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Effect of Cereal Type and Enzyme Addition on Performance, Pancreatic Enzyme Activity, Intestinal Microflora and Gut Morphology of Broilers

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Abstract

The effects of grain and carbohydrase enzyme supplementation were investigated on digestive physiology of chickens. A total of 625 one-day-old chicks (Ross 308) were randomly assigned to five treatments in a completely randomized design. Treatments included two different types of grains (wheat, and barley) with or without a multi-carbohydrase supplement. A corn-based diet was also considered to serve as a control. Feeding barley-based diet with multi-carbohydrase led to higher feed intake ($P < 0.01$) than those fed corn- and wheat-based diets. Birds fed on barley and wheat diets had lower weight gain despite a higher feed conversion ratio ($P < 0.01$). Total count and number of different type of bacteria including Gram-negative, *E. coli*, and Clostridia increased after feeding wheat and barley but the number of Lactobacilli and Bifidobacteria decreased ($P < 0.01$). Feeding barley and wheat diets reduced villus height in different parts of the small intestine when compared to those fed on a corn diet. However, enzyme supplementation of barley and wheat diets improved weight gain and feed conversion ratio and resulted in reduced number of *E. coli* and Clostridia and increased number of Lactobacilli and Bifidobacteria, and also restored the negative effects on intestinal villi height ($P < 0.01$). The activities of pancreatic α-amylase and lipase were ($P < 0.01$) increased in chickens fed wheat and barley diets when compared to the control fed on a corn diet. Enzyme supplementation reduced the activities of pancreatic α-amylase and lipase ($P < 0.01$). In conclusion, various dietary non-starch polysaccharides without enzyme supplementation have an adverse effect on digesta viscosity, ileal microflora, villi morphology, and pancreatic enzyme activity.

Introduction

Corn is considered as the main ingredient of poultry diet. However, increased demand for ethanol fuel products has shifted corn grain from animal nutrition to biofuel in the major corn producing areas of the world. These two factors lead to the unprecedented rise of corn price for poultry nutrition (Donohue and Cunningham, 2009). Wheat and barley as

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alternative cereals could replace corn in poultry feed formulation and successfully grow in most areas with lower water requirement (Ravindran *et al*., 1999; Lin *et al*., 2010).

After corn, other major energy sources for poultry diets are wheat and barley, but these two cereals have considerable amount of non-starch polysaccharides (NSPs) (Olukosi *et al*., 2007; Mirzaie *et al*., 2012), which impede the normal intestinal functions due to anti-nutritional factors (Jamroz *et al*., 2002). The content and structure of NSP polymers vary between different grains, which consequently affect the nutritive value of grains (Olukosi *et al*., 2007). The major NSPs of wheat are arabinoxylan polymers, whereas NSPs in barley comprises of polymers of (1→3) (1→4)-β-glucans (Choct, 1997; Yin *et al*., 2000; Jamroz *et al*., 2002). It has been previously shown that combined multi-enzyme is required to degradation of NSPs in poultry diets for optimum digestion and performance (Ravindran *et al*., 1999; Olukosi *et al*., 2007; Slominski, 2011; Khajali and Slominski, 2012). Lower growth rate (Yin *et al*., 2000; Mirzaie *et al*., 2012), higher bacterial fermentative activity (Langhout *et al*., 1999; Olukosi *et al*., 2007), prevent the normal activity of intestinal enzymes (Zhao *et al*., 2007; Lin *et al*., 2010), and remodeling of gut enterocyte (Iji *et al*., 2001; Saki *et al*., 2011) are the main undesirable consequences due to NSPs.

Few researchers have shown the magnitude of the adverse effect of equal fractions of NSP from different sources (Ravindran *et al*., 1999; Yin *et al*., 2000; Lin *et al*., 2010).There is limited information on the efficacy of multi-carbohydrase supplements under such circumstances. Therefore, in the present study, equal NSP fractions of wheat and barley were balanced in experimental diets supplemented with exogenous multi-enzyme to compare the impacts on growth, ileal microbial population, gut morphology, and pancreatic enzyme activity of α-amylase and lipase compared to corn-based diet.

Materials and Methods

Animals, management, and treatments

A total of 625 one-day-old Ross-308 broiler chicks (initial weight 45±4 g) were randomly allocated to five treatments and five replicates with 25 chicks each. Each replicate was kept in a 1.5 × 2.2 m² floor pen located in the animal science research institute of Karaj, Iran. Three experimental diets based on corn (control), wheat and barley were formulated for the starting (1 to 3 wk of age) and growing (3 to 6 wk of age) stages according to NRC (1994) recommendations. Two additional diets were prepared by supplementing exogenous multi-carbohydrases to the wheat- (Wheat + Enzyme) and barley- (Barley + Enzyme) based diets. The enzyme supplement (COMBO® enzyme blend produced by American Biosystem Co) contained 480 U/g multi-glycanase (including 200 U/g xylanase, 200 U/g β-glucanase, and 80 U/g hemicellulase) and 1000 U/g phytase and was used at a level of 1 g/kg in the experimental diets. Wheat and barley samples were analyzed before the experiment for NSP constituents, depicted in Table 1 (AOAC, 2005).

Table 1. Chemical analysis of wheat and barley grain (%)

Sample	Cellulose[1]	Hemi-Cellulose[2]	Lignin[3] (ADL)	Total NSP	Soluble NSP	Insoluble NSP	DF[4]
Wheat	1.8	10.4	1.59	13.11	2.45	10.66	14.7
(±*SE*)	(±0.1)	(±0.52)	(±0.18)	(±0.64)	(±0.12)	(±0.53)	(±0.73)
Barley	4.4	23.62	1.97	16.73	4.12	12.61	18.7
(±*SE*)	(±0.24)	(±1.42)	(±0.24)	(±0.81)	(±0.15)	(±0.65)	(±0.94)

[1]Cellulose=ADF-ADL; [2]Hemi-Cellulose=NDF-ADF; [3]Lignin (ADL)=ADF-Cellulose; [4]Dietary Fiber=Total NSP +ADL.

The composition of the experimental diets during the starting and growing stages is shown in Table 2. All diets were formulated to have the same contents of metabolizable energy, crude protein, and dietary electrolyte balance (DEB). Wheat- and barley-based diets were formulated to have equal fractions of soluble NSP.

Feed and water were provided with free access. Temperature and lighting schedule followed the management guideline of the Ross-308 strain. The experimental animals were kept, maintained, and treated in accepted standards and protocols were approved by the Institutional Animal Care and Use Committee of Shahrekord University.

Table 2. Ingredients and chemical composition of diets during the starting and growing stages

Ingredients(%)/Treatments	Starter (1- 21 days)					Grower (22- 42 days)				
	Control	Wheat	Barley	Wheat + Enzyme	Barley + Enzyme	Control	Wheat	Barley	Wheat + Enzyme	Barley + Enzyme
Corn	56.00	44.55	45.00	44.55	45.00	58.00	40.00	42.41	40.00	42.41
Soybean meal 44%	36.80	35.10	33.90	35.10	33.90	32.00	30.50	29.60	30.50	29.60
Soy oil	2.00	1.35	2.00	1.35	2.00	2.90	2.85	3.47	2.85	3.47
Wheat	-	15.00	-	15.00	-	-	20.00	-	20.00	-
Barley	-	-	15.00	-	15.00	-	-	20.00	-	20.00
DCP	1.83	1.78	1.78	1.78	1.78	1.81	1.74	1.71	1.74	1.71
Calcium Carbonate	1.12	1.14	1.00	1.14	1.00	1.13	1.14	1.13	1.14	1.13
Sodium Chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Potassium Carbonate	0.10	0.13	0.13	0.13	0.13	0.12	0.12	0.11	0.12	0.11
DL-Methionine	0.17	0.15	0.15	0.15	0.15	0.25	0.25	0.05	0.25	0.05
L-Lysine HCL	0.10	-	0.10	-	0.10	0.15	0.10	0.05	0.10	0.05
Vitamin Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Inert‡	1.08	-	0.14	-	0.14	2.84	2.50	0.67	2.50	0.67
Chemical Composition										
ME (Kcal/kg)	2900	2900	2900	2900	2900	2950	2950	2950	2950	2950
Protein (%)	21.00	21.00	21.00	21.00	21.00	19.00	19.00	19.00	19.00	19.00
Methionine + Cystine (%)	0.86	0.84	0.82	0.84	0.82	0.85	0.85	0.84	0.85	0.84
Lysine (%)	1.20	1.19	1.18	1.19	1.18	1.20	1.19	1.11	1.19	1.11
Calcium (%)	0.95	0.94	0.92	0.94	0.92	0.95	0.95	0.87	0.95	0.87
Available Phosphorus (%)	0.43	0.43	0.45	0.43	0.45	0.45	0.42	0.43	0.42	0.43
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Chloride (%)	0.22	0.23	0.24	0.23	0.24	0.22	0.23	0.23	0.23	0.23
Potassium (%)	0.95	0.95	0.96	0.95	0.96	0.87	0.87	0.88	0.87	0.88
(Na+K)-Cl (meq/kg)	247.85	247.90	247.81	247.90	247.81	231.23	231.56	231.54	231.56	231.54
Total NSP Analyzed (%)	12.43	12.89	12.96	12.89	12.96	11.62	12.11	12.65	12.11	12.65
Soluble NSP Analyzed (%)	1.25	1.37	1.58	1.37	1.58	1.10	1.45	1.60	1.42	1.60

