresponding improvements in feed conversion efficiency were showed in this treatment. Negative effect of hulled barley inclusion to the diets was not showed in this study. The reason for this aspect partly is related to including hulled barley to the diet after 2 weeks of age. During the first 2 weeks of age, the gastrointestinal tract especially the small intestine epithelium is not completely mature (cellularity and enzymology) for this reason the chicks could not observed any inconvenience material such as non-starch polysaccharides (NSP) in their diets (Henning, 1979; McNab and Smithard, 1992; Petersen *et al.*, 1976). The processed barley in treatment no (3) has been shown same results to compare treated barley by commercial enzyme for weight gain, feed intake and feed conversion. The improvement in nutritive value of processed barley partly is related to rumen

Table 2: Growth performance of broilers fed corn or barley-based diets from 14 to 42 or 56 days of age

	Treatment <sup>1</sup>					SEM	Sex		
	(1)	(2)	(3)	(4)	(5)		Male	Female	P*
Days 14 to 42									
Weight gain, g	1377	1346	1412	1429	1436	10.4	1510	1291	0.0001
Feed intake, g	2518	2598	2591	2665	2618	12.9	2724	2467	0.0001
Feed conversion	1.83	1.93	1.84	1.87	1.82	.012	1.80	1.91	0.0058
Days 14 to 56									
Weight gain, g	2277 <sup>ab</sup>	2185 <sup>b</sup>	2252 <sup>ab</sup>	2325 <sup>a</sup>	2372 <sup>a</sup>	42.5	2436	2128	0.0001
Feed intake, g	5094	4980	5052	5086	5183	71.4	5333	4825	0.0001
Feed conversion	2.25	2.29	2.25	2.19	2.19	.040	2.19	2.27	0.0291

<sup>1</sup>(1) = corn, (2) = hulled barley, (3) hulled barley treated by rumen fluid, (4) = hulled barley treated by enzyme, (5) = hulless barley.

<sup>ab</sup>Means in row with no common superscript differ significantly (P<0.05). SEM: standard error of the mean. \* Probability

microbial action and partly is related to soaking it in the fluid. Gohl (1977) has presented evidence to indicate that stimulation of endogenous β-glucanase and its subsequent action on β-glucan, as happens during malting (Bamforth, 1982), is one of the factors contributing to the increase in nutritive value of barley as result of water treatment. Beneficial alterations to the structure of the starch (Potter *et al.*, 1965) have also been proposed as contributory factors.

In this study hulless barley has shown interest result to compare other treatments, but the β-glucan content in this treatment (5) is higher than other diets (data is not showed). The result of this study suggested that the β-glucan is not unique factor for negative effects of barley. The combination of β-glucan along with insoluble fiber (hull) may acts as a synergic status for produce negative effects. Newman *et al.* (1985) reported that the alkaline viscosity of the unpearled barley was 2.27 cp, and of the pearled, 1.99 cp. By removing one of the factors such as β-glucan [adding enzyme (3) or (4)] or insoluble fiber (hulless barley) better performance will expect. This result is in contrast to Scott *et al.* (1998) who has reported that the hulless barley cultivars were significantly reduced the excreta dry matter (DM), feed intake, 17-d body weight (BW), and increased feed to gain ratio. They have mentioned that enzyme having a greater effect on the hulless variety. In agreement with results of present study Classen *et al.*, (2000) has been reported that hulless barley has a higher apparent metabolizable energy (AME) and protein content than hulled barley because of the diluting effect of the fibrous hull. They also mentioned that within hulless barley cultivars, low fiber content has been suggested to further enhance nutritional value for broiler chickens.

The feed conversion of female broilers will usually be higher (less efficient) than male birds of corresponding weight, after about 30 days of age. The reason for this finding is that female birds tend to deposit proportionally more fat in the carcass (Ryley, *et al.*, 1970).

