Growth and Carcass Performance in Broiler Chickens Supplemented with β-Glucanase from Aerobic Fungi *Aspergillus Niger* and *Trichoderma Longibrachiatum*

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A variety of factors can affect feed efficiency in poultry; among them is β-Glucan. β-Glucan in feeds is often poorly digest and has deleterious effects on nutrient absorption. Supplementation of diets with proper enzyme can enhance nutrient digestion and absorption. The aim of this study was β-Glucanase production from *Aspergillus niger* and *Trichoderma longibrachiatum* (GPβ-Glucanase), as well as its *in vitro* and *in vivo* assessment. Yeast biomass was produced using *Zapek* medium with Glucose and inoculated in Erlenmeyer flasks (repressed conditions) with above fungi. The mycelia then transferred to Erlenmeyer flasks containing *Zapek* medium supplemented with 2% barely β-Glucan and incubated (induction conditions), and enzyme recovered from the medium. The mean activities of GPβ-Glucanase and commercial β-Glucanase from *Bacillus subtillis* (ZYβ-Glucanase) were recorded as 1348 ± 5.12 and 1251 ± 5.31 BGU^1/g respectively. Chick model was used for *in vivo* assessment. 540 Broiler chicks were fed one of nine diets in a 3×3 factorial arrangement from 1 to 49 days of age. The factors were: enzyme addition (0, ZYβ-Glucanase and GPβ-Glucanase) and level of hull-less barley (HB) in diet (0, 40, and 60%). The results showed that supplementation of diets containing 40% HB either with commercial or produced enzyme led to significant ($P < 0.05$) increase in weight gain and feed intake, whereas significant ($P < 0.05$) decrease feed efficiency, however no significant differences were observed between two kinds of enzyme. Conclusion was that GPβ-Glucanase is of great potential and comparable to ZYβ-Glucanase for β-Glucan hydrolysis.

Key words: aspergillus, β-glucanase, broiler, trichoderma

Introduction

The major objective of any poultry diet formulation is providing nutrients, to meet a specific set of nutrient requirements, which in turn affecting efficiency of feed utilization. A variety of factors can affect the bioavailability of nutrient; among these are non starch polysaccharides, such as β-Glucans. Efficiency of feed requires efficient digestion of complex β-Glucan substrates that are present in non starch polysaccharides diets. β-Glucan in feed is often poorly digest (Ankrah *et al.,* 1999) and diets containing β-Glucan have deleterious effects on nutrient absorption and may promote intestinal disturbance by enteric pathogens (Choct *et al.,* 1996). In Tangarone *et al.* (1989) and Mokar *et al.* (1991) studies on properties of β-Glucanases from *Trichoderma longibrachiatum* and *Aspergillus niger* the optimal activity of those enzymes was at pH of 5.5 and temperature of 37 C. Yu *et al.* (1998) reported that the nutritive value of barley can be considerably improved by the dietary inclusion of β-Glucanase

preparation from *Aspergillus niger* and *Trichoderma longibrachiatum.* Addition of enzyme to diet containing β-Glucan improved growth and feed conversion Ratio (FCR) in broiler chicken (Almirall *et al.,* 1995; van der Klis *et al.,* 1995). The implication of such findings is that enzyme supplementation led to reduce viscosity in gastrointestinal tract, thereby increase passage rate of feed and body weight (Ouhida *et al.,* 2000; Bedford and Partridge, 2001). Coenen *et al.* (1995) showed that β-Glucanase preparation from *Trichoderma reesei* is safe when included in broiler diets. However, some occupational health precautions should be taken to avoid skin contact and inhalation, as it is a case for almost all enzyme proteins. Fuents *et al.* (1998) observed that enzyme addition preparation from *Aspergillus niger* and *Trichoderma longibrachiatum* to barley based diets increased dietary apparent metabolisable energy values by 12% on average. In an attempt the effects of two kinds of commercial enzyme preparation were examined on nutrients digestibility in broiler chicks fed xylan diets. The responses in apparent metabolisable energy and, nitrogen and amino acid digestibilities were similar for both enzymes (Hew *et al.,* 1998).

The purpose of this trail was to produce and evaluate the β-Glucanase activity from two different sources.

Material and Methods

Aspergillus niger (*A. niger*) and *Trichoderma longibrachiatum* (*T. longibrachiatum*) were obtained from the Iranian Research organization for Science and Technology (IROST) culture collection (Tehran, Iran) and maintained on agar medium. To compare produced β-Glucanase from *A. niger* and *T. longibrachiatum,* ZYβ-Glucanase (Lohman Animal Health Co, Germany) prepared from *bacillus subtillis* was used as a reference. Yeast biomass was produced using *Zapek* medium (FeSO$_4$ 7H$_2$O, MgSO$_4$ 7H$_2$O, KH$_2$PO$_4$, NaNO$_3$) with Glucose, and inoculated in Erlenmeyer flasks for 4 days at 29 C (repressed conditions) with the above fungi. The mycelia were then washed under sterile condition, transferred to Erlenmeyer flasks containing *Zapek* medium supplemented with 2% barely β-Glucan and incubated as described above (induction conditions) and crude β-Glucanase then recovered from the medium. β-Glucanase activity was assayed by incubating 0.5 mL of 50 mg.mL^{-1} β-Glucan (Merck) in 10 mM potassium acetate buffer, pH 5.5, with 0.5 mL of enzyme (either GPβ-Glucanase or ZYβ-Glucanase) solution appropriately diluted in the same buffer. Mixture then was incubated at 37 C for 30 min. One β-Glucanase unit (BGU) is defined as that quantity of enzyme in g that will liberate 1 μmol of reducing sugar (as glucose equivalence) per min under the standard assay conditions (Cruz and Liobell, 1999; Oriana *et al.,* 2001). Chick model was used for *in vivo* assessment. 540 Broiler chicks (equal numbers of males and females) were fed one of nine diets (Table 1) in a 3×3 factorial arrangement from 1 to 49 days of age. The factors were: enzyme addition (0, ZYβ-Glucanase and GPβ-Glucanase) and level of hull-less barley (HB) in diet (0, 40, and 60%). Therefore, the arrangement of treatments were T1, corn-soybean based diet (control diet); T2, control diet plus ZYβ-Glucanase; T3, control diet plus GPβ-Glucanase; T4, 40% HB; T5, 60% HB; T6, 40%HB plus ZYβ-Glucanase; T7, 60% HB plus ZYβ-Glucanase; T8, 40% HB plus GPβ-Glucanase; T9, 60% HB plus GPβ-Glucanase (Table 1). The enzymes were used at the inclusion level recommended by their manufacturers (0.05% diet). Each dietary treatment was fed to 6 replicates of 10 broilers per replicate. The feeding program consisted of three diets, starter (1 to 21 d), grower (21 to 42) and finisher (42 to 49 d). The broilers were housed in floor pens (1.1×1.0 m^2) and pine shaving served as litter material. Each pen was equipped with a hanging pan feeder and a bell-type waterer. Broilers were exposed to 24 h of light for the first 7 d, then to a light:darkness cycle of 23 h light:1 h darkness until 49 d of age. Room temperature was maintained at 33 C for the first 7 d and then was gradually reduced to 21 C at 49 d of age. Criteria used to measure response were body weight gain (BWG), feed intake (FI), FCR, dressed carcass, edible carcass, abdominal fat, breast, drumstick and visceral. Body weight and FI of broilers from all pens were measured every week. Mortality of each pen was recorded on a daily basis. FCR was adjusted according to the FI of the dead broilers. All diets were recorded for *ad-libitium* consumption in mash form and broiler had free access to water. At 49 days of age, two broilers per pen (one male and one female), representative of the mean body weight, were killed for carcass cut analysis.

Table 1. **Composition of the experimental diets treatment 1 to 9 (T1 to T9)**

Item	Starter (1 to 21 d)			Grower (21 to 42 d)			Finisher (42 to 49 d)		
Ingredient%[1]	T1	T6 and T8	T7 and T9	T1	T6 and T8	T7 and T9	T1	T6 and T8	T7 and T9
Enzyme	0	0.05	0.05	0	0.05	0.05	0	0.05	0.05
Hull-less barley	0	40	60	0	40	60	0	40	60
Corn	56.7	20.78	3.81	64	27.68	10.6	65.9	30.42	13.38
Soybean meal	29	26.6	22.24	27.9	22.13	17.9	24.07	21.05	16.77
Fish meal	5.1	3.89	5.06	1.59	2.23	3.32	1	0.11	1.24
Soybean oil	3.3	5	5.5	2.67	4.47	5	3.52	4.98	5.5
Wheat bran	2	0	0	0	0	0	2	0	0
Dicalcium phosphate	0.84	1	0.88	0.88	0.82	0.71	0.72	0.83	0.72
Oyster shells	1.2	0.94	0.76	1.33	1.02	0.85	1.29	1.01	0.84
Salt	0.31	0.33	0.31	0.26	0.25	0.23	0.19	0.2	0.18
Vitamin and mineral mix[2]	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Anticoccidial agent	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	0.07	0.1	0.1	0.04	0.05	0.05	0.01	0.03	0.03
Composition (calculated)									
ME, Kcal/kg	2950	2950	2950	3000	3000	3000	3050	3050	3050
Crude protein%	21.2	21.2	21.2	18.7	18.7	18.7	17.15	17.15	17.15
Crude fiber%	3.5	3.5	3.5	3.3	3.3	3.3	3.3	3.3	3.3
Calcium%	0.92	0.92	0.92	0.84	0.84	0.84	0.76	0.76	0.76
Available phosphorus%	0.41	0.41	0.41	0.33	0.33	0.33	0.28	0.28	0.28

[1] Composition of treatments 2 and 3 are similar treatment 1 but with ZYβ-Glucanase and GPβ-Glucanase, respectively. Composition of treatments 4 and 5 are similar treatments 6 and 7 but without ZYβ-Glucanase enzyme, respectively.

[2] Vitamin and mineral mix combinations in diet (%): mineral mix[3], 0.25; vitamin mix[4], 0.25; vitamin A, D₃, E, K and B complex, either 0.15.

[3] Mineral combination (mg/kg mineral mix): manganese, 100000; iron, 50000; zinc, 100000; copper, 10000; iodine, 1000; selenium, 200.

[4] Vitamin combination (IU or mg/kg vitamin mix): vitamin A, 9000000 IU; vitamin D₃, 2000000 IU; vitamin E, 18000; vitamin K₃, 2000 mg; vitamin B₁, 18000; vitamin B₂, 6600; vitamin B₃, 10000; vitamin B₅, 30000; vitamin B₆, 300; vitamin B₉, 1000; vitamin B₁₂, 15 mg; biotin, 100 mg; choline chloride, 250000.

Results

The crude enzyme preparation contained β-Glucanase was provided from the culture medium of *A. niger* and *T. longibrachiatum* and named as GPβ-Glucanase. In the present work, GPβ-Glucanase has shown optimal activity at pH 5.5 and at temperature of 37C (See introduction). The mean GPβ-Glucanase and ZYβ-Glucanase enzyme activity were recorded as 1348±5.12 and 1251±5.31 BGU/g, respectively. No significant differences ($P > 0.05$) was observed between two enzyme preparations. Table 2 shows the effect of enzyme supplementation on broiler performance. Supplementation of diet containing HB showed significant effect ($P < 0.05$) on BWG in the starter (1 to 21 d) and grower periods (21 to 42 d). However no significant difference was observed between two enzyme preparations. Neither enzyme nor HB levels had significant effect on BWG during 42 to 49 d. Treatment 6 (T6) and T8 (40% HB+either enzyme) were found with higher ($P < 0.05$) BWG in the starter and grower periods when compared with T4 (40% HB, containing no enzyme).

FI and FCR were significantly ($P < 0.05$) affected by the addition of enzyme and HB from 1 to 49 d. There was no significant interaction between enzyme and HB for BWG, FI and FCR (Table 2). Table 3 shows the effect of enzyme on chicken organs relative weight. Addition of either GPβ-Glucanase or ZYβ-Glucanase significantly ($P < 0.05$) affected dressed carcass, edible carcass, abdominal fat, breast and visceral, whereas no significant differences was obtained for drumstick.

