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Effects of Barley Cultivar and Dietary Supplemental enzyme on Performance, Egg Quality Traits, and Selected Blood Parameters of Laying Hens

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Abstract

The effect of supplementing two commercial multienzyme to diets included two barley cultivars (Sararood [71.99%] and Valfajr [66.39%]) on performance, egg quality, and blood parameters of laying hens was investigated in an 8-wk (65 to 73 wk of age) experiment. The commercial multienzymes were Grindazym™ (with mainly β-glucanase and xylanase activity) and Hemicell® (with mainly β-mannanase activity). Each dietary treatment consisted of five replicates of six hens. Barley cultivar had no significant effect on the measured criteria and there was no interaction between barley cultivar and enzyme throughout the study. Diet supplementation with enzymes reduced feed intake ($P < 0.05$). Hens receiving Grindazym-supplemented diets produced more eggs than those receiving diets without enzyme or supplemented with Hemicell ($P < 0.05$). Conversely, egg weight was higher for hens receiving the Hemicell-supplemented diets than for those fed the other diets ($P < 0.05$). Hens receiving the Grindazym-supplemented diet showed higher egg mass than those fed the unsupplemented diets ($P < 0.05$) and egg mass of hens receiving the Hemicell-supplemented diets was intermediate between these two groups. Feed conversion ratio was improved by enzyme supplementation throughout the study ($P < 0.05$). Serum concentration of triiodothyronine was higher in hens receiving the Grindazym-supplemented diets than that in hens receiving the diets with no enzyme or supplemented with Hemicell ($P < 0.05$). Overall, the nutritive value of barley could be improved by enzyme supplementation. However, the two enzyme sources had different effects on performance of laying hens probably due to different mechanisms of action.

Introduction

The use of barley in poultry diets is limited because of having low metabolizable energy and its negative effects on bird productivity as well as its tendency to increase the incidence of sticky droppings (García *et al*., 2008). These undesirable features have been attributed to β-glucans, a group of non-starch polysaccharides (NSPs), which consists of units of glucose joined by β-1,3 and β-1,4 bonds (Storsley *et al*., 2003). β-glucans form gels in the digestive tract of birds that are not broken down because of the lack of appropriate enzymes and the rapid rate of passage in poultry (Leeson *et al*., 2000). In addition to β-glucans, the most notable components of barley fiber are arabinoxylans (Ferrari *et al*., 2009). Though the soluble

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arabinoxylan content in barley is relatively low, it increases the total concentration of soluble NSP, which ultimately determines the extent of the anti-nutritive effects (Jeroch and Dänicke, 1995). It has also been reported that water-soluble polysaccharides from barley grain contain small amounts of β-mannans (Fincher, 1975; Hrmova *et al*., 2006) which have anti-nutritional properties that hinder the full utilization of nutrients in barley by monogastric animals (Choct, 2015). β-mannan is a mannose polysaccharide that galactose, glucose, or both, often found attached to its backbone (Hsiao *et al*., 2006).

The feeding value of barley for broiler chickens may be enhanced by dietary supplementation of enzymes including β-glucanase and xylanase (García *et al*., 2008; Onderci *et al*., 2008) which reduce intestinal viscosity because of increasing breakdown of β-glucans and, to a lesser extent, arabinoxylans (Jeroch and Dänicke, 1995; Jacob and Pescatore, 2012; Chotinsky, 2015). However, enzyme supplementation of diets for laying hens has been inconsistent (Jacob and Pescatore, 2012). Berg (1959) and Brenes *et al*. (1993) reported no effect of enzyme supplementation on egg weight, shell quality, interior quality, or mortality. Al Bustany and Elwinger (1988) reported that laying hens fed on wheat and barley yielded similar performance values, and thus, the addition of β-glucanase to diets containing barley had no significant beneficial effects. In contrast, Lázaro *et al*. (2003) reported that cereals containing soluble fiber (wheat, barley, and rye) can successfully replace corn in laying hen diets and that enzyme (β-glucanase and/or xylanase) supplementation improves digestibility and productive traits.

Though largely unclear, the discrepancies among results of enzyme supplementation probably depend on the characteristics of the barley, the enzyme sources, and their associated activities. Starch is the main component of barley (Mathlouthi *et al*., 2003) but its nutritional contribution to the diet depends on the proportion of amylose and amylopectin as well as the amount of resistant starch present (McCleary, 2003). In addition, the NSP content of barley, especially of β-glucans, is variable depending on factors such as cultivar (Villamide *et al*., 1997), environmental factors (Scott and Boldaji 1997), and storage condition after harvest (Fuente *et al*., 1998). Janmohammadi *et al*. (2009) reported that Iranian barley cultivars are variable in their nitrogen corrected apparent metabolizable energy (AME_n), starch, crude protein and other chemical content. Villamide *et al*. (1997) reported that starch content of barley ranged between 54.7 and 63.1%, crude protein between 11.2 and 16.5%, and NSP between 10.2 and 16.5% in eight different cultivars. Furthermore, AME_n increased by 7.8% in these cultivars (Villamide *et al*., 1997), whereas Ravindran *et al*. (2007) reported AME_n improvements ranging from 5.5 to 23.1% in different barley cultivars with β-glucanase supplementation. To our knowledge, there is no study about the effect of exogenous β-mannanase on the nutritive value of barley or on the performance of laying hens given diets containing barley. Importantly, β-mannans are also found in several other feed ingredients, including soybean meal, which is almost universally used as protein sources in poultry feed. Therefore, β-mannan is present in the overwhelming majority of poultry diets currently used around the world (Hsiao *et al*., 2006). There are reports in the literature demonstrating performance improvement of laying hens fed β-mannanase supplemented corn-soybean meal-based diets (Jackson *et al*., 1999; Wu *et al*., 2005).

Changes in regulation of metabolism and functioning of the growth-related endocrine system are believed to underlie the changes in nutritional status and productive performance of birds fed enzyme-supplemented barley diets (Gao *et al*., 2008). For example, triiodothyronine (T_3) and thyroxine (T_4) in peripheral blood of laying hens facilitate the differentiation, growth, and development of tissue, promote the formation of protein and enzymes, increase the utilization of carbohydrates, and enhance the disintegration of fats (Ooi *et al*., 2004). Nutritional status is an important factor in the regulation of plasma hormones and intermediary metabolism in laying hens (Swennen *et al*., 2005). So, it is hypothesized that the effects of enzyme supplementation on productive performance may be associated with changes in the concentration of metabolic hormones and metabolites in laying hens fed barley diets. Until now, there has not been a study determining the effects of enzyme supplementation of barley diets on circulating hormone and metabolite levels in laying hens.

The objective of the present study was to examine the effects of recommended inclusion

levels of two commercial enzyme preparations (β-glucanase/xylanase, Grindazym™ and β-mannanase, Hemicell®) on the performance, egg quality traits and blood parameters of laying hens fed diets based on two barley cultivars.

Materials and Methods

Birds and experimental design

All procedures used in this study were approved by the Animal Ethics Committee of Razi University and complied with the guidelines for the care and use of animals in research (FASS, 2010). A total of 180 laying hens (65 wk of age; Lohmann LSL, white egg) were randomly allocated to 30 cages (40 × 40 cm; six hens per unit). Each cage was equipped with an individual feeder and two nipple drinkers. Light was provided for 16 hrs daily and temperature was maintained at 23±3°C throughout the experiment.

Table 1. Ingredients and composition of the basal experimental diets

Item (% unless noted)	Sararood diet	Valfajr diet
Ingredients		
Barley	71.99	66.39
Soybean meal (44% CP)	13.89	17.19
Soybean oil	2.74	2.74
Oyster shell	4.50	4.50
Limestone	4.81	4.79
Dicalcium phosphate	0.67	0.67
Common salt	0.27	0.27
Sand	0.04	2.36
Vitamin premix [1]	0.25	0.25
Mineral premix [2]	0.25	0.25
DL-Methionine	0.09	0.09
Ladyfinger [3]	0.50	0.50
Nutrient composition		
Metabolizable energy (Kcal/kg)	2725	2725
Crude protein	14.58	14.58
Calcium	3.75	3.75
Sodium	0.16	0.16
Nonphytate phosphorus	0.29	0.29
Lysine	0.65	0.65
Methionine	0.30	0.30
Methionine + Cystine	0.56	0.56
Linoleic acid	2.15	2.15

[1] Vitamin premix supplied the following per kg of diet: vitamin A, 18,000 IU; vitamin D_3, 4,000 IU; vitamin E, 36 mg; vitamin K, 4 mg; vitamin B_{12}, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.
[2] Mineral premix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4 mg; Zn, 169.4 mg.
[3] Ladyfinger was added as a source of pigments.

Hens were fed one of six diets, arranged in a factorial design: there were two cultivars of barley (Sararood and Valfajr), which was or was not supplemented with one of two commercial enzyme sources, Grindazym™ GVMG (0.3 g/kg of diet) or Hemicell® (0.5 g/kg of diet). Each diet had five replicates during an 8-wk trial period (Table 1). All diets were provided in mash form and formulated to meet or exceed the nutrient requirements of the Management Guide of Lohmann LSL-Lite (Lohmann Tierzucht, 2005). Grindazym™ GVMG was obtained from Grindsted Products (Danisco Ingredients, Brabrand, Denmark) and was added to supply 3330 units of β-glucanase activity/kg and 1330 units of xylanase activity/kg of diet. Hemicell was provided by ChemGen Co., Ltd. (Shanghai, China) and was supplemented to supply 8250 units of β-mannanase activity/kg of diet. One unit of β-glucanase is defined as the amount of enzyme that liberates 0.27 µmol reducing sugars from β-glucan, measured as glucose equivalents, under the conditions of the assay. One unit of xylanase is defined as the amount of enzyme that liberates one µmol reducing sugars from xylan, measured as xylose equivalents, under the conditions of the assay (Mirzaee *et al*., 2014). One unit of β-mannanase is defined as the amount of enzyme which liberates one µmol of total reducing sugar (glucose equivalence) per min at the optimal enzymatic reaction conditions of pH 3.8 and 65°C (Wu *et al*., 2005).

The two barley cultivars used in the study were both grown in the Agricultural Research Stations of Sararood, Kermanshah, Iran. Proximate analysis of the barley samples was carried out following standard methods of analysis (AOAC, 1995). The contents of total NSP (AOAC, 1995), β-glucans (Aman and Graham, 1987), arabinoxylans (Rouau and Surget, 1994) and β-mannans (Englyst and Cummings, 1984) were measured by colorimetric and chromatographic methods as indicated and AME_n contents were estimated according to the equation developed by Janssen *et al*. (1979). All measurements were completed in 10 replicates.

Performance production and egg quality traits

Productive performance of the laying hens was measured from 65 to 73 wk of age. Egg production and egg weight were recorded daily for each replicate, and the total number of eggs laid per bird and the average egg weight per bird were calculated at the end of the experiment. Egg mass per bird was calculated as number of eggs produced in a replicate × average egg weight. Feed intake was measured weekly and mortality was recorded daily. Data on feed intake and egg mass were used to calculate feed conversion ratio (feed intake/egg mass, g/g). The number of shell-less, soft-shelled, cracked, and broken eggs was also recorded daily by replicate. The incidence of dirty eggs was not measured because it was extremely low and uniform between the groups. All the hens were weighed by replicate at the beginning and end of the experiment and body weight changes were determined.

All the eggs laid during the last three days of the experiment were collected to measure traits of egg quality. Egg specific gravity, eggshell weight, eggshell thickness, albumen height and yolk color were measured on ten eggs from each treatment (two eggs per replicate). Egg specific gravity was determined using 11 gradient saline solutions varying in specific gravity from 1.060 to 1.100 at 0.005-unit increments (Holder and Bradford, 1979). Eggshell thickness was measured using an FHK device (Fujihira Industry Co. Ltd., Tokyo, Japan). Albumen height was documented at three different sites using a spherometer, and Haugh units (HU) –an indicator of interior egg quality– were calculated by the formula $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$ (Eisen *et al*., 1962). Yolks were separated using an egg separator, then weighed. Albumen weight was calculated by subtracting the yolk and eggshell weight from the total egg weight. The yolk index was determined as the ratio of the yolk height to the yolk width and yolk color was compared to the Roche yolk color fan, which ranges from a pale yellow (score 1) to a dark orange (score 15) (Vuilleumier, 1969).

