Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
http://dx.doi.org/10.5424/sjar/2014124-5929

Spanish Journal of Agricultural Research 2014 12(4): 1071-1081
ISSN: 1695-971X
eISSN: 2171-9292

RESEARCH ARTICLE

OPEN ACCESS

Effects of wheat cultivar, metabolizable energy level, and xylanase supplementation to laying hens diet on performance, egg quality traits, and selected blood parameters

Masoud Mirzaee[1], Mehran Torki[1]* and Mahmood Habibian[1,2]

[1] Department of Animal Science. College of Agriculture and Natural Resources Razi University. Kermanshah 6715685418, Iran. [2] Department of Animal Science. Faculty of Agriculture. University of Kurdistan. Sanandaj 661715175, Iran

Abstract

A 2 × 2 × 2 factorial arrangement of treatments was conducted to evaluate the effects of two dietary apparent metabolizable energy (AME) levels (2,720 and 2,580 kcal kg^{-1} diet) and enzyme (0 and 0.3 g kg^{-1} diet, Grindazym® GP 15,000 with mostly xylanase activity) supplementation on the performance of laying hens fed diets based on two wheat cultivars (Marvdasht and Sardari). Experimental diets were formulated to have a constant energy to protein ratio and were fed to 65-wk-old Lohmann LSL-Lite laying hens for 7 wk. The lower level of AME reduced egg production and egg mass ($p < 0.05$) and increased feed conversion ratio ($p < 0.05$). Enzyme addition increased feed intake of the birds fed a diet with Sardari cultivar ($p < 0.05$) but had no effect on feed intake of the birds fed a diet with Marvdasht cultivar ($p > 0.05$). Nevertheless, birds receiving diets based on Marvdasht cultivar had higher feed intake and egg mass than that of those receiving diets based on Sardari cultivar ($p < 0.05$). The birds fed diets based on Marvdasht cultivar produced less undesired eggs and had better yolk color as compared with the birds fed diets based on Sardari cultivar ($p < 0.05$). The serum concentration of glucose increased by enzyme supplementation when birds receiving lower AME level ($p < 0.05$). These results indicate that enzyme supplementation may have a positive effect on the feed intake of laying hens when fed on wheat-based diets; however, this effect is cultivar dependent and does not necessarily mean that enzyme supplementation always benefit production.

Additional key words: *Triticum aestivum;* feed intake; egg production; serum glucose; leukocyte profile.

Introduction

Feed is the largest single cost in poultry production with the energy content being a major consideration given that birds eat to satisfy an energy requirement (Stilborn & Waldroup, 1990; De Lange & Birkett, 2005). At present, corn (*Zea mays* L.) is the predominant cereal grain used as energy source in poultry diets (Wang *et al.,* 2005). However, it is not always available at a reasonable price in many countries. In such situations, wheat (*Triticum aestivum* L.) may be a more practical alternative, especially when the price difference between corn and wheat is favorable. Wheat has slightly lower energy content than corn, but provides more protein and is richer in many other nutrients including amino acids such as lysine, methionine, arginine, phenylalanine and tryptophan (Çiftci *et al.,* 2003b; Ghobadi & Karimi, 2012). However, it has some negative effects on feed intake, growth rate, and feed conversion ratio (FCR) where inclusion levels exceed 30% (Gutiérrez-Álamo *et al.,* 2008a). This is largely attributed to the physiochemical properties of the non-starch polysaccharides (NSP), which are believed to interfere with the digestion and absorption processes of the small intestine, depressing the availability of nutrients, especially fat and energy procurement

* Corresponding author: torki@razi.ac.ir
Received: 17-03-14. Accepted: 06-10-14.

Abbreviations used: AME (apparent metabolizable energy); BGU (β-glucanase unit); CF (crude fiber); CP (crude protein); DM (dry matter); EE (ether extract); FCR (feed conversion ratio); FXU (fungal xylanase unit); HDL (high density lipoprotein cholesterol); HU (Haugh unit); LDL (low density lipoprotein cholesterol); NSP (non-starch polysaccharides); T3 (triiodothyronine); T4 (thyroxine).

(Choct & Hughes, 1999). However, several other physical and chemical factors can influence energy available from wheat and as a result animal performance. Among them hardness (Carré *et al.,* 2002), pelleting (Ghobadi & Karimi, 2012), starch, crude protein (CP) and ether extract contents (Steenfeldt, 2001; Svihus & Gullord, 2002) can be underlined. Parsaie *et al.* (2006) reported that Iranian wheat cultivars are variable in their apparent metabolizable energy (AME, 1,893 to 3,062 kcal kg^{-1}), NSP (9.6 to 14.9%), CP (9.5 to 14.0%) and other chemicals content. Rafuse *et al.* (2005) reported that CP of five varieties of Canadian wheat varies between 11.4 and 15.5%. In a survey of 18 wheat cultivars, Kim *et al.* (2003) reported that starch content ranged between 58.5 and 73.7%, CP between 9.7 and 19.1%, and NSP between 7.8 and 11.0%. In addition, Mollah *et al.* (1983) found AME ranging between 2,670 and 3,860 kcal kg^{-1} of dry matter (DM) when 22 wheat samples were fed to growing broiler chickens. Moreover, some wheat cultivars considered to be of high quality have produced broilers with unexpectedly low performance (Gutiérrez-Álamo *et al.,* 2008b).

The exogenous xylanase is used to reduce or to eliminate the negative effects of the soluble NSP on performance of young chickens (Bedford & Schultz, 1998; Choct *et al.,* 2004; Gutiérrez-Álamo *et al.,* 2008b; Zhang *et al.,* 2012; Zou *et al.,* 2013), but the effects are limited and inconclusive for laying hens. It has been reported that addition of xylanase to wheat-based layer diets improves egg production rate (Pan *et al.,* 1998). Additionally, Pan *et al.* (1998) reported significant improvements in AME and feed conversion ratio (FCR) with supplementation of a commercial enzyme preparation containing β-glucanase, cellulase, and xylanase, when diets containing 80% wheat or 65% rye were fed to 22-wk-old hens. Mathlouthi *et al.* (2002) found that xylanase supplementation improved egg mass of laying hens fed diets containing 70% wheat and wheat-barley (49% wheat and 20% barley).

As mentioned above, enzyme supplementation can change the nutritional status and improve productive performance of birds fed wheat-included diets, but which are also closely related to the regulation of metabolism and functioning of the growth-related endocrine system. For example, triiodothyronine (T3) and thyroxine (T4) in peripheral blood of laying hens played their physiological functions in many ways such as facilitating the differentiation, growth and development of tissue, promoting the formation of protein and enzymes, increasing the utilization of carbohydrate, and enhancing 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; Xiao-Ying *et al.,* 2010). So we 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 wheat-based diets.

The objective of the present study was to examine the effects of two levels of AME and enzyme supplementation on the performance, egg quality traits and selected blood parameters of laying hens fed diets based on two wheat cultivars.

Material and methods

Birds and experimental design

All procedures used in this 7-wk experiment were approved by the Animal Ethics Committee of Razi University and complied with the guidelines for the care and use of animals in research (Federation of Animal Science Societies, 2010). A total number of 240 65-wk-old Lohmann LSL-Lite laying hens (after production peak) with similar body weight (1,450 ± 14 g) and egg production rate (80.3 ± 3.8%) were randomly distributed in 40 cages (6 hens/cage) in eight experimental groups. The hens were placed in wire-floored cages arranged in a single tier within a conventional open-sided house. The cages were located in an environmentally controlled room with the room temperature kept at 21-23°C and the photoperiod set at 16 h of light (incandescent lighting, 10-lx) and 8 h dark. As is presented in Table 1, eight experimental diets including two wheat cultivars (Marvdasht and Sardari) and two levels of AME (2,720 and 2,580 kcal kg^{-1}) with or without a commercial enzyme (0.0 and 0.3 g kg^{-1} Grindazym® GP 15,000: 36,000 FXU g^{-1} endo-1, 4-β-xylanase EC 3.2.1.8., 15,000 BGU g^{-1} endo-1, 4-β-glucanase EC 3.2.1.4. per g) fed to hens with 5 replicates per diet during 7-wk trial period. One unit of xylanase (FXU) is defined as the amount of enzyme that liberates 1 μmol reducing sugars from xylan, measured as xylose equivalents, under the conditions of the assay. One unit of β-glucanase (BGU) is defined as the amount of enzyme that liberates 0.27 μmol reducing sugars from β-glucan, measured as glucose equiva-

Table 1. Ingredients and nutrients composition of experimental diets (%, unless indicated otherwise)

Wheat cultivar	Marvