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Original Article

Broiler Chicken Growth Performance, Ileal Microbial Population and Serum Enzyme Activity Affected By Dietary Source of NonStarch Polysaccharides As Supplemented With or Without Enzymes

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ABSTRACT

An experiment was conducted to evaluate the effect of different dietary source of Non-starch polysaccharides with or without enzymes on growth performance, ileal microbial population, and serum enzyme activity of broiler chickens. A total number of 625 unsexed broiler chicken (Ross-308) were randomly assigned to 5 treatments with 5 replicates and 25 birds per each unit, using a CRD statistical design. Treatments were included control, wheat (W), wheat+ enzyme (WE), barley (B), and barley+ enzyme (BE). Feed intake and body weight gain were significantly increased, as well as feed conversion ratio decreased by diets supplemented with enzymes rather diets without enzymes ($P<0.05$). The inclusion of W and B in diets led to significantly increased the total intestinal bacteria or gram negative and coliform bacteria as well as decreased the\number of lactic acid bacteria at 42 days of age ($P<0.05$). Serum enzyme activity of amylase and lipase were significantly increased after feeding chickens by diets contained W and B rather control or WE and BE at 42 days of age ($P<0.05$). The results of present study have shown that supplementation of W and B with enzymes completely restored the situation and neutralized the negative effects of W and B on growth performance, intestinal microbial population and serum enzyme activity of broiler chickens.

Keywords: broiler, enzyme activity, growth, micro biota, NSP.

INTRODUCTION

Broiler feeding with wheat and barley are common practice in many countries because they are good energetic cereals which grow on site and reduce feed costs (Ahmadi *et al*., 2013). The major corn-producing countries such as the United State and Brazil have been recently shifted using of corn to ethanol fuel producing. This diversion along with increased world's demand for this cereal has resulted in rise of corn price and consequently feed cost for

poultry industry (Donohue and Cunningham, 2009). Wheat and barley as alternative cereals can be easily replaced for corn in poultry diets. These cereals can locally grow in most parts of the world as well as they have remarkably lower water requirement than corn (Yin *et al*., 2000; Lin *et al*., 2010).

Wheat and barley as major sources of energy in poultry diets have considerable amount of non-starch polysaccharides (NSPs), which generally considered as anti-nutritional factors (Mirzaie *et al*., 2012; Ahmadi *et al*., 2013). The major component of NSPs is water soluble NSPs (arabinoxylan in wheat and β- glucans in barley), as which reduces digestion efficiency and production proficiency. (Choct, 1997; Moharrery *et al*., 2005). The NSPs content in these cereal grains may have adverse effects on the utilization of nutrients which thus limits their inclusion level in poultry rations. Increase feeding levels of NSPs in diets directly influences on growth, gut microbial characteristics as affected other physiological characteristics such as enzyme activities (Olukosi *et al*., 2007; Mirzaie *et al*., 2012). Reports indicate that a complex blend of NSPs degrading enzymes requires obtaining satisfactory gain and other performances (Ravindran *et al*., 1999; Slominski, 2011). In the present study, equal amount of wheat and barley and nearly similar fractions of NSP from those were included in broiler diets with and without multi-enzyme to compare the effects on growth performance, ileal bacterial population and serum enzyme activity.

MATERIAL AND METHODS

Experimental Design and Birds

A total of 625 unsexed broiler chicken (Ross-308) were randomly divided to 5 treatments with 5 replicates of 25 birds in each. Treatments were included of control (corn-soy basal diet), and the inclusion of wheat (W), wheat+ enzyme (WE), barley (B), and barley+ enzyme (BE) at levels of 15 and 20 percent in starter and grower periods respectively. Diets were designed as starter (1 to 21 days of age) and grower (22 to 42 days of age) based on NRC (1994) recommendations to meet their nutrient requirements (Table 1). Combo® multi-enzyme was used contained 1000 unit phytase and 180 unit multi-glycanase activities. Feed and water offered ad libitum in all period of experiment. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality were measured. The lighting schedule was 23 h light / 1 h darkness at 32^{o}C the first day. This was subsequently reduced 3oC each week until third week. Thereafter it was constant.