*Each kg of premix contained: 44000 IU A, 7200 IU D_3, 440 mg E, 40 mg K_3,70 mg B_{12}, 65 mg B_1, 320 mg B_2, 290 mg Pantothenic acid, 1220 mg Niacin, 65 mg B_6, 22 mg Biotin, 270 mg Choline Chloride.

**Each kg of premix contained: 950 mg Mn, 450 mg Zn, 320 mg Fe, 100 mg Cu, 65 mg Se, 68 mg I and 45 mg Co.

‡Inert contained sand plus enzyme and enzyme supplementation included 1 kg per 1000 kg of diet for all treatments and contained 1000 active units of Phytase and 480 active units of Multi-glycanase units per gram.

Measurements

Feed intake, weight gain, and feed to gain ratio were calculated for the 1-21 day, 22-42 day, and 1-42 day periods. At the end of the trial (42 days of age), three birds per pen (15 chickens from each treatment) were randomly selected and slaughtered. Intestinal microbiota was enumerated according to protocols from Langhout *et al*. (1999) with some modifications. In brief, aqueous digesta samples (3 mL) were taken from the distal segment of ileum and immediately transferred to sterile bottles containing 15 mL of anaerobic transport medium (TRM; pH=7.0), then weighed and stored at 4°C for further examination. Samples were homogenized and 1 mL of each sample was serially diluted 10- fold. An aliquot (0.1 mL) of each diluted sample was then cultivated on specific media and transferred to an incubator set at 37°C for 24 hrs. At the end of incubation period, bacterial colonies were counted. Specific media (described below) were used to culture different types of bacteria including Nutrient Agar (NA) for total bacterial count, Eosin Methylene Blue (EMB) Agar for Gram-negative bacteria, MacConkey Agar (MCA) for coliforms, Rogosa Agar (RA) for lactic acids, Eugon Agar (EA) for bifidobacteria, and Reinforced Clostridial Agar (RCA) for clostridium bacteria.

Small intestinal morphology including villus height and width, crypt depth, and villus height to crypt depth ratio were determined in the duodenum, jejunum, and ileum. Segments of 2-3 cm from the midpoint of the duodenum (duodenum), the midpoint between the bile duct entry and Meckel's diverticulum (jejunum), and the distal end of the ileum (ileum) were dissected. The segments were flushed with ice-cold phosphate buffered saline (PBS, pH=7.2) and fixed in 10% neutral buffered formalin solution for further histological study. Formalin-fixed tissues were dehydrated, cleared, and impregnated in paraffin wax and cut into 6-µm sections with an LEICA RM 2145 microtome. These sections were mounted on 10% Poly-L-Lysine coated slides (Langhout *et al*., 1999) and stained with hematoxylin and eosin. Histological indices were determined using a computer-aided light microscopic image analyzer (Sigma Scan, San Rafael, CA. USA) according to the method

reported by Saki *et al*. (2011). The mean of 10 measures per section was used for the analysis.

