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Effects of Xylanase and β-Glucanase on Performance and Humoral Immune Response of Broilers Fed Wheat-Corn-Soy Based Nutritionally Marginal Diets

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Abstract: A total of 240 1-day-old chicks were divided into five treatment groups, with four replicates per treatment. All diets from 0 to 6 weeks were differing only in the concentration of dietary Ca and nonphytate P. The treatments were: 1) adequate level of Ca and nonphytate P (positive control), 2) reduced levels of Ca and nonphytate P (negative control), 3) negative control plus 42 units/kg xylanase and 60 units/kg β-glucanase, 4) negative control plus 70 units/kg xylanase and 100 units/kg β-glucanase, 5) negative control plus 98 units/kg xylanase and 140 units/kg β-glucanase. Body weight gain and feed consumed per pen basis were recorded weekly. Blood samples were collected weekly and their sera were separated to analysis for the level of Newcastle disease virus vaccine antibody. Supplementation with xylanase and β-glucanase improved the body weight gain and feed conversion ratio of birds ($P < 0.01$). For the low Ca-nonphytate P diets, antibody production against Newcastle disease virus vaccine was significantly increased by dietary xylanase and β-glucanase supplementation ($P<0.01$). Results of present study show that addition of nonstarch polysaccharides hydrolyzing enzyme preparations (xylanase and β-glucanase) to wheat-corn-soy based diets, can improve the performance and immunity in broiler chickens.

Key words: Calcium Phosphate · Xylanase · B-glucanase · Newcastle Disease Virus Vaccine · Broiler

INTRODUCTION

With attention to role of wheat as an important source of energy in poultry diets, its high level of xylans and β-glucans, the principal water-soluble non-starch polysaccharides (NSP), limits its use. The presence of xylans and β-glucans increases the viscosity of the digesta, interfering the digestion and absorption of nutrients and causing poor performance [1]. It has been proven that supplementation with exogenous xylanase and β-glucanase is an effective solution to lower the viscosity of intestinal contents and improve digestibility of nutrients in broilers, leading to greater apparent metabolisable energy (AME) of wheat-based diets [2]. It is known that the effectiveness of the enzyme addition is dependent on the source of xylans and β-glucans and the quantity of wheat included in the diet. As a common field observation and based on the summary of several research reports, the application of xylanase and β-glucanase in wheat-based diets can improve broiler performance by 4-6% in terms of feed conversion ratio and increase the AME content of wheat by up to 6% [3-5]. Cowieson *et al*. [4] reported that wheat-based diets supplemented with exogenous xylanase and β-glucanase could deliver identical, or even better, growth and feed conversion rate than unsupplemented corn-based diets. The improvement in performance is a result of improvement in nutrient and energy application from the diet. Energy utilization in poultry is usually expressed in terms of AME which accounts for energy loss in the excreta. Most research on the effects of xylanase and β-glucanase on energy utilization are based on the AME system, which does not consider the energy partition for production and heat production. It is expected that the net energy (NE) system will be more sensitive in measuring the response of broilers to xylanase and β-glucanase application [6].

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The mode of action of NSP-hydrolysing enzymes is still not completely understood [7]. The basis of action appears to be the partial hydrolysis of NSP in the upper digestive tract, leading to a decrease of digesta viscosity in the small intestine [8] and elimination of the nutrient encapsulating effect of the cell wall polysaccharides [9]. In addition to effects on feed digestion and absorption, morphological and histological changes and metabolic reactions of the intestinal tissues, as well as quantity and composition changes of the intestinal microflora, included in the overall response to the NSP-hydrolysing enzymes [10, 11]. However, animal growth is also closely related to the regulation of metabolism and immune system. Until now, few studies have been conducted to determine the effects of xylanase and β-glucanase supplementation to wheat-based diets with low levels of Ca and nonphytate P (nPP) diets on performance and immunity of poultry. Therefore, the objectives of this study were to examine the effects of xylanase and β-glucanas supplementation on broiler performance and antibodies against Newcastle disease virus (NDV) vaccine up to 42 d of age.

MATERIALS AND METHODS

A completely randomized experimental design was used and chicks (Ross 308) were divided into five treatment groups, with four replicates per treatment and 12 chicks per replicate. A total of 240, 1-d-old broiler chicks (Ross 308) were raised in floor pens with ad libitum access to feed and water and controlled ventilation. Chicks of a uniform body weight were placed in individual pens and average initial body weight was 48 g. Temperature was maintained at 32°C for the first 4 d and then gradually reduced.