**Protein metabolism:** Table 3 shows excreta and

digestibility data in this experiment. Chicks on the corn diet (1) produced the least amount of moisture in excreta (P<0.05). The enzyme treated barley (4) and hulless barley (5) produced similar percentage of excreta moisture and was slightly drier to compare untreated (2) and processed barley (3) (P>0.05). This finding could be partly explained by the capacity of NSPs to bind water (Langhout and Schutte, 1996). The increase in water digesta is thus the primary mechanism by which water-soluble NSPs exert anti-nutritive properties (Bedford and Classen, 1993). Cellulose, hemicellulose, pectin, lignin, and digestion resistant starch have modifying effects on water digesta in the non-ruminant intestinal track (Craig *et al.*, 1998). Inclusive of these fiber fractions are the soluble and insoluble β-glucans, which is found in higher percentage in barley diet to compare corn based diet.

The adjusted crude protein digestibility shows highest value for hulless barley (5) and lowest value for untreated barley (2) (P<0.05). Volatile fatty acids and polyamines produced by gut bacteria have stimulatory effects on the proliferation rate and secretory activity of intestinal mucosa (Furuse *et al.*, 1991; Osborne and Seidel, 1989; Sakata, 1987). Before correcting for the concentration of uric acid in the excreta of barley treatments (2, 3, 4, 5) no significant difference was observed between these treatments and corn-based diet (1), but after correction for the uric acid concentration significant difference was observed (P<0.05). The reason for this result partly explained by excretion of nitrogen through uric acid. In this regard, declining uric acid by enzyme supplementation could be related to more availability and digestibility of protein and therefore eliminated nitrogen excretion as a main material of uric acid production. Consequently reduction in uric acid excretion may reduce the environmental contamination. In future this could lead to the lower rate of crude protein in broiler ration which treated by enzyme supplementation. On the other hands, in barley-fed birds, with an increase in microbial fermentation there would be more loss of nitrogen, leading to a reduction of

Table 3: Mean values of excreta moisture and digestibility coefficients of diets

	Treatments <sup>1</sup>					SEM
	(1)	(2)	(3)	(4)	(5)	
Excreta moisture	53.85 <sup>b</sup>	75.30 <sup>a</sup>	75.85 <sup>a</sup>	72.59 <sup>a</sup>	71.82 <sup>a</sup>	0.356
Digestibility coefficients:						
Protein <sup>2</sup>	42.81 <sup>ab</sup>	38.46 <sup>b</sup>	53.16 <sup>a</sup>	46.45 <sup>ab</sup>	53.83 <sup>a</sup>	0.034
Corrected protein <sup>3</sup>	75.24 <sup>bc</sup>	74.75 <sup>c</sup>	79.60 <sup>ab</sup>	75.75 <sup>bc</sup>	81.73 <sup>a</sup>	0.013

<sup>1</sup>(1) = corn, (2) = hulled barley, (3) hulled barley treated by rumen fluid, (4)= hulled barley treated by enzyme, (5) = hullless barley.

<sup>a,b</sup>Means within a row lacking a common superscript differ (P<0.05). SEM: standard error of the mean. <sup>2</sup> (Nitrogen \* 6.25). <sup>3</sup>fecal nitrogen in the excreta was corrected as total nitrogen minus nitrogen in the uric acid.

apparent nitrogen digestibility, as was seen. This result agrees with the finding of Smits *et al.* (1997) who demonstrated a significant reduction of apparent nitrogen digestibility after feeding higher NSP to the birds. Angkanaporn and Coct. (1994) demonstrated that water-soluble pentosans in the diet significantly raised the endogenous losses in broiler birds.

**Serum amylase:** The data related to serum glucose and amylase activity is shown in Table 4. There was no significant difference between treatments in serum glucose. No differences among sex in serum glucose concentration were found (P>0.05). But, amylase activity showed significant difference among the treatments. Higher serum amylase activity has been shown for corn based diet, and lower serum amylase activity was belonged to enzyme treated barley (P<0.05). No significant difference (P>0.05) was observed between corn based diet (1) and processed barley (3) for serum amylase activity. The significant reduction in serum amylase activity, which is occurred in broiler fed untreated barley (2) diet to compare (2 and 5) regimens suggested that the NSPs may be the main reason, because the NSPs content in barley was degraded by action of processing with rumen fluid before feeding. In this regard, the reason for lower serum amylase activity in enzyme treated barley (4) is not clear. This possibility may be bring up, which the grace period that is passed digesta through the first part of duodenum (where the pancreas duct is opened to there), is not enough for degrading NSPs by exogenous enzyme. Therefore, in this part no difference can be observed between enzyme treated barley and untreated barley for NSPs concentration. This results is in agreement to Kapica and Puzio (2004) who has reported that phytase supplementation diet limited the high level of the secretion of stomach and pancreas enzymes in broiler chickens, which were fed diet rich in plant proteins containing phytate. On the other hands NSPs could reduce enzyme activity such as amylase and lipase by effecting on pancreas and small intestine which were emphasized by Almiral *et al.* (1995).