Digesta samples were obtained from selected birds to measure intestinal pH and viscosity. Ileal digesta were individually collected, homogenized at 4˚C, and immediately measured for pH and viscosity (Brookfield viscometer, Model DV-II, MA, USA) according to Langhout *et al*. (1999). Pancreas was harvested to measure the activities of α-amylase and lipase enzymes. Sample sections (3 cm in length) were taken from the middle of the pancreas, rinsed with 0.01 *M* PBS (pH=7.2), and stored in liquid nitrogen at -80˚C until analysis. The pancreatic samples were homogenized in ice-cold 0.2 mol/L Tris - HCl buffer and 0.05 mol/L NaCl as described by Li *et al*. (2004). The homogenates were centrifuged at 3000 × *g* for 15 min at 4˚C and the supernatants were used for enzyme assay. The activity of α-amylase (EC 3.2.1.1) was determined using a validated kit from Parsazmun Chemical Company (Parsazmun Co., Karaj, Iran TS.M.91.4.5). The activity of lipase (EC 3.1.1.3) was measured by a validated kit from ZiestChem Chemical Company (ZiestChem Co., Tehran, Iran Ver. Lipase. 10558). Total protein (g/dL) of samples was performed using a validated kit from Parsazmun Chemical Company (Parsazmun Co., Karaj, Iran TS.M.91.45.4) according to the manufacturer's instruction. The activities of amylase and lipase are expressed as unit per milligram of pancreatic protein content.

Statistical Analyses

The results were statistically analyzed by GLM procedure of SAS software (SAS Institute Inc., 2003). The statistical scheme was based on completely randomized design (CRD). Samples within pens (3 per each unit) were subjected to analysis. The statistical model used for growth data was $Y_{ij}= \mu+ T_i + e_{ij}$ and for sampling observation within pens was $Y_{ijk} = \mu+ T_i + e_{ij} + Se_{ijk}$ where Y_{ij} and Y_{ijk} are observations; μ is the overall mean; T_i is the effect of treatments (different diets); e_{ij} is random error, and e_{ijk} is the effect of sampling error. Duncan's multiple range tests were used to separate the means.

Results

Growth performance

Table 3 depicts the results of feed intake, bodyweight, and feed conversion ratio of chickens during 1 to 21 and 22 to 42 days of the trial.

Table 3. Effect of different types of cereal grains and enzyme supplementation on broiler growth performance

Dietary treatments	Feed intake (g/bird/day)	Weight gain (g/bird/day)	FCR (g/g)
During 1-21 days			
Corn (Control)	45.90[a]	37.10[a]	1.24 [ab]
Wheat	41.50[b]	32.80[b]	1.27 [a]
Wheat + Enzyme	45.20[a]	38.50[a]	1.17 [b]
Barley	43.20[b]	33.70[b]	1.28 [a]
Barley + Enzyme	45.10[a]	38.20[a]	1.18 [b]
SEM	1.11	1.03	0.03
P-value	0.001	0.001	0.001
During 22-42 days			
Corn (Control)	147.40 [a]	67.90[a]	2.17 [ab]
Wheat	133.70[c]	61.10[c]	2.19 [ab]
Wheat + Enzyme	138.90[b]	63.20[bc]	2.20 [a]
Barley	142.40[ab]	64.50[b]	2.21 [a]
Barley + Enzyme	144.10[a]	67.40[a]	2.14 [b]
SEM	3.23	1.05	0.03
P-value	0.007	0.002	0.001
During 1-42 days			
Corn (Control)	92.70[a]	52.50[a]	1.77[b]
Wheat	87.60[c]	46.90[b]	1.86[ab]
Wheat + Enzyme	92.10[b]	50.40[ab]	1.83[ab]
Barley	93.80[ab]	49.10[ab]	1.91[a]
Barley + Enzyme	95.10[a]	51.30[a]	1.85[ab]
SEM	2.19	1.25	0.08
P-value	0.006	0.001	0.001

[a-c] Means with different superscript letters within columns have a significant difference ($P < 0.01$).

Birds fed wheat significantly reduced feed intake compared to other dietary treatments, but supplementation with multiple carbohydrases to this diet remarkably restored feed intake so that the difference was insignificant with the control group fed on corn. On the other hand, birds fed wheat or barley had lower feed intake than other treatments but multiple carbohydrase supplement significantly increased feed intake compared to the corn diet. Nevertheless, birds fed barley and wheat diets had a significantly lower weight gain but a higher FCR than chickens on corn diets. Enzyme supplementation of barley and wheat diets improved FCR ($P < 0.01$).

Intestinal microbial and gut morphology

Total bacterial population and Gram-negative count including *E. coli* and clostridia in the intestinal content were higher in birds fed wheat and barley than the control ($P < 0.01$) (Table 4).

Table 4. Effect of different types of cereal grains and enzyme supplementation on ileal bacterial population in broiler chickens (log CFU/g digesta)

Dietary treatments	Total bacterial count	Total gram negative	*E. Coli*	Clostridia	Lactic acid bacteria	Bifidobacteria
Corn (Control)	6.67^{b}	5.31^{b}	5.07^{b}	4.86^{b}	4.91^{b}	5.40^{a}
Wheat	7.13^{a}	6.33^{a}	6.32^{a}	5.69^{a}	3.87^{c}	4.06^{b}
Wheat + Enzyme	5.33^{c}	5.21^{b}	5.21^{b}	4.83^{b}	5.20^{a}	5.67^{a}
Barley	7.17^{a}	6.24^{a}	6.13^{a}	5.86^{a}	3.93^{c}	3.51^{c}
Barley + Enzyme	5.75^{c}	5.26^{b}	4.56^{c}	4.78^{b}	5.49^{a}	5.76^{a}
SEM	0.17	0.13	0.12	0.17	0.14	0.13
P-value	<0.001	0.001	<0.001	0.001	<0.001	<0.001

$^{a\text{-}c}$Means with different superscript letters within columns have a significant difference ($P < 0.01$).

On the other hand, the number of lactic acid bacteria and bifidobacteria were significantly lower in birds fed wheat and barley diets compared to the control. The inclusion of the multiple carbohydrases to the wheat and barley diets caused changes in gut microflora so that the number of lactic acid bacteria and bifidobacteria were significantly increased. Meanwhile, no significant differences were found between the enzyme-supplemented wheat and barley diets as well as the corn diet.

Intestinal morphometric indices including villus height and width, as well as crypt depth of the duodenum, jejunum, and ileum were determined (Table 5).

Villus height of the three intestinal segments was lower but villus width and crypt depth were higher in birds fed wheat and barley than the control or enzyme supplemented diets ($P < 0.01$). On the other hand, the villus height: width and villus height: crypt depth ratios were significantly lower ($P < 0.01$) in birds that received wheat and barley diets compared to the control or enzyme supplemented diets. Inclusion of the multiple carbohydrases to the wheat and barley diets restored the situation so that no significant difference was found between the wheat and barley diets supplemented with the multi-enzyme and the corn diet.

Digesta pH, digesta viscosity and pancreatic enzyme activity

Digesta viscosity of chicks fed wheat and barley were significantly ($P < 0.01$) higher than a control group fed on corn (Table 6). Inversely, digesta pH of chicks fed wheat and barley were significantly lower than the control group ($P < 0.01$). Pancreatic α-amylase and lipase activity of chicks fed wheat and barley were significantly ($P < 0.01$) increased compared to the control group fed on corn. Supplementation of carbohydrase enzyme mixture to the wheat and barley diets significantly reduced the digesta viscosity and activities of α-amylase and lipase (Table 6).

Discussion

Negative effects of soluble NSP of wheat and barley have been well demonstrated by other researchers (Yin *et al*., 2000; Olukosi *et al*., 2007; Mirzaie *et al*., 2012). However, the comparative effects of NSP type on broiler performance and physiology have not been adequately addressed. Results reported herein indicate that arabinoxylan polymers of wheat NSP have a more deleterious impact on voluntary feed intake of broiler chickens than (1→3)(1→4)-β- glucans of barley NSP. Birds fed wheat diets consumed lower feed than those fed barley, corn or enzyme supplemented diets throughout the trial.