Discussion

The activities of β-glucanases from different preparation have been reported previously. Optimum pH and temperature for β-glucanase isolated from *T. harzianum*, was determined as 4.4 of 45 C, respectively (Noronha and Ulhoa, 1996). Sharma and Nakas (1987) observed the highest β-Glucanase activity prepared from *T. longibrachiatum* at pH 3.5‒5.0 and temperature ranged from 30 to 70 C. The

Table 2.   **Effect of enzyme supplementation on the performance of broilers**

Diet		Weight gain (g)			Feed intake (g)			Feed conversion ratio (g/g)		
Enzyme	HB[1]	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
0	0	435[ab]	1200[a]	545	722[a]	1798[bc]	1073[ab]	1.44[a]	1.63[ab]	1.63[a]
0	40	333[cd]	1037[c]	460	652[bc]	1643[d]	839[c]	1.84[c]	1.96[c]	2.01[c]
0	60	314[d]	999[d]	403	646[c]	1503[e]	768[d]	2.02[d]	2.06[d]	2.09[d]
ZYβ-Glucanase	0	453[a]	1194[a]	551	736[a]	1895[ab]	1145[ab]	1.49[a]	1.61[a]	1.64[a]
ZYβ-Glucanase	40	417[ab]	1165[ab]	526	712[ab]	1781[bc]	994[b]	1.58[ab]	1.75[ab]	1.81[ab]
ZYβ-Glucanase	60	369[bc]	1048[c]	531	702[abc]	1705[cd]	938[c]	1.65[ab]	1.87[bc]	1.92[b]
GPβ-Glucanase	0	467[a]	1211[a]	568	735[a]	1965[a]	1214[a]	1.55[ab]	1.6[a]	1.68[a]
GPβ-Glucanase	40	431[ab]	1161[ab]	549	716[ab]	1759[cd]	1003[b]	1.64[ab]	1.73[ab]	1.78[a]
GPβ-Glucanase	60	355[c]	1091[bc]	527	685[abc]	1728[cd]	846[c]	1.68[b]	1.86[b]	1.94[b]
Probability of greater F-value in analysis of variance[2]										
Source of variance										
E[1]		**	*	NS	**	**	*	**	*	*
HB		**	*	NS	*	*	*	*	*	*
E×HB		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b,c]: Means within column with no common superscripts differ significantly ($p < 0.05$).
[1] HB: Hull-less barley, E: Enzyme.
[2] NS: $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3.   **Relative weights of dressed carcass, edible carcass, abdominal fat, breast, drumstick and visceral of broiler chickens fed enzyme-supplemented diets containing hull-less barley**

Diet		Relative weight (g/1000 g body weight×100)					
Enzyme	HB[1]	Dressed carcass[2]	Edible carcass[3]	Abdominal fat	Breast	Drumstick	Visceral
0	0	87.6[a]	71[a]	4.55[a]	27.21[ab]	27.29	15.59[ab]
0	40	86[cd]	68.5[c]	3.64[c]	25.22[c]	26.62	18.16[cd]
0	60	84.65[d]	67.8[c]	3.53[c]	24.97[c]	26.07	19.05[d]
ZYβ-Glucanase	0	87.4[a]	71.5[a]	4.54[a]	27.65[a]	27.84	15.12[ab]
ZYβ-Glucanase	40	87.05[ab]	69.9[ab]	4.36[ab]	27.12[ab]	27.08	16.58[abc]
ZYβ-Glucanase	60	86.8[abc]	69.6[b]	3.96[b]	26.4[b]	26.97	16.91[c]
GPβ-Glucanase	0	87.4[a]	71.8[a]	4.98[a]	27.6[a]	27.81	14.74[ab]
GPβ-Glucanase	40	86.9[ab]	69.8[ab]	4.27[ab]	26.44[ab]	27.09	15.76[ab]
GPβ-Glucanase	60	86.6[bc]	69.5[b]	3.87[bc]	26.36[bc]	26.83	16.95[c]
Probability of greater F-value in analysis of variance[4]							
Source of variance							
E[1]		*	*	**	*	NS	*
HB		NS	*	NS	*	NS	NS
E×HB		NS	NS	NS	NS	NS	NS