The avian pancreas consists of three lobes. The dorsal

and ventral lobes are supported and separated by the pancreatic artery within the duodenal loop, and the splenic lobe runs more laterally up to the spleen, as an extension of the ventral lobe. The pancreas has both endocrine and exocrine functions. While the amount of endocrine tissue is proportionally greater than that of mammals, (Hazelwood, 2000) over 99% of the pancreatic mass has an exocrine function (Fudge, 2000). The exocrine pancreas consists of compound tubuloacinar glands divided into lobules. These glands secrete amylase, lipase, proteolytic enzymes and sodium bicarbonate into the ascending duodenum via pancreatic ducts (Denbow, 2000). Pancreatic secretion, which is at a higher rate than that of mammals, is controlled by both neural and hormonal mechanisms. Immediately a bird starts eating, pancreatic secretion begins, apparently via a vagal reflex. Distension of the proventriculus stimulates a hormonal response involving vasoactive intestinal polypeptide that results in pancreatic secretion. Diet can also affect the rate of secretion, with diets high in fat and carbohydrates increasing the activity of amylase and lipase (Denbow, 2000).

Amylase is secreted in saliva, intestinal fluid and pancreatic juices (Denbow, 2000). In mammals and birds, pancreas-derived amylase makes up only a small part of serum amylase, but with acute pancreatitis and leakage of pancreas-derived amylase, total serum amylase levels rise significantly (Williams, 1996).

**Visceral organs:** The relative weight (g per 100 g carcass weight) of liver, gizzard and intestine were not influenced by the experimental diets (Table 5). Also, no significant difference between sex and no interaction was observed between broiler sex and diets. Generally, the organs associated with nutrient derivation may not respond to the nutrition due to the fact that such organs are preferentially developed in early life (Iji *et al.*, 2001; Tan *et al.*, 1999). But, Leeson and Zubair (1997) observed an increase in the weight of the liver in chickens that were fed a high protein diet. In some instances, feed restriction has been observed to lead to an increase in the weights of visceral organs (Zubair

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Table 4: Some blood serum parameters of chickens fed corn or barley based diets

	Treatments <sup>1</sup>						Sex		
	(1)	(2)	(3)	(4)	(5)	SEM	Male	Female	P*
Amylase activity <sup>2</sup>	168 <sup>a</sup>	102 <sup>b</sup>	153 <sup>a</sup>	99 <sup>b</sup>	138 <sup>ab</sup>	15.63	130	133	0.8067
Glucose (µg.ml <sup>-1</sup> )	242.6	270.1	274.6	255.5	286.5	14.4	265.3	266.5	0.9285

<sup>1</sup>(1) = corn, (2) = hulled barley, (3) hulled barley treated by rumen fluid, (4) = hulled barley treated by enzyme, (5) = hulless barley.

<sup>2</sup>Micogram glucose. min<sup>-1</sup>.ml<sup>-1</sup> of serum. SEM: standard error of the mean.\* Probability.

Table 5: The effect of different diets on the weight (g per100 g carcass weight) of visceral organs in chickens

	Treatments <sup>1</sup>						Sex		
	(1)	(2)	(3)	(4)	(5)	SEM	Male	Female	P*
Fresh carcass <sup>2</sup>	70.27	71.40	70.0	270.84	70.04	1.654	70.54	70.49	0.9701
Small intestine	4.75	4.78	4.72	4.69	4.78	0.053	4.72	4.77	0.3354
Gizzard	2.14	2.25	2.32	2.31	2.29	0.128	2.20	2.32	0.3172
Liver	3.14	3.22	3.07	3.01	3.22	0.119	3.08	3.18	0.3362

<sup>1</sup>(1) = corn, (2) = hulled barley, (3) hulled barley treated by rumen fluid, (4) = hulled barley treated by enzyme, (5) = hulless barley.