Consequently, birds fed wheat diet had lower body weight gain compared to those fed barley, corn or enzyme supplemented diets, primarily due to the differences in NSP constituent, the size of molecules, and the degree of digestion which can affect digesta viscosity and passage rate of gut content (Choct, 1997; Choct *et al*., 2006). The growth performance data are consistent with the digesta pH, digesta viscosity and pancreatic enzyme activity as depicted in Table 6. These observations indicated that every change in the gut environment due to different dietary NSP sources could affect the physicochemical properties of the intestinal and consequently performance and/or physiology of birds.

Table 5. Effect of different types of cereal grains and enzyme supplementation on gut morphology at three parts of intestine in chickens

Dietary treatments	Duodenum				
	VH[1] (µm)	VW[2] (µm)	CD[3] (µm)	H/W ratio[4]	H/CD ratio[5]
Corn (Control)	1530.33^{ab}	115.00^{c}	108.33^{c}	13.31^{a}	14.39^{a}
Wheat	1360.00^{c}	120.00^{b}	114.67^{b}	11.33^{b}	11.86^{b}
Wheat + Enzyme	1668.00^{a}	123.67^{ab}	108.00^{c}	13.49^{a}	15.44^{a}
Barley	1440.04^{b}	117.00^{c}	119.00^{a}	12.31^{ab}	12.10^{b}
Barley + Enzyme	1589.82^{ab}	125.33^{a}	109.00^{c}	12.68^{ab}	14.59^{a}
SEM	63.80	2.18	2.39	0.54	0.52
P-value	0.001	0.003	0.001	<0.001	<0.001
	Jejunum				
Corn (Control)	1273.67^{a}	138.67^{b}	109.67^{b}	9.18^{a}	11.61^{ab}
Wheat	1190.00^{ab}	150.00^{a}	116.00^{a}	7.93^{b}	10.26^{ab}
Wheat + Enzyme	1297.00^{a}	136.00^{b}	108.00^{b}	9.54^{a}	12.01^{a}
Barley	1025.23^{b}	125.00^{c}	110.33^{b}	8.20^{ab}	9.29^{b}
Barley + Enzyme	1191.33^{ab}	135.00^{b}	95.00^{c}	8.83^{ab}	12.54^{a}
SEM	69.62	3.14	4.72	0.61	0.97
P-value	0.001	0.001	0.005	<0.001	<0.001
	Ileum				
Corn (Control)	1285.33^{ab}	87.00^{d}	105.00^{b}	14.94^{a}	12.50^{a}
Wheat	1140.67^{b}	128.33^{b}	115.00^{a}	8.68^{c}	9.88^{b}
Wheat + Enzyme	1408.00^{a}	102.67^{c}	109.67^{ab}	13.33^{ab}	12.61^{a}
Barley	1172.00^{b}	142.33^{a}	114.33^{a}	8.23^{c}	10.25^{b}
Barley + Enzyme	1308.00^{a}	107.00^{c}	108.00^{ab}	12.22^{b}	12.12^{a}
SEM	67.67	3.88	3.14	0.71	0.69
P-value	0.001	0.001	0.002	<0.001	<0.001

$^{a-c}$Means with different superscript letters within columns have a significant difference ($P < 0.01$).
[1] Villus Height, [2] Villus Width, [3] Crypt Depth, [4] Villus Height to Villus Width ratio, [5] Villus Height to Crypt Depth ratio.

Table 6. Effects of different types of cereal grains and enzyme supplementation on digesta pH, digesta viscosity, and pancreatic enzyme activity in chickens

Dietary treatments	Digesta Viscosity (CP[1])	Digesta pH	pancreatic enzyme activity (U/mg CP) [2]	
			α-amylase	Lipase
Corn (Control)	1.59^{c}	6.89^{a}	0.71^{c}	0.24^{b}
Wheat	2.17^{a}	5.93^{b}	1.37^{a}	0.42^{a}
Wheat + Enzyme	1.60^{b}	6.39^{a}	0.88^{b}	0.28^{b}
Barley	1.95^{a}	5.67^{b}	1.43^{a}	0.42^{a}
Barley + Enzyme	1.60^{b}	6.41^{a}	0.91^{b}	0.26^{b}
SEM	0.04	0.16	0.06	0.03
P-value	0.005	0.001	0.001	0.005

$^{a-c}$Means with different superscript letters within columns have a significant difference ($P < 0.01$).
[1] centipoise; [2] Units of enzymes per one milligram of pancreatic crude protein.