[a, b, c]: Means within column with no common superscripts differ significantly ($p < 0.05$).
[1] HB: Hull-less barley, E: Enzyme.
[2] Dressed carcass: carcass with neck, feet but feathers and head removed.
[3] Edible carcass: Dressed carcass without feet and visceral.
[4] NS: $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

biological property is also as an important parameter in enzyme activity, because they make it possible to establish such conditions of application that do not decrease the action of lytic enzymes (Fayad *et al.,* 2001). When compared to ZYβ-Glucanase prepared from *Bacillus subtillis,* GPβ-Glucanase from *A. niger* and *T. longibrachiatum* have shown with about 7.8% increased activity. Though, no signifi-

cant difference ($P > 0.05$) was observed between two enzyme preparations. Results obtained from this study suggest that both produced and commercial enzymes are able to degraded β-Glucan and liberate glucose in *in vitro* assay. The selection of suitable source of microorganism has a particular important role in the enzyme production process (Jurgen *et al.,* 1998). β-Glucan in HB is known to affect nutrient

utilization adversely and to give rise to highly viscose conditions in the small intestine (Choct et al., 1996). These effects can be overcome by including exogenous enzyme preparations in the diet (Almirall et al., 1995). In growth assay, supplementation of HB diets with enzyme from either sources significantly ($P<0.05$) promote both BWG and FI, whereas inclusion of enzyme to corn based diets had no beneficial effect on BWG and FI, which are in agreement with those reported earlier (Almirall and Esteve-Garcia, 1995; Ouhida et al., 2000; Bedford and Partridge, 2001). Enzyme supplementation of HB diets resulted in an increase in FI, probably related to a reduction in digesta viscosity, as reported by Hesselman and Aman (1986). On the other hand, this increase in FI resulted in an increase in broiler BWG as previously noted by Svihus et al. (1997). The improvements of performance caused by the β-Glucanase preparations from either source were due to a reduction in β-Glucan concentration in digestive tract of broiler chickens. In the present study, inclusion of HB to diets without enzyme, negatively affected chicken performance as found by Graham and Pettersson (1992); Nahas and Lefrancois (2002). Supplementation with the β-Glucanase did not significantly improved BWG of the finishing broilers. This finding suggests that inclusion of HB in the starting and growing periods influence broiler gains more than in the finishing period. The mature chickens may better adapt to the barley diet. This may be due to the adaptability of intestinal microbes to secrete enzymes for the hydrolysis of β-Glucan (Yu et al., 1998). The presence of a more developed digestive system in mature, compared with immature, birds presumably enables the birds to utilize more efficiently diets rich in viscose polysaccharides (Brake et al., 1997). Rotter et al. (1990) suggested that the gel-forming effect of β-Glucan had a greater influence in the gut of young chicks than older chickens. Inclusion of HB in diets negatively affected FCR as found by Nahas and Lefrancois (2002). β-Glucan caused increase viscosity in digestive tract followed by reduces absorption due to a decrease in the convective transport of nutrients in broiler chicks (Hesselman and Aman, 1986). In addition, β-Glucan caused a thickening of the unstirred water layer, which is considered to be rate limiting in relation to absorption (Bedford and Partridge, 2001). The

viscous intestinal environment and the resultant slower rate of digesta passage and presence of significant amounts of undigested material also lead to the proliferation of microflora in the small intestinal (Almirall et al., 1995). The larger visceral observed in broilers fed on HB as compared with visceral of birds fed HB diets supplemented with either enzyme were in accordance with results reported earlier (Brenes et al., 1993). In this study, decrease in relative visceral weight in HB-fed birds with either enzyme compared with those without enzyme may also contribute to increased carcass yield as previously noted by Brenes et al. (1993). It is likely also that β-Glucanase modify microbial metabolism in the hindgut by reducing the amount of β-Glucan and nitrogen passing into the hindgut. By fermenting and utilizing carbohydrates and protein, the microflora competed effectively with the host for nutrients (Bedford, 1995; Choct et al., 1996). In this study, enzyme addition to diets containing HB further increased nutrient digestibility as indicated by the improved efficiency of feed utilization and increased relative weight of abdominal fat as reported by Brenes et al. (1993). From the results in present study, it appeared that β-Glucanase from either source degraded β-Glucan to low-molecular weight components and decrease digesta viscosity leading to improved nutrient digestion and absorption, which is in good agreement with that reported earlier (Hew et al., 1998).

Conclusion was that GPβ-Glucanase is of great potential and comparetable to ZYβ-Glucanase enzyme for β-Glucan hydrolysis, and can enhance broiler chick performance fed, diet containing 40% HB.

Acknowledgments

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