Blood biochemical parameters

Blood samples were collected from wing vein of six randomly selected birds per treatment (one hen per replicate) at the end of the experiment. Blood was collected into sample bottles absent of anticoagulant and then centrifuged at 3000 × *g* for 15 min. The sera were removed and stored at -20˚C until further analysis. Serum glucose, total cholesterol, high-density lipoprotein (HDL-C) cholesterol, and low-density lipoprotein (LDL-C) cholesterol were analyzed using the diagnostic kit (Parsazmun, Tehran, Iran) and enzymatic methods while serum T_3 and T_4 were measured using ELISA kits (Pishtaz Teb, Tehran, Iran). All measurements were conducted in the same assay in triplicate in order to reduce the influence of unavoidable interassay variances.

Statistical analysis

The nutrient contents of the two cultivars were compared using Student's t-test. Data from the *in vivo* experiment were subjected to ANOVA in a completely randomized design with 2 × 3 factorial arrangements of treatments using GLM procedure of SAS software (SAS, 2003). The mean values were compared by Duncan's multiple-range test. All statements of significance were based on a $P < 0.05$. Percentage mortality data were transformed using arcsine method before analysis, and final data were presented as natural numbers.

Results

Barley nutrients contents

The nutrient analysis of the barley samples is given in Table 2. The contents of dry matter, ash, organic matter, starch, ether extract, and crude protein were similar for both barley cultivars. However, nitrogen free extract and metabolizable energy content of Valfajr cultivar were higher ($P < 0.05$) than those of Sararood cultivar. Conversely, crude fiber, total NSP, and total arabinoxylans content were lower in Valfajr than in Sararood cultivar ($P < 0.05$). The content of total β-glucans and total β-mannans were not different between cultivars.

Table 2. Nutrient analysis of the two barley cultivars used in the study (as dry matter basis)

Item (%unless noted)	Cultivar		*SEM*	*P*-values
	Sararood	Valfajr		
Dry matter	89.16	89.63	3.733	0.246
Ash	4.11	3.05	0.501	0.441
Organic matter	95.89	96.95	4.252	0.320
Starch	52.10	52.90	0.974	0.287
Ether extract (EE)	1.46	1.54	0.031	0.251
Crude protein (CP)	11.69	10.50	0.368	0.158
Nitrogen free extract (NFE)	66.17[b]	72.00[a]	1.296	0.002
Crude fiber (CF)	5.73[a]	4.20[b]	0.341	0.037
Total NSP	15.97[a]	12.37[b]	0.955	0.039
Total β-glucans	3.26	3.57	0.111	0.263
Total arabinoxylans	5.44[a]	4.53[b]	0.202	0.041
Total β-mannans	0.27	0.26	0.011	0.929
AME_n (Kcal/kg)	3039[b]	3185[a]	36.2	0.041

NFE = 100 – (% Humidity + % EE + % CF + % CP + % Ash), AME_n = 3,078 - 90.4 × CF + 9.2 × Starch (Janssen *et al*., 1979), total β-glucans was determined according to the method of Aman and Graham (1987), total arabinoxylans was determined as described by Rouau and Surget (1994), total β-mannans was measured by the method of Englyst and Cummings (1984), the other nutrients levels were analyzed following standard methods of analysis (AOAC, 1995).

Productive performance

The effects of dietary treatments on productive performance of laying hens are presented in Table 3. There were no differences detected in feed intake, egg production, egg weight, egg mass and feed conversion ratio between the two barley cultivars. Overall, enzyme supplementation reduced feed intake throughout the experiment ($P < 0.05$). Hens that received the Grindazym-supplemented diets had higher egg production than those fed the diets without enzyme or supplemented with Hemicell ($P < 0.05$). Conversely, egg weight was higher for hens fed the Hemicell-supplemented diet than for those that received the other diets ($P < 0.05$). Hens that received the Grindazym-supplemented diets showed higher egg mass than those fed the unsupplemented diets ($P < 0.05$), and the egg mass for hens receiving the Hemicell-supplemented diets was intermediate between these two groups ($P > 0.05$). Feed conversion ratio was improved by enzyme supplementation ($P < 0.05$). Dietary treatments had no effect on body weight change and mortality percentage during the experiment ($P > 0.05$).

Table 3. Effects of dietary treatments on productive performance of laying hens over eight weeks

Dietary treatments	Feed intake (g/hen/d)	Egg production (%)	Egg weight (g)	Egg mass (g/hen/d)	Feed conversion ratio[4]	Body weight change(g)	Mortality (%)
Barley cultivar							
Sararood	111.9	79.1	64.2	50.8	2.21	47.5	2.07
Valfajr	112.1	77.3	64.4	49.8	2.26	66.6	4.09
Enzyme							
No enzyme	114.5[a]	75.4[b]	63.4[b]	47.9[b]	2.40[a]	43.3	3.88
Grindazym[1]	110.9[b]	82.2[a]	63.5[b]	52.3[a]	2.12[b]	72.5	2.52
Hemicell[2]	110.6[b]	77.0[b]	65.9[a]	50.7[ab]	2.19[b]	55.4	2.83
SEM[3]	0.48	0.97	0.22	0.63	0.031	7.71	0.14
Sources of variation	*P*-values						
Barley cultivar (B)	0.60	0.36	0.51	0.45	0.36	0.22	0.20
Enzyme (E)	<0.0001	0.02	<0.0001	0.03	0.001	0.32	0.65
B × E	0.42	0.64	0.93	0.64	0.71	0.64	0.40

[a, b]Means within a column with different superscripts are significantly different ($P < 0.05$).
[1] 0.3 g/kg of diet Grindazym ™ GVMG (supplied 3330 units of β-glucanase/kg and 1330 units of xylanase activity/kg of diet).
[2] 0.05 g/kg of diet Hemicell® (provided 8250 units of β-mannanase activity/kg of diet).
[3] Standard error of the means.
[4] as g feed/g egg.

Table 4. Effects of treatments on percentage of abnormal eggs (from 1 to 8 wk) and egg quality traits (wk 8) of laying hens

Dietary treatments	Abnormal egg[1] (%)	Shape index	Haugh unit	Yolk index	Yolk color	Specific gravity (g/cm^3)	Shell weight (%)	Shell thickness (mm×10^{-2})
Barley cultivar								
Sararood	3.1	75.3	84.4	43.0	6.73	1.073	5.99	38.0
Valfajr	3.0	75.6	84.0	42.7	6.86	1.074	5.82	37.5
Enzyme								
No enzyme	3.7	75.4	83.6	42.8	6.80	1.077	6.01	37.5
Grindazym[2]	3.0	75.6	84.5	42.9	6.90	1.074	5.81	37.6
Hemicell[3]	2.6	75.5	84.6	42.8	6.70	1.071	5.90	38.3
SEM[4]	0.25	0.35	0.68	0.40	0.148	0.0015	0.060	0.36
Sources of variation	*P*-values							
Barley cultivar (B)	0.85	0.68	0.79	0.77	0.69	0.69	0.19	0.52
Enzyme (E)	0.24	0.97	0.83	0.99	0.88	0.36	0.43	0.70
B× E	0.65	0.30	0.45	0.52	0.96	0.26	0.10	0.13

[1] The percentage of all cracked, broken soft-shelled and shelled egg of total laid eggs.
[2] 0.3 g/kg of diet Grindazym ™ GVMG (supplied 3330 units of β-glucanase/kg and 1330 units of xylanase activity/kg of diet).
[3] 0.05 g/kg of diet Hemicell® (provided 8250 units of β-mannanase activity/kg of diet).
[4] Standard error of the means.

Egg quality traits

Data on egg quality traits are shown in Table 4. Dietary treatments had no significant effect on the percentage of abnormal eggs. Similarly, dietary treatments had no effect on shell weight, shell thickness, specific gravity, shape index, Haugh unit, yolk index, and yolk color ($P > 0.05$).

Serum biochemical parameters

The effects of dietary treatments on serum biochemical concentrations are presented in Table 5. The serum concentration of T_3 was higher for hens receiving the Grindazym-supplemented diets than others ($P < 0.05$). The serum concentrations of T_4, glucose, triglycerides, total cholesterol, HDL-C and LDL-C were not influenced by the dietary treatments ($P > 0.05$).