Birds fed wheat had the greatest viscosity of intestinal digesta, which was significantly ($P < 0.01$) greater than the control group fed corn. Barley also increased digesta viscosity in the intestine. Increased viscosity creates an ideal environment for maximal proliferation of

anaerobic and Gram-negative bacteria as observed in this study. These conditions lead to increased production of volatile fatty acids which could decrease digesta pH due to production of short chain fatty acids in the lumen. These observations are in line with Jaroni *et al*. (1999) and Langhout *et al*. (1999). Stagnant intestinal digesta and low oxygen conditions due to non-degradable NSP provide a quiet environment for fermentative anaerobic bacteria proliferation (Langhout *et al*., 1999). Reduction in nutrient availability and production of detrimental by-products can result in microbial changes in the gut (Choct *et al*., 2006). The water soluble fraction of wheat and barley NSPs has a deleterious impact on intestinal physicochemical properties and microbial proliferation of chickens (Choct, 1997; Choct *et al*., 2006). Results of this experiment also indicate that NSP polymers of wheat and barley decreased the population of lactic acid bacteria and Bifidobacteria in the intestinal digesta. These bacteria are associated with beneficial effects on birds and are known as probiotic growth promotants. The impaired live performance of birds fed on wheat and barley can partly be explained by a decrease in population of Gram-positive bacteria including Lactobacilli and Bifidobacteria. The probiotic-type bacteria modulate innate immune system of the host animal (Christensen *et al*., 2002) and they are necessary for the development of gut-associated lymphoid tissue (GLUT) (Rhee *et al*., 2005).

The negative effects of NSP on the proliferation of bacteria in the intestine were significantly ameliorated after supplementation of wheat and barley diets with exogenous multiple enzymes (especially on probiotic-type bacteria). These results are in agreement with previous reports (Yin *et al*., 2000; Choct *et al*., 2006; Mirzaie *et al*., 2012). Degradation of NSP of wheat and barley by carbohydrases has been successful and promising in broilers (Olukosi *et al*., 2007; Slominski, 2011). Glycanase enzymes including xylanases and β- glucanases release the encapsulated nutrients and reduce digesta viscosity. These processes are further facilitated by the action of phytases (Ravindran *et al*., 1999; Olukosi *et al*., 2007). As birds do not possess endogenous glycanases to degrade NSP, the application of exogenous NSP-degrading enzymes seems to be necessary when wheat and barley replace corn.

Reduced villus height, and in contrast, increased villus width and crypt depth can result from an increase in digesta viscosity. This leads to quick changes in the intestinal mucosa due to the proximity of the mucosal surface to the intestinal viscose content (Saki *et al*., 2011). The crypt can act as villus factory and a large crypt indicates a fast tissue turnover and a high demand for new tissue. Therefore, the addition of a viscose matter (such as NSP) to the diet can produce deeper crypts with a high rate of cell proliferation and tissue renewal (Iji *et al*., 2001). Therefore the shorter villus height induced by wheat and barley diets is related to NSP viscosity and is associated with a reduction in absorptive potential through the intestine, further growth efficiency, and normal physiological conditions (Saki *et al*., 2011).

Pancreas α-amylase and lipase activities of broiler chickens were significantly increased in birds fed wheat and barley diets compared to those fed a corn diet or a wheat and barley diet supplemented with enzymes. This finding reflects the fact that water-soluble NSP of wheat and barley impede pancreatic α-amylase and lipase activities (Li *et al*., 2004). This finding may indicate needs for greater secretion of pancreatic enzymes (Williams, 1996; Denbow, 2000). Intestinal enzyme activity depends on the source of dietary nutrient, quantity and/or quality of anti-nutrients in the gut (Li *et al*., 2004; Mirzaie *et al*., 2012). Diet type also affects the rate of secretion from the pancreas. Diets with high in fat or carbohydrates increase the secretion rate and serum concentration of amylase and lipase (Brenes *et al*., 1993; Zhao *et al*., 2007; Lin *et al*., 2010). Amylase is secreted in saliva, intestinal fluid, and pancreatic juices while lipase is secreted in stomach and pancreatic juices (Denbow, 2000). In normal conditions, pancreas-derived amylase and lipase contribute a small portion of serum enzymes, but with abnormal conditions such as a change in diet cereal type and anti-nutritional factors, acute pancreatitis and leakage of enzymes can occur, increasing the total serum concentration of enzymes (Williams, 1996).

Conclusion

Solubility of different sources of NSP and their impact on digesta viscosity play an important role in growth efficiency and physiology of broilers. Various NSP components of wheat and

barley have adverse effects on digesta viscosity, villi morphology and bacterial population of the gut and subsequent transmission of hydrolyzed products to the enterocyte cells and nutrient absorption. NSP polymers of wheat and barley increase the pancreatic activities of α-amylase and lipase. Such changes are remarkably restored by supplementing NSP-degrading enzymes to broiler diets.

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تاثیر نوع غله و افزودن آنزیم بر عملکرد، فعالیت آنزیمی لوزالمعده، جمعیت میکروبی و ریخت‌شناسی روده در جوجه‌های گوشتی

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چکیده

تاثیر نوع غله و مکمل کردن آنزیم کربوهیدراز بر فیزیولوژی هضم در جوجه‌های گوشتی مورد مطالعه قرار گرفت. تعداد ۶۲۵ قطعه جوجه یک‌روزه از سویه راس ۳۰۸ به یک طرح کاملاً تصادفی با ۵ تیمار اختصاص داده شدند. تیمارها شامل ۲ نوع مختلف دانه غله (گندم و جو) با یا بدون مکمل آنزیمی از نوع مولتی‌کربوهیدراز بودند. علاوه بر تیمارهای قبلی یک جیره شاهد بر پایه ذرت–سویا نیز به عنوان گروه شاهد در نظر گرفته شد. تغذیه جوجه‌ها با جیره دارای جو به همراه آنزیم در مقایسه با جیره شاهد یا جیره دارای گندم باعث افزایش مصرف خوراک شد ($P < ۰/۰۱$). پرندگان تغذیه شده با جیره‌های دارای گندم و جو از افزایش وزن کمتر و ضریب تبدیل غذایی بالاتری نسبت به بقیه جیره‌ها برخوردار بودند ($P < ۰/۰۱$). شمارش کلی باکتری‌ها و جمعیت گونه‌های مختلف باکتری شامل گرم منفی، اشرشیا کولای و کلستریدیا با تغذیه از جیره‌های گندم و جو افزایش، ولی جمعیت باکتری‌های گونه لاکتوباسیل و بیفیدوباکتریا کاهش یافت ($P < ۰/۰۱$). جیره‌های گندم و جو در مقایسه با جیره شاهد باعث کاهش ارتفاع پرز در قسمت‌های مختلف روده کوچک شدند ($P < ۰/۰۱$). هرچند، مکمل سازی این جیره‌ها با آنزیم باعث بهبود رشد، کاهش ضریب تبدیل غذایی و کاهش جمعیت اشرشیا کولای و کلستریدیا و در عوض افزایش جمعیت لاکتوباسیل و بیفیدوباکتریا، و نیز باعث افزایش اندازه پرزهای روده کوچک شد ($P < ۰/۰۱$). فعالیت آنزیمی آلفا–آمیلاز و لیپاز لوزالمعده بدنبال تغذیه با جیره‌های گندم و جو در مقایسه با جیره شاهد افزایش یافت ($P < ۰/۰۱$). مکمل سازی جیره‌های گندم و جو با آنزیم مولتی‌کربوهیدراز باعث کاهش فعالیت آنزیمی لوزالمعده شد ($P < ۰/۰۱$). در کل، جیره‌های دارای منابع مختلف کربوهیدرات‌های غیرنشاسته‌ای بدون مکمل سازی آنزیمی، باعث ایجاد بروز نتایج منفی بر گران‌روی محتویات هضمی، جمعیت میکروبی و ریخت-شناسی پرزهای روده و فعالیت آنزیمی لوزالعمده می‌شوند.

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