Microbial Sampling and Incubation

On day 42 of the experiment, two birds from each replicate were slaughtered by cervical dislocation and ileum contents were collected. Contents were gently removed into sterile sampling tubes and immediately transferred on ice to the laboratory. Serial dilutions of 1 g sample (10-4 to 10-7) were made. Selective media of Nutrient Agar, MacConkey Agar, Eosin methylene Blue Agar, Rogosa Agar, and Reinforced Clostridial Agar were included to detect the total number of bacteria, coliforms, gram-negative, lactic acid bacteria, and clostridium, respectively. Total number of bacteria, coliforms and lactic acid bacteria were counted after aerobic incubation for 24 h at 37^{o}C. Gram-negative bacteria were counted after incubation for 48 h at 37^{o}C and clostridium were counted after anaerobic incubation for 24 h at 37^{o}C.

Serum Enzyme Activity

At 42 days of age, two birds from each replicate were randomly selected and blood samples were taken via wing vein. Blood samples were transferred to vial tubes containing sodium heparin. The tubes were centrifuged at 5,000 × g for 20 min, and the supernatant was discarded. Serum amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) activity were determined by use of specific kits (Biosystem Company, Spanish).

Table 1. Diet composition at different periods of the experiment

Diets	experimental diets during 1-21days					experimental diets during 22-42days				
Ingredients(%)/Treatment	control	wheat	Barley	Wheat + enzyme	Barley + enzyme	control	wheat	Barley	Wheat + enzyme	Barley + enzyme
Corn grain	56	44.6	45	44.6	45	58	40	42	40	42
Soybean meal (45% CP/kg)	36.8	35.05	34	35.05	34	32	30.5	29.3	30.5	29.3
Soybean oil	2	1.35	2	1.35	2	2.9	2.85	3.47	2.85	3.47
Wheat	-	15	-	15	-	-	20	-	20	-
Barley	-	-	15	-	15	-	-	20	-	20
Enzyme[1]	-	-	-	+	+	-	-	-	+	+
Dicalcium phosphate	2	2	2	2	2	2.5	2.5	2.5	2.5	2.5
Oyster shell	1	1	1	1	1	1	1	1	1	1
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Potassium carbonate	-	0.05	0.05	0.05	0.05	0.1	0.1	0.1	0.1	0.1
DL-Methionine	0.17	0.15	0.15	0.15	0.15	0.25	0.25	0.25	0.25	0.25
L-Lysine HCL	0.1	-	-	-	-	0.1	0.1	0.1	0.1	0.1
Premix[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Inert	1.13	-	-	-	-	2.35	1.90	0.48	1.90	0.48
Calculated Analysis										
Metabolizable energy (kcal/kg)	2900	2900	2900	2900	2900	2980	2980	2980	2980	2980
Crude protein	21	21	21	21	21	19	19	19	19	19
Met + Cys	0.95	0.84	0.83	0.84	0.83	0.85	0.85	0.84	0.85	0.84
Lysine	1.32	1.19	1.18	1.19	1.18	1.20	1.20	1.11	1.20	1.11
Calcium	0.98	0.94	0.94	0.94	0.94	1.03	1.02	1.02	1.02	1.02
Available phosphorus	0.53	0.53	0.50	0.53	0.50	0.65	0.62	0.61	0.62	0.61
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	0.87	0.87	0.87
DEB=(Na+K)-Cl (meq/kg)[3]	230	230	230	230	230	231	231	231	231	231
Total NSP	12.43	12.89	12.96	12.89	12.96	11.62	12.11	12.65	12.11	12.65

[1]Enzymes contained 1000 unit Phytase and 180 unit Multi-glycanase and added at level 0.1% on top of ingredients in enzyme supplemented diets.[2]Supplied the following per kilogram of diet: vitamin A 44,000 IU, vitamin D3 17,000 IU, vitamin E 440 mg, vitamin K3 40 mg, vitamin B12 70 mg, vitamin B1 65 mg, vitamin B2 32 mg, Pantothenic acid 49 mg, Niacin 122 mg, vitamin B6 65 mg, Biotin 22 mg, Choline Chloride 27 mg: 650 mg of Mn, 250 mg of Zn, 125 mg of Fe, 110 mg of Cu, 60 mg of Se, 68 mg of I, and 21 mg of Co. [3]DEB: Dietary Electrolyte Balance

Statistical Analyses

All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure of GLM procedure of SAS software (SAS Inst. Inc., Cary, NC). A completely randomized design was employed. Pen was used as the experimental unit and data were analyzed by GLM procedure. Logarithmic (log 10) transformation was applied for microbial colony forming unit (CFU). Duncan's multiple range test were used for comparison of means ($P<0.05$).

RESULTS

The effect of dietary treatments on broiler chicken performance is shown at Table 2. The results indicated that the diets contained W or B led to significantly decreases in FI and BWG as well as significantly increases in FCR rather other diets ($P<0.05$). WE and BE diets which supplemented with enzymes resulted in significantly increases in FI and BWG as well as significantly decreases in FCR rather W and B diets ($P<0.05$).

Table 2. Effect of diets on broiler chicken performance at 42 days of age

Treatment	FI[1] (g/d per bird)	BWG[2] (g/d per bird)	FCR[3]	Mortality(%)
Control	92.70[a]	53.23[a]	1.74[b]	4.45
W[4]	87.63[b]	46.92[b]	1.88[a]	4
WE[5]	92.10[a]	51.40[a]	1.79[b]	3.67
B[6]	87.83[b]	47.01[b]	1.88[a]	4
BE[7]	92.17[a]	51.31[a]	1.78[b]	3.45
SEM	1.51	1.32	0.054	1.22
P-value	0.037	0.041	0.016	0.110

Means with common letters in the same column are not significantly different ($P<0.05$). SEM: Standard error of the means. [1]Feed intake, [2]Body weight gain, [3]Feed conversion ratio, [4]Wheat, [5]Wheat+enzyme, [6]Barley [7]Barley+Enzyme.

Table 3 showed the effect of diets on ileal micro flora population at 42 days of age. Diets contained W or B caused to significantly increases in total number of bacteria, gram-negative, coliforms, and clostridium bacteria as well as significantly decreases in lactic acid bacteria population rather other diets (P<0.05). WE and BE diets caused to significantly decreases in total number of bacteria, gram-negative, coliforms, and clostridium bacteria as well as significantly increases in lactic acid bacteria population rather other diets (P<0.05).

Table 3. Ileal microflora in response to diets at 42 days of age (Log 10 cfu/g of digesta)

Treatment	Total number of bacteria	Gram Negative	Coliforms	Lactobacillus	Clostridium
Control	6.67[b]	5.31[b]	5.07[b]	4.91[b]	4.86[b]
W[1]	7.13[a]	6.33[a]	6.32[a]	3.87[c]	5.69[a]
WE[2]	5.33[c]	5.21[b]	5.21[b]	5.20[a]	4.83[b]
B[3]	7.17[a]	6.24[a]	6.13[a]	3.93[c]	5.86[a]
BE[4]	5.75[c]	5.27[b]	4.56[c]	5.49[a]	4.78[b]
SEM	0.156	0.129	0.121	0.144	0.172
P-value	0.021	0.051	0.018	0.029	0.015

Means with common letters in the same column are not significantly different (P<0.05). SEM: Standard error of the means. [1]Wheat, [2]Wheat+enzyme, [3]Barley,and [4]Barley+Enzyme.

The effects of diets on the serum amylase and lipase enzyme activity are presented at Table 4. The inclusion of W and B in diets led to significantly increases in serum enzyme activity rather control (P<0.05). WE and BE diets which supplemented with enzymes led to significantly decreases in serum amylase and lipase enzyme activity rather W and B diets (P<0.05).

Table 4. Effect of diets on the serum enzyme activity of broilers at 42 days of age

Treatment	Amylase (U/L)	Lipase (U/L)
Control	22.94[c]	10[c]
W[1]	48.60[a]	21.84[a]
WE[2]	35.23[b]	17.63[b]
B[3]	46.32[a]	20.84[a]
BE[4]	37.71[b]	18.73[b]
SEM	2.62	1.44
P-value	0.012	0.003

Means with common letters in the same column are not significantly different (P<0.05). SEM: Standard error of the means. [1]Wheat, [2]Wheat+enzyme, [3]Barley,and [4]Barley+Enzyme.

DISCUSSION

Soluble NSPs of wheat and barley have negative effects on broiler performance (Yin *et al*., 2000; Lin *et al*., 2010; Mirzaie *et al*., 2012). Results reported in Table 2, indicated that W and B diets have more deleterious impact on voluntary feed intake of broiler chickens than other diets. Birds fed on W or B diet consumed lower feed intake, consequently they had lower BWG compared to other diets due to presence of soluble NSPs in their constituent NSP. The growth performance indices are consistent with the viscosity of ileal digesta, which negatively affected by soluble NSPs (Silva and Smithard, 1996; Jamroz *et al*., 2002; Jadalla *et al*., 2014). Results showed that depolymerization of the NSP constitutes of W and B diets were successful, which subsequently led to reduce digesta viscisity and significant improvements in FI and BWG of broilers. Multi-enzymes (including xylanases, β- glucanases and cellulose), release the encapsulated nutrients and reduce digesta viscisity. These processes are further facilitated by the action of phytases (Ravindran *et al*., 1999; Slominiski, 2011).

Results of this experiment indicated that W and B diets increased the total number of bacteria and the population of gram negative, coliform and clostridium bacteria, conversely decreased the population of lactic acid bacteria in the intestinal digesta compared to other diets. Inversely, birds fed on corn or WE and BE diets had higher number of lactic acid bacteria (table 3). Water soluble NSPs increase digesta viscosity in the gut which creates ideal

environment for maximum proliferation of bacteria, especially for anaerobic species such as clostridium (Jaroni *et al*., 1999; Langhout, 1999; Choct *et al*., 2006). A slow moving digesta with low oxygen tension could provide a stable media where fermentative microbes such as anaerobic bacteria can establish. These microbial changes result in reduced nutrients available for host and produces of detrimental byproducts (Choct *et al*., 2006). Supplementation of W and B diets with multi-enzymes significantly reduced the negative effects of soluble NSPs on viscosity and proliferation of bacteria in the intestine through breakdown of NSP polymers. These findings are in accordance with several reports. (Lin *et al*., 2010; Mirzaie *et al*., 2011 ; 2012).

Serum α-amylase and lipase activities of broiler chickens significantly increased after consuming of W and B diets compared to control or WE and BE diets (table 4). Presence of adequate enzymes in the blood is very important for digestibility of nutrients . But increasing gut viscosity because of viscous nature of water soluble NSPs impedes enzyme capability to hydrolyze nutrients and transmission of hydrolyzed products to the intestinal mucosa (Moharrery *et al*., 2005; Olukosi *et al*., 2007; Mirzaie *et al*. 2011). This in turn, increases the output of hepatic and pancreatic juice and enzyme activity either in intestine or in serum in mono-gastric animals (Li *et al*., 2004). Researchers have shown that enzyme activity depends on dietary nutrient source and presence of anti-nutrients such as NSPs in the gut (Brenes *et al*., 1993a; Zhao *et al*., 2007; Lin *et al*., 2010). Hence it can be concluded that the inclusion of W and B to broiler diets result in increased of soluble NSP content and consequently increased the digesta viscosity and the activity of enzymes both in intestine and in serum (Jaroni *et al*., 1999; Zhao *et al*., 2007; Lin *et al*., 2010). Enzyme supplementation of W and B diets modulates these changes. These observations were in line with relevant reports (Silva and Smithard, 1996; Li *et al*., 2004; Zhao *et al*., 2007; Lin *et al*., 2010).

CONCLUSION

In conclusion, results of the present experiment indicated that the adverse effects of W and B diets on broiler chicken performance. Total number of bacteria, number of gram negative, coliform and clostridium in intestinal digesta increased, but conversely decreased the number of lactic acid bacteria in birds fed on W and B diets. Besides, have been increased the serum α-amylase and lipase activities in birds fed on W and B diets. These changes remarkably restored by supplementing W and B diets with NSP-degrading multi-enzymes.

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