Table 5. Effects of dietary treatments on serum biochemical parameters of laying hens

Dietary treatments	T_3 [1] (ng/mL)	T_4 [2] (ng/mL)	Glucose (mmol/L)	Triglycerides (mmol/L)	Total cholesterol (mmol/L)	HDL-C [3] (mmol/L)	LDL-C [4] (mmol/L)
Barley cultivar							
Sararood	2.5	12.7	14.3	15.2	9.50	2.48	3.22
Valfajr	2.4	12.1	14.4	16.1	10.72	2.31	3.33
Enzyme							
No enzyme	2.1[b]	11.2	14.0	15.4	9.72	2.24	3.20
Grindazym [5]	3.1[a]	12.2	14.9	16.1	10.86	2.49	3.34
Hemicell [6]	2.0[b]	13.7	14.1	15.4	9.77	2.43	3.30
SEM [7]	0.21	0.80	0.53	1.29	0.627	0.088	0.090
Sources of variation	*P*-values						
Barley cultivar (B)	0.52	0.81	0.92	0.76	0.45	0.44	0.53
Enzyme (E)	<0.0001	0.65	0.77	0.97	0.79	0.61	0.81
B × E	0.46	0.46	0.89	0.39	0.87	0.95	0.80

[a, b]Means within a column with different superscripts are significantly different ($P < 0.05$).
[1] triiodothyronine; [2] thyroxine; [3] high-density lipoprotein cholesterol; [4] low-density lipoprotein cholesterol.
[5] 0.3 g/kg of diet Grindazym ™ GVMG (supplied 3330 units of β-glucanase/kg and 1330 units of xylanase activity/kg of diet).
[6] 0.05 g/kg of diet Hemicell® (provided 8250 units of β-mannanase activity/kg of diet).
[7] Standard error of the means.

Discussion

The contents of dry matter, ash, organic matter, starch, ether extract, and crude protein were similar between Valfajr and Sararood cultivars and within the range of data reported in the literature (NRC, 1994; Villamide *et al*., 1997). However, another study reported higher values of dry matter, ether extract, and crude protein for different barley cultivars (Janmohammadi *et al*., 2009). The content of nitrogen free extract and metabolizable energy of Valfajr cultivar were higher than those of Sararood cultivar, but remain within the range reported by NRC (1994) and Villamide *et al*. (1997) and they are lower than those reported by Janmohammadi *et al*. (2009). The content of crude fiber, total NSP, and total arabinoxylans were lower in the Valfajr cultivar than in the Sararood cultivar, but total β-glucans and total β-mannans were similar. Nonetheless, all measured values were similar to the ones previously reported (Villamide *et al*., 1997; Choct, 2015).

Barley cultivar did not have a significant effect on the measured criteria throughout the study. This may be explained by the low variation of β-glucan content between the cultivars. β-glucan is the main antinutritional factor of barley due to its location in the endosperm of the grain (Henry, 1987). Perez-Vendrell *et al*. (1993) indicated that the variation of β-glucan content between the cultivars is related to the growing and harvest conditions, such as weather, geographical location, and agronomic practices than to the cultivar itself. The barley cultivars we used in these experiments –Valfajr and Sararood– were grown in the same region and were therefore subjected to the same climate factors including temperature, rainfall and soil conditions.

Grindazym and Hemicell supplementation both reduced feed intake. Singh *et al*. (2006) reported that, in broiler chickens, enzyme supplementation reduces intestinal viscosity and

decreases retention time of digesta in the gut, allowing for higher feed intake and therefore improving growth and indirectly feed conversion ratio. Moreover, reduced viscosity increases contact between nutrients and digestive enzymes and thereby improves digestibility (Langhout *et al*., 1997). Lázaro *et al*. (2003) proposed that in laying hens only reduce of intestinal viscosity seems to be effective since they found β-glucanase and xylanase supplementation improved digestibility of most nutrients in barley diets but did not affect feed intake. Other researchers have also indicated no response in feed intake of laying hens with enzyme supplementation of barley diets (Brufau *et al*., 1994; Vukic Vranjes and Wenk, 1996). Such a decrease in feed intake due to β-mannanase supplementation has also not been previously reported (Jackson *et al*., 1999; Zangeneh and Torki, 2011). However, Wu *et al*. (2005) demonstrated that degradation of β-mannanase resulted in more energy available for production in laying hens. Similarly, Radcliffe *et al*. (1999) observed increased apparent energy digestibility in swine with β-mannanase addition to corn-soybean meal based diets.

Enzyme supplementation significantly improved egg mass and feed conversion ratio. Interestingly, egg production was higher in hens that received Grindazym-supplemented diets than for those received diets without enzyme supplementation or diets supplemented with Hemicell. Conversely, egg weight was remarkably higher for hens that received Hemicell-supplemented diets than for those that received the other diets. These results show that Grindazym and Hemicell have different effects on the performance of laying hens and probably exert their effects *via* different modes of action. Lázaro *et al*. (2003) reported that the addition of an enzyme premix containing β-glucanase and xylanase to a laying barley diet resulted in 6.3% improvement in dry matter digestibility, 3.5% increase in fat digestibility and 75% improvement in NSP digestibility. Other researchers have reported a positive response to β-glucanase supplementation on energy and fat digestibility in barley diets, too (Vukic Vranjes and Wenk, 1996). To our knowledge, there is no study that reports the effect of exogenous β-mannanase on digestibility of nutrients in laying hens. However, Azarfar (2013) reported a 22.1% improvement in fat digestibility in broilers when β-mannanase was supplemented to a corn-soybean meal based diet. These reports along with our results suggest that enzyme supplementation increases energy availability of barley diets as well as fat and linoleic acid uptake, which can improve egg production and especially egg weight (Grobas *et al*., 2001). However, these observations do not explain why the two enzymes differed with respect to their effect on laying performance.

The energy content and nutritional composition of feed have been documented to modify the activity of deiodinase (Klandorf and Harvey, 1985) and to regulate the peripheral metabolism of the thyroid hormones in poultry species (Darras *et al*., 2000). In the present study, the serum concentration of T_4 was not affected by dietary treatments. However, hens that received the Grindazym-supplemented diets exhibited the highest serum concentration of T_3, which was a similar trend to that observed for egg production. Sechman *et al*. (2009) reported that the serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were correlated with the serum concentrations of T_3. The oviduct is the place where egg white and eggshell form, and its development and functions can influence the productivity of laying hens. The oviduct is also the target organ for LH and FSH hormones and they can maintain higher secretion of the oviduct, therefore, improving the quantity of egg laying, reducing the feed conversion ratio, and lengthening the span of egg laying (Zuelke and Brackett, 1993). LH concentration in peripheral circulation is directly correlated with the ovulation of layers. Also, FSH can influence the development and maturation of ovarian follicle, which has a synergistic impact with LH on ovulation (Ooi *et al*., 2004). Further studies are needed to verify the results and to clarify the mechanism of actions.

The increase in T_3 concentration following enzyme supplementation is likely to be triggered by changes in the concentrations of energy substrates in the blood (Tivey *et al*., 1993; Hajati, 2010). The results of this study, however, showed no effect of enzyme supplementation on the serum concentrations of glucose, triglycerides, total cholesterol, HDL-C, and LDL-C. Similarly, Gao *et al*. (2008) observed that enzyme supplementation in broiler chickens increased the blood concentration of T_3 and

insulin without affecting blood concentration of glucose. Therefore, the regulation of insulin, which functions in the animal as one of the most important anabolic hormones, may be involved (Fernandes *et al*., 1996). Insulin regulates the rate of glucose entry into body cells, energy metabolism, muscle tissue deposition, fat metabolism and cholesterol metabolism.

The results confirm that enzyme supplementation improves productive traits when added to barley diets. The highest egg production was observed in hens given Grindazym-supplemented diets, whereas the highest egg weight was seen in hens fed on Hemicell-supplemented diets. Hens received diet supplemented with Grindazym had the highest serum concentrations of T_3, suggesting that changes in egg production are probably mediated by changes in thyroid hormone economy. Based on our results, use of Grindazym or Hemicell for barley–soybean meal based diets depends on farmer targeting to have more egg production or heavier egg.

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