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Full Length Research Paper

Influence of non starch polysaccharide-degrading enzymes on the meat yield and viscosity of jejunal digesta in broilers fed wheat/barley-based diet

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This study was conducted to investigate the effect of four commercial multi-enzymes on the performance, meat yield, water intake, litter moisture and jejunal digesta viscosity of chicks fed wheat/barley based diet. A total of 195 1-d-old male broiler chicks (Ross 308) were allocated to 5 treatment groups, with 3 replicates per treatment group and 13 birds per replicate pen and all data were analyzed in a randomized complete block design. During the starter period, feed conversion ratio (FCR) was significantly (P < 0.05) decreased by only enzymes A, B and D. Moreover, FCR was significantly (P < 0.05) decreased by all enzymes in grower and entire periods. The relative weight of the breast as proportion of live weight was significantly (P < 0.05) decreased by only enzyme A. The relative weight of the abdominal fat as proportion of live weight was also significantly (P < 0.05) increased by enzyme D. Enzyme supplementation decreased viscosity of jejunal contents of chicks at day 28, but only enzymes A and D reduced significantly (P < 0.05) the viscosity of jejunum compared to control diet. In conclusion, there were similar improvements on FCR of birds fed diets with enzyme supplementation and choice preference of enzyme supplementation has to be base on its economic value.

Key words: β-glucanase, xylanase, broilers, performance, meat yield, water intake, litter moisture, viscosity.

INTRODUCTION

More cereal grains, particularly wheat and barley, although rich in starch, contain significant quantities of non-starch polysaccharides (NSPs), which act as anti-nutritional factors by increasing digesta viscosity leading to a decreased digestibility of starch, protein and fat (Choct and Annison, 1990). Xylanase and β-glucanase are enzymes that degrade NSPs (Choct et al., 2004) and have been shown to improve the nutritive value of wheat and barley based diets for birds (Juanpere et al., 2005) by reducing the anti-nutritional effects of non-starch polysaccharides (Choct et al., 2004). The most important NSPs fraction in wheat and barley has been reported to be β-glucans and pentosans (Annison, 1991; Bedford, 2000). The hydrolysis of the polysaccharide structure of the cell wall components causes an improvement of carbohydrate and protein availability as well as a reduction in digesta viscosity and so drier droppings and litter (Rotter et al., 1989a). Overall, the results of previous studies have demonstrated that the viscous carbohydrates in wheat and barley reduce nutrient digestibility and chick performance. These negative effects can be overcome by addition of enzymes to wheat and barley based diets. There are varieties of commercial enzymes available and producers are presented with a challenge to choose the most suitable enzyme for improvement of their flock performance. Therefore, in the present study, the effects of four enzymes in wheat/barley-based diet on broilers

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Table 1. Ingredient composition (g/kg) of the experimental diets given to broiler chickens.

Ingredient	Starter	Grower	Finisher
Wheat	300.0	300.0	300.0
Barley	300.0	300.0	300.0
Wheat bran	------	------	75.3
Soybean meal	200.1	214.4	104.6
Gluten meal	102.6	67.4	95.5
Soya Oil	44.2	70.7	78.0
L-Lys-HCL	0.6	0.3	0.4
DL-Met	2.1	1.5	0.2
Dicalcium phosphate	20.4	17.8	17.6
Limestone	21.9	19.8	20.4
Sodium chloride	3.1	3.1	3.0
Vitamin premix [1]	2.5	2.5	2.5
Mineral premix [2]	2.5	2.5	2.5
Enzyme A	0. 1	0. 1	0. 1
Enzyme B	0. 5	0. 5	0. 5
Enzyme C	0. 1	0. 1	0. 1
Enzyme D	0. 4	0. 4	0. 4