<sup>2</sup>carcass yields as a percentage of live weight. SEM: standard error of the mean. \* Probability.

Table 6: Effect of different diets on morphometry of the intestinal mucosa

	Treatments <sup>1</sup>					
	(1)	(2)	(3)	(4)	(5)	SEM
<b>Duodenum</b>						
Villus length (µm)	808 <sup>b</sup>	450 <sup>d</sup>	513 <sup>cd</sup>	592 <sup>c</sup>	954 <sup>a</sup>	28.2
Villus width (µm)	232 <sup>c</sup>	236 <sup>a</sup>	235 <sup>b</sup>	234 <sup>b</sup>	231 <sup>c</sup>	0.51
Villus surface area (mm <sup>2</sup> )	0.186 <sup>b</sup>	0.106 <sup>d</sup>	0.122 <sup>cd</sup>	0.138 <sup>c</sup>	0.219 <sup>a</sup>	0.007
<b>Jejunum</b>						
Villus length (µm)	570 <sup>a</sup>	429 <sup>c</sup>	453 <sup>c</sup>	511 <sup>b</sup>	564 <sup>a</sup>	16.2
Villus width (µm)	234 <sup>d</sup>	236 <sup>a</sup>	235 <sup>ab</sup>	235 <sup>bc</sup>	234 <sup>dc</sup>	0.26
Villus surface area (mm <sup>2</sup> )	0.133 <sup>a</sup>	0.101 <sup>c</sup>	0.106 <sup>c</sup>	0.122 <sup>b</sup>	0.133 <sup>a</sup>	0.004
<b>Ileum</b>						
Villus length (µm)	391 <sup>a</sup>	310 <sup>b</sup>	415 <sup>a</sup>	417 <sup>a</sup>	347 <sup>b</sup>	15.2
Villus width (µm)	137 <sup>b</sup>	139 <sup>a</sup>	136 <sup>b</sup>	136 <sup>b</sup>	138 <sup>a</sup>	0.45
Villus surface area (mm <sup>2</sup> )	0.053 <sup>ab</sup>	0.043 <sup>c</sup>	0.056 <sup>ab</sup>	0.057 <sup>a</sup>	0.050 <sup>b</sup>	0.002

<sup>1</sup> (1) = corn, (2) = hulled barley, (3) hulled barley treated by rumen fluid, (4) = hulled barley treated by enzyme, (5) = hulless barley. <sup>a</sup>

<sup>b</sup>Means within a row lacking a common superscript differ (P<0.05). SEM: standard error of the mean.

and Leeson, 1994), probably as a result of birds trying to increase their potential for digestion and absorption.

**Intestine morphometry:** Villus length, width, and surface were reduced (P< 0.05) in the birds fed the barley based diet compared with those fed the corn based diet (Table 6). The results of the current study showed that the type of diet (Table 6) affected villus depth and surface. The villus width showed negative correlation with villus length in three parts of the intestine (duodenum, jejunum and ileum). The addition of enzyme or processed barley by rumen fluid increased (P< 0.05) villus length and surface in broiler chickens fed the barley based diet, and this was similar to villus size in those fed the corn based diet in ileum section. The villus length and surface gradually decreased from duodenum to ileum section in all diets. However, the villus width

did not showed any difference between duodenum and jejunum sections, but in ileum the width of villus sharply reduced to compare first two sections.

The morphological results of this study, demonstrating that intestinal mucosa was modified in chickens receiving barley alone, but not in those receiving processed barley or barley plus enzymes. In this regard the hulless barley without any treatment showed superior results as same as corn based diets. In the barley-based diets, damage to the small intestinal mucosa may be caused indirectly by the viscous characteristics of NSPs (Stanogias and Pearce, 1985). Sakata (1987) demonstrated that an increase in bacterial activity in the gastrointestinal tract was associated with a change in the morphology of the gut wall. This reinforces the idea that exogenous enzymes might exert their beneficial action by influencing the

intestinal microflora (Bedford, 2000). The results of present study are in agreements to Mathlouthi *et al.* (2002) who has mentioned that addition of enzyme to rye based diets increased villus size and the villus height to crypt depth ratio. On the other hand, Yasar and Forbes (2000), reported that the decrease in digesta viscosity after exogenous enzyme addition is most likely associated with an improvement in small intestine wall morphology.

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