According to normal management practices until a temperature of 22°C was achieved at d 28. The lightning regimen was 23 hours of light and 1 hour of dark. All diets were formulated to provide 3200 (control group) and 2900 kcal of ME/kg and to meet the amino acid ratios and all other nutrients as suggested by the NRC (1994) for broilers from 0 to 6 wk of age (Table 1), differing only in the concentration of dietary Ca and nPP. During the experiment, no antibiotics were added to broilers diet or water. A positive control, adequate (Adq) in Ca and nPP without xylanase and β-glucanase and negative control, Low in Ca and nPP without xylanase and β-glucanase were used. The treatments were: diet 1) adequate level of Ca and nPP (Adq Ca-nPP) as positive control (CTL+) diet, 2) reduced levels of Ca and nPP (low Ca-nPP) as negative control (CTL-) diet, 3) negative control diet plus 42 units/kg xylanase and 60 units/kg β-glucanase, 4) negative control diet plus 70 units/kg xylanase and 100 units/kg β-glucanase, 5) negative control diet plus 98 units/kg xylanase and 140 units/kg β-glucanase. Xylanase and β-glucanase source was NAT (Freedzome 2000, Agil, England).

Body weight gain (BWG) and feed consumed per pen basis were recorded weekly. Mortalities were recorded daily. At the age of 9 days, all chicks were vaccinated with Hitcher B1 NDV vaccine by eye dropper and bivalent killed vaccine (NDV plus AI) by inoculation according to the recommendation of the manufacturer (Newpasol 102, Inactivated W/O Emulsion ND + AI Vaccine, Pasouk Biological Co). Blood samples were collected every week from the wing veins of individual chickens in all groups and their sera were separated and inactivated at 56°C for 30 min and kept at -20°C until analysis for the level of NDV antibody. Serum Antibody titer was measured by hemagglutination-inhibition test as described by Alexander *et al*. [11] on d7, 14, 21, 28, 35 and 42.

Statistical Analysis: When the chicks reached 42 d of age, the feeding trial was terminated. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software [13]. The treatment means with significant differences were compared by using Duncan's new multiple range tests. In present study differences were based on significance at $P<0.05$.

RESULTS

The chickens were healthy throughout the experiment, with a mortality of less than 1% that was unrelated to dietary treatment. Following clinical examinations no bacterial or viral disease was detected. The effects of Ca and nPP concentrations and xylanase and β-glucanase supplementation on growth performance are summarized in Table 2. During the starter and grower phase, except some insignificant changes, feed intake did not change. However, in the overall, broilers fed the inadequate Ca and P diet had increased feed intake ($P < 0.01$) compared with those fed the adequate Calcium and Phosphorus diets. Inadequate Ca and P diet, caused reduction ($P < 0.01$) in BWG during the starter and grower phases but addition of xylanase and β-glucanase to said diet, returned its levels to normal ($P < 0.01$). FCR levels were affected negatively ($P < 0.01$) during study by inadequate Ca and P diet and xylanase and β-glucanase addition, improved its levels. Totally, supplementation

Table 1: Composition of experimental diets[1]

	Starter (0-21 d)					Finisher (22-42)				
Ingredients %	T 1	T 2	T 3	T 4	T 5	T 1	T 2	T 3	t 4	t 5
Wheat	30	30	30	30	30	30	30	30	30	30
Corn	24.64	26.64	26.64	26.64	26.64	25.08	34.45	34.45	34.45	34.45
Corn gluten	10	3.02	3.02	3.02	3.02	7.37	0	0	0	0
Wheat bran	0	5.21	5.21	5.21	5.21	7.21	5.10	5.10	5.10	5.10
Soybean	24.97	28.52	28.52	28.52	28.52	19.17	24.61	24.61	24.61	24.61
Soybean oil	5.64	3	3	3	3	7.00	2.16	2.16	2.16	2.16
Oyster shell	1.77	1.62	1.62	1.62	1.62	1.79	1.73	1.73	1.73	1.73
Ca phosphate	1.75	1.41	1.41	1.41	1.41	1.30	1.08	1.08	1.08	1.08
Salt	0.41	0.35	0.35	0.35	0.35	0.28	0.24	0.24	0.24	0.24
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lys	0.23	0	0	0	0	0.28	0.05	0.05	0.05	0.05
Met	0.09	0.13	0.13	0.13	0.13	0.02	0.08	0.08	0.08	0.08
Xylanase	0	0	42	70	98	0	0	42	70	98
β-glucanase	0	0	60	100	140	0	0	60	100	140
Nutrient composition Calculated										
ME, kcal/kg	3200	2900	2900	2900	2900	3200	2900	2900	2900	2900
Crude protein %	23	21	21	21	21	20	18.1	18.1	18.1	18.1
Ca %	1.0	0.9	0.9	0.9	0.9	0.9	0.85	0.85	0.85	0.85
Available P %	0.45	0.4	0.4	0.4	0.4	0.35	0.32	0.32	0.32	0.32
P (Total) %	0.702	0.691	0.691	0.691	0.691	0.41	0.41	0.41	0.41	0.41
Na %	0.20	0.18	0.18	0.18	0.18	0.150	0.135	0.135	0.135	0.135
Lys%	1.1	0.99	0.99	0.99	0.99	0.91	0.91	0.91	0.91	0.91
Met %	0.50	0.45	0.45	0.45	0.45	0.380	0.351	0.351	0.351	0.351
Met + Cys %	0.90	0.81	0.81	0.81	0.81	0.62	0.60	0.60	0.60	0.60
Trp %	0.223	0.242	0.242	0.242	0.242	0.204	0.212	0.212	0.212	0.212
Thr %	0	0.7182	0.718	0.718	0.718	0.660	0.617	0.617	0.617	0.617
Analyesed										
P (total)	0.71	0.53	0.53	0.53	0.53	0.47	0.47	0.47	0.47	0.47
Ca	1.01	0.77	0.77	0.77	0.77	0.95	0.72	0.72	0.72	0.72

[1]Calculated from NRC (1994).

[2]provides per kilogram of diet: Cu (CuSO4_5 H2O), 4.0 mg; I (potassium iodate), 1.0 mg; Fe (ferrous sulfate_7 H2O), 60 mg; Mn (manganese sulfate_H2O), 60 mg; Se (sodium selenite), 0.1mg; Zn (zin sulfate_7H2O), 44 mg; and Ca (calcium carbonate), 723 mg. For experiment 3, provides per kilogram of diet: Cu (CuSO4_5 H2O), 7.0 mg; I (potassium iodate), 1.0 mg;Fe (ferrous sulfate_7 H2O), 50 mg; Mn (manganese sulfate_H2O), 100 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate_7H2O), 75 mg.

[3]For experiments 1 and 2, provides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D3, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 2.4 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; pyridoxine (pyridoxine_HCl), 6.4 mg; riboflavin, 15 mg; and thiamin (thiamin_HCl), 15.2 mg. For experiment 3, provides per kilogram of diet: vitamin A (vitamin A palmitate), 8,000 IU; vitamin D3, 3,000 IU; vitamin E (vitamin E acetate), 25 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.1 mg; folacin (folic acid), 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin (thiamin(HCl), 3 mg.

with xylanase and β-glucanase improved the body weight gain (BWG) and feed conversion ratio (FCR) of birds ($P < 0.01$). These data indicate that xylanase and β-glucanase supplementation improves performances of chicks fed with deficient in Ca and nPP in diets for broiler chicks.

The effects of treatments on antibody production against NDV in broilers from day 7 to day 42 are presented in Table 3. On the 7th day of the study, there was no difference among antibody titers of experimental groups. Chickens of CTL- treatment showed reduction in antibody titers against NDV as compared to the CTL+. For the low- Ca-nPP diets, antibody production against NDV was significantly increased by dietary xylanase and β-glucanase supplementation ($P<0.01$). However, for antibody titers, xylanase and β-glucanase supplementation was more effective in the deficient Ca and P diet than in the CTL+ diet. The increase in enzymes supplementation dose rate (from 70 to 98 xylanase and 100 to 140 β-glucanase FTU/kg) of feed did not show any

Table 2: Effects of Ca-nPP concentrations and xylanase and β-glucanase supplementation on the performance of broilers fed nutritionally marginal diets[1]

	Treatment				
Item	1	2	3	4	5
Starter (0 to 21d)					
FI (g)	925.78	950.20	955.05	950	962.03
BWG (g)	617.35[a]	545.75[bc]	575.75[abc]	540.50[c]	607.75[ab]
FCR (g/g)	1.500[b]	1.743[a]	1.660[a]	1.759[a]	1.597[ab]
Grower (22 to 42 d)					
FI (g)	3347[b]	3560.50[a]	3567.25[a]	3596.13[a]	3539.63[ab]
BWG (g)	1722.50[a]	1655[b]	1737[a]	1749.75[a]	1763.50[a]
FCR (g/g)	1.943[b]	2.151[a]	2.054[ab]	2.055[ab]	2.008[b]
Overall (0 to 42 d)					
FI (g)	4272.78[b]	4510.65[a]	4522.30[a]	4546.13[a]	4501.65[a]
BWG (g)	2339.85[a]	2200.75[b]	2312.75[b]	2290.25[a]	2371.20[a]
FCR (g/g)	1.826[c]	2.050[a]	1.955[ab]	1.985[ab]	1.900[bc]

a-c values within a row with no common superscript differ significantly (P < 0.05)

Table 3: Effect of xylanase and β-glucanase supplementation level on NDV vaccine antibody titer of broilers

	Treatment				
Age (day)	T 1	T 2	T 3	T 4	T 5
7	7.05	6.95	7.35	7.30	7.225
14	5.075	4.975	5.150	5.225	5.225
21	4.550	4.325	4.550	4.475	4.400
28	5.550	4.550	5.625	5.800	5.400
35	6.250[ab]	5.375[b]	6.425[ab]	6.375[ab]	6.8750[a]
42	5.875[ab]	5.475[b]	5.875[ab]	6.875[a]	6.625[ab]

a-c values within a row with no common superscript differ significantly (P < 0.01)

further improvements in anti-NDV antibodies. These results demonstrated the positive influence of xylanase and β-glucanase supplementation on the response to vaccination of the chickens' immune system.

DISCUSSION

The present study showed that the addition of xylanase and β-glucanase to wheat-based diets significantly improved body weight gain and feed conversion efficiency, accordance with previous results [14]. The improved feed conversion ratio in birds fed the wheat-based diet supplemented with xylanase and β-glucanase was due to an increase in the weight gain. This beneficial effect of exogenous enzymes has previously been reported by numerous studies [15]. It is likely that these enzymes markedly increase the nutritive value of wheat in broiler chicken. This improved performance of birds fed wheat-based diet by xylanase and β-glucanase supplementation is due to prevent the formation of viscous digesta [16]. Non-starch polysaccharides (NSP) in wheat are generally believed to be responsible for the majority of the anti-nutritive activity in poultry by virtue of their capacity to increase intestinal viscosity and modulate gut microflora [17]. Increased viscosity of the intestinal contents decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction, leading to significant modifications of the structure and function of the digestive organs [18]. To adapt to these changes, the activities of the intestinal secretory mechanisms may be enhanced possibly leading to hypertrophy of the digestive organs. This increased size of the digestive organs could be an adaptive response to an increased need for enzymes [19]. When xylanase and β-glucanase is supplemented to a wheat-based diet, a greater proportion of NSP may be hydrolyzed, which might attenuate the secretory function of the responding organs and then the organ sizes may decrease. In addition, xylanase and β-glucanase can effectively reduce digesta viscosity, presumably by cleaving the large molecules into smaller fragments.

The primary lymphoid organs in birds include the thymus and the bursa of-fabricius, which are the places where lymphocytes develop and the T-cell and B-cell receptor genes are rearranged. The lymphocytes then migrate to secondary lymphoid organs such as the spleen. The spleen is an immune defence organ that comprises part of the peripheral lymphoid tissue. It acts as the main

site of lymphocyte differentiation (B-cells) and proliferation (B and T-cells) and these B- and T-cells are involved in the production of humoral and cell-mediated immune responses [20]. Studies showed that enzyme supplementation to wheat-based diets significantly increased the relative weight of the spleen, suggesting that enzyme supplement accelerated the development of the immune organ [21]. Serum antibody titre to NDV is the immune parameter widely used to measure the humoral immune responses. In this study, we found that enzyme supplementation increased serum antibody titres to NDV during 5^{th} and 6^{th} weeks of study, suggesting that enzyme supplement enhanced the humoral response. To our knowledge, limited studies have been conducted to determine the effects of enzyme supplementation to wheat based diets on the immunity of poultry. There is much evidence demonstrating that inappropriate diet intake negatively influences the development of immune organs and normal immune responses and nutrients can modulate immune function in human and animals. Energy, proteins, lipids, vitamins, minerals and nucleic acids play an important role in the regulation of cellular and humoral immune responses. Therefore, it may be that the addition of the enzyme enhanced the digestion of feed and the absorption of nutrients, which in turn could have an effect on body immunity [21]. Results of present study show that, NSP-hydrolyzing enzyme preparations (xylanase and β-glucanase), when added to wheat-corn-soy based diets, can improve the performance in broiler chickens. This improvement is achieved through the enzyme's influence on digestion, absorption, metabolism and immunity of broiler chickens.

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