[1]Provided the following (per kg of diet): vitamin A (transretinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,000 IU; vitamin E (all-rac-tocopherol acetate), 18 IU; vitamin K (bisulfate menadione complex), 2 mg; riboflavin, 6.6 mg; pantothenic acid (D-calcium pantothenate), 10 mg; pyridoxine (pyridoxine_HCl), 3 mg; folic acid, 1 mg; thiamin (thiamin mononitrate), 1.8 mg; vitamin B12 (cyanocobalamin), 15 µg; D-biotin, 0.1 mg; niacin, 30 mg; choline (choline chloride),500 mg and ethoxyquin, 0.1 mg.
[2]Provided the following (per kg of diet): Se (Na_2SeO_3), 0.2 mg; I (KI), 1 mg; Cu ($CuSO_4.5H_2O$),10 mg; Fe ($FeSO_4.7H_2O$), 50 mg; Zn (ZnO), 85 mg and Mn ($MnSO_4.H_2O$),100 mg.

performance were examined.

MATERIALS AND METHODS

Birds and housing

One hundred and ninety five (195) male day-old broiler chicks of commercial strain (Ross 308) were randomly allocated to 5 treatment groups, with 3 replicates per treatment and 13 birds per floor pen replicate. The average initial body weight of chicks in each pen was 42 g. Room temperature was kept at 34°C during the first 3 days of the trail and was then reduced gradually according to age until reaching 22°C at 21 d. The light was continuous during the first 3 d and then the lighting regimen was 23 h/d. This experiment was conducted in the early November to middle of December.

Dietary treatments

Once birds were randomly distributed among pens, each pen was assigned to one of five feed treatments formulated to meet or exceed all nutrient recommendations published in the Ross rear guideline. The five dietary treatments consisted of five rations containing 60% wheat/barley supplemented with and without enzyme (enzymes A, B, C and D). These enzymes contained β-glucanase and xylanase activities mainly. The β-glucanase and xylanase activities as determined by the manufacturers, were endo-1,4-β-glucanase activity: min 800 units/kg diet; endo-1,3(4)-β-glucanase activity: min 1800 units/kg diet; endo-1,4-β-xylanase activity: min 2600 units/kg diet for enzyme A, endo-1,3(4)-β-glucanase: 100 AGL/kg diet; endo-1,4-β-xylanase: 70 AXC units/kg diet for enzyme B, endo-1,4-β-glucanase: 1500 BGU/kg diet; endo-1,4-β-xylanase: 3600 FXU/kg diet for enzyme C and β-glucanases: 1420 units/kg diet; xylanases: 660 units/kg diet for enzyme D, respectively. One diet had no enzymes which was called control diet. The ingredient composition of the experimental diets is shown in Table 1 and the nutrient composition in Table 2. Mash feed and water were made available for *ad libitum* consumption. Prior to formulation, all major dietary ingredients were analyzed for AMEn, amino acid (AA) profiles (according to prediction formula existing in NRC, 1994), crude protein (CP), crude fiber (CF) ether extract (EE) and macro minerals content as described by AOAC International (AOAC, 2000) methods. Starch of barley was determined by enzymatic method (Conan and Carre, 1989).

Productive performance traits

Mortality 0 to 42 d was determined for each pen. Body weight (BW) of chicks and feed consumption were determined by cage at 0, 10, 30 and 42 d of age, then feed conversion ratio (FCR) was calculated from these data by period and globally. Feed intake (FI) was adjusted for mortality. Birds that died during the experiment were weighed, and their BW was included in calculations of FCR. Feed wastage was recorded by replicate for each period.

Table 2. Nutrient composition of the experimental diets.

Calculated analysis	Starter	Grower	Finisher
AME, kcal/kg	2,860	3,000	3,030
Crude protein, g/kg	224.1	209.5	190.0
Lysine, g/kg	13.68	11.43	9.47
Methionine + cystine, g/kg	10.36	8.95	7.58
Crude fiber, g/kg	41.96	41.98	45.28
Calcium, g/kg	9.50	8.58	8.52
Available phosphorous, g/kg	4.75	4.29	4.26
Sodium, g/kg	1.52	1.52	1.52

Meat yield characteristics

At 42 d of age, two birds per pen were wing-banded, slaughtered and eviscerated in order to determine carcass weight and carcass yield as well as legs, breast (including skin and bone), liver (without gallbladder), gizzard and abdominal fat weights as a proportion of live weight.

Water intake and litter moisture

Water intake of chicks and feed consumption were recorded by cage at 5, 16, 24, 33 and 40 d of age in a 24 h interval. Then water to feed ratio (W: F) was calculated from these data by days 5, 16, 24, 33 and 40 days of age. Litter moisture percentage was measured by collection of four samples for each pen from each corner of pens on 42 d of age.

Intestinal viscosity

At 28 d of age, 2 birds per pen with body weights near the mean of each pen were selected and euthanized by carbon dioxide. The jejunum (defined as the region from the pancreas to Meckel's diverticulum) was dissected aseptically, and the digesta contents were collected and pooled by replicate as described by Lazaro et al. (2004). The digesta was homogenized and two Eppendorf tubes were filled (1.5 g of sample) and centrifuged (12,500 × g, 3 min). The viscosity (in centipoises, cps) of a 0.5-mL aliquot obtained from the supernatant solution was determined at 28 d of age with a digital viscosimeter (model DV-III, Brookfield Engineering Laboratories Inc., Stoughton, MA) at 25°C. Each sample was read four times and the average value was used for the statistical analysis.

Statistical analysis

In this research, ANCOVA was applied to analyze the data collected on grower and finisher performances. Because initial body weights were different, therefore, it was coverable applied in the model. In model developing, backward stepwise, the covariate effect was omitted due to the fact that it was not significant. Therefore, ANOVA was used to analyze the data. All data was analyzed through the general linear model (GLM) procedure of SAS (SAS, Institute; 2002) for a randomized complete block design, with pen representing the experimental unit. Significant differences among treatments were separated by Duncan's multiple range tests, with a 0.05 level of probability.

RESULTS AND DISCUSSION

Productive performance

Mortality for all groups was within the expected range and there was no significant difference in mortality of all treatments. Effects of dietary treatments on the performance of broiler chicks at all periods are shown in Table 3.

Only diet containing enzyme C significantly ($P < 0.05$) increased FI in the starter period, which is in agreement with previous observation (Garcia et al., 2008). In grower, finisher and entire periods, FI was not significantly affected by enzyme supplementation ($P > 0.05$). These findings are in agreement with results of Leeson et al. (2000). It has been proposed that the viscous NSPs (that is, β-glucan and arabinoxylan) are responsible for the poor feeding value of rye, oats, barley and some varieties of wheat for poultry since high digesta viscosity may be responsible for impaired digestion and absorption of nutrients and FI reduction (Annison, 1991).

In starter, grower and finisher periods, BW of broilers fed diets with enzyme were not significantly ($P > 0.05$) improved as compared to control diet. This result is in agreement with those reported by Garcia et al. (2008). In entire period, BW was significantly ($P < 0.05$) increased by all enzymes. This finding agreed with those reported by Leeson et al. (2000), Senkoylu et al. (2004) and Garcia et al. (2008).

In starter period, FCR of birds fed diets with enzymes A, B and D was significantly ($P < 0.05$) improved as compared to control diet. These results are in agreement with those reported by Leeson et al. (2000), Gracia et al. (2003) and Garcia et al. (2008). In grower and entire periods, FCR was significantly ($P < 0.05$) decreased when diets containing enzymes were compared to control diet and there were no significant differences among diets containing each of the enzymes. These results are in agreement with those reported by Abdulilah (1995), Leeson et al. (2000) and Garcia et al. (2008). However, in finisher period, FCR was not significantly ($P > 0.05$) affected by enzyme supplementation and there were no significant differences among all treatments. This result is

Table 3. Effect of dietary treatments on the performance of broiler chicks at all periods.

Treatment	Starter (0 - 10 days)			Grower (11 – 30 days)			Finisher (31 - 42 days)			Entire (0 - 42 days)		
	BW	FI	FCR	BW	FI	FCR	BW	FI	FCR	BW	FI	FCR
Control	228	269^{b}	1.18^{a}	1,092	1,903	1.75^{a}	866	2,138	2.48	2,287^{b}	4,475	1.96^{a}
Enzyme A	248	272^{ab}	1.10^{b}	1,235	1,941	1.57^{b}	899	2,129	2.40	2,495^{a}	4,510	1.81^{b}
Enzyme B	247	258^{b}	1.04^{b}	1,249	1,924	1.54^{b}	890	2,100	2.36	2,500^{a}	4,448	1.78^{b}
Enzyme C	249	290^{a}	1.16^{a}	1,250	1,955	1.57^{b}	922	2,149	2.33	2,527^{a}	4,539	1.80^{b}
Enzyme D	251	270^{b}	1.07^{b}	1,281	1,968	1.54^{b}	838	2,012	2.48	2,487^{a}	4,420	1.78^{b}
SEM	5.272	5.925	0.017	44.845	43.025	0.045	47.801	51.563	0.100	46.945	77.731	0.017

$^{a-b}$Means within a column without common superscripts differ significantly ($P < 0.05$).

Table 4. Effect of dietary treatments on the carcass characteristics of broiler chicks at 42 d.

Treatment	Carcass Weight (g)	Carcass Yield	Breast Weight	Legs Weight	Abdominal Fat	Liver Weight	Gizzard Weight
		(% of live weight)					
Control	1,549.17	69.60	32.51^{ab}	28.49	1.31^{b}	2.55	1.72
Enzyme A	1,611.67	68.56	30.63^{c}	29.29	1.76^{ab}	2.52	1.67
Enzyme B	1,648.33	68.06	32.36^{ab}	28.71	1.70^{ab}	2.59	1.68
Enzyme C	1,680.83	70.30	33.33^{a}	28.14	1.76^{ab}	2.70	1.77
Enzyme D	1,668.33	69.87	31.42^{bc}	28.56	2.04^{a}	2.60	1.95
SEM	51.837	0.735	0.403	0.324	0.139	0.206	0.121

$^{a-c}$Means within a column without common superscripts differ significantly ($P < 0.05$).

also in agreement with the findings reported by Garcia et al. (2008).

Numerous researchers have reported increased growth and improved FCR because of NSPs enzymes inclusion in animal diets based on barley, especially for poultry (Almirall et al., 1995; Jamroz et al., 2002). The improvement that occurred in FCR may result from improvement in the use of metabolizable energy and digestibility of fat, protein and carbohydrates. Such findings, confirmed by other researchers (Rotter et al., 1989b), suggested that the addition of enzymes caused a breakdown of the β-glucans and pentosans surrounding the starch molecules. This breakdown led to an increase in the availability of carbohydrates and other nutrients in wheat and barley, consequently increasing its nutritive value.

Meat yield characteristics

As shown in Table 4, carcass weight was not significantly ($P > 0.05$) affected in the birds fed diets containing different enzymes compared to control diet. In addition, carcass yield was also not significantly ($P > 0.05$) affected by enzyme addition. These results are consistent with those reported by Biswas et al. (1999).

Relative weight of the legs as proportion of live weight was not significantly ($P > 0.05$) affected by enzyme supplementation. This result agreed with Shirzadi et al. (2009). However, relative weight of the breast as proportion of live weight was significantly ($P < 0.05$) decreased by only enzyme A compared to control diet. The reason for this is not clear, but it seems that feeding birds with diet containing enzyme A, redounded to increase of weights of head and neck and back sections of carcass. It seems that enzyme A had more effect on decrease of jejunal contents viscosity of birds in comparison with other enzymes (Table 5) and low viscosity led to decrease in deconjugation of bile salts via decrease in gut microflora, especially *Streptococcus faecium* and *Clostridium perfringens* (Knarreborg et al., 2002). Consequently, absorption of lipids and soluble vitamins in lipid especially vitamin D was increased by small intestinal brush border. Therefore, increased absorption of vitamin D led to increase of broiler's bone mineral density and weights of the head, neck and back can be increased in comparison with weight of breast. Hence, this ordinance redounded to decrease of breast weight as proportion to live weight.

In addition, abdominal fat was significantly ($P < 0.05$) increased by enzyme D compared to control diet, but there were no significant differences among diets containing enzyme. This result agreed with the findings of Brenes et al. (1993) and Shirzadi et al. (2009). It is reported that the NSPs fraction of cereal protects lipids, starch and protein via decreasing the access of digestive enzymes to dietary components, but enzyme supple-

Table 5. Effect of treatments on water intake, intestinal viscosity, and litter moisture.

Item	Control	Enzyme A	Enzyme B	Enzyme C	Enzyme D	SEM
Water : feed ratio, ml/g						
5 d	2.96	2.54	2.72	2.52	2.64	0.105
16 d	2.07	1.88	1.91	1.89	1.95	0.073
24 d	2.47	2.21	2.21	2.09	1.98	0.130
33 d	2.38	2.20	2.10	2.15	2.06	0.081
40 d	2.18	1.91	2.08	2.15	1.94	0.082
Viscosity (cps), day 28	2.51^{a}	1.43^{c}	2.10^{ab}	2.27^{ab}	1.88^{bc}	0.176
Litter moisture (%), day 42	16.04	14.43	14.09	14.80	16.39	0.864

$^{a-c}$Means within a column without common superscripts differ significantly ($P < 0.05$).

mentation with hydrolysis cell wall components, specifically the NSPs fraction, can increase the access of digestive enzymes to dietary components and also the potential energy of diet to convert extra energy to abdominal fat. In the case of relative weight of liver and gizzard as proportion of live weight, there were no significant differences among all treatments. These findings are consistent with results of Alam et al. (2003) and Shirzadi et al. (2009).

Water intake and litter moisture

Effect of treatments on water intake and litter moisture of broiler chickens is shown in Table 5. Water-feed ratio of the chicks at 5, 16, 24, 33 and 40 d was not significantly ($P > 0.05$) reduced by enzyme addition and there were no significant differences among all treatments. These findings are consistent with results of Leeson et al. (2000). In the case chick's litter moisture at 42 d, the enzymes were not significantly ($P > 0.05$) affected, this result is in agreement with Leeson et al. (2000). However, enzymes did not significantly affect water-feed ratio and litter moisture, but anyway they tended to be lower. The reason of insignificant effect of enzymes on these characteristics is not clear, but it might be related to low NSPs content in wheat and barley and in fact, NSPs content was not enough, to significantly affect these items.

Intestinal fluid viscosity

The results demonstrated that the viscosity of jejunal contents of chicks was significantly ($P < 0.05$) reduced only by enzymes A and D compared to the control diet (Table 5). However, no significant ($P > 0.05$) difference was observed among enzymes B, C and D. This result confirmed the results of Leeson et al. (2000). Garcia et al. (2008) reported that enzyme supplementation reduced viscosity of jejunum in broilers fed barley-based at 7, 28 and 42 days of age. In addition, Gracia et al. (2003) reported similar effect at 4, 8, 15 and 21 days of age. Similar effects have been reported with rye grain (Lazaro et al., 2004) and wheat grain (Gao et al., 2007). Sieo et al. (2005) demonstrated that viscosity of jejunal contents in broiler chickens fed barley-based diet was reduced by supplementing β-glucanase-producing transformed lactobacillus strains. When supplemental enzyme were added to the control diet, it might partially degrade these larger molecular polysaccharides into smaller ones, even oligosaccharides and at the same time, the enzymes might alleviate the viscous property of digesta (Choct et al., 1999; Mathlouthi et al., 2003). The breakdown of NSPs into smaller polymers prevents them from forming viscous networks (Malathi and Devegowda, 2001). Bedford et al. (1991) reported that viscosities were significantly correlated only with carbohydrate fractions that existed as complexes greater than 500,000 Da. The addition of enzymes significantly reduced the size of the complexes and thus, reduced viscosity. Fuente et al. (1998) found that the digesta viscosity of chickens fed a barley-based diet was reduced by 50% with the addition of a commercial multi-enzyme complex containing β-glucanase and xylanase activities.

Conclusions and applications

The results of this study showed that during the starter and grower periods, it is required to add enzymes to control diets and maybe in finisher period, it could be unnecessary to supplement diets with enzymes. Also, the results led to the conclusion that there were similar improvements in performance of birds fed diets with enzyme supplementation and choosing more suitable enzyme should be based on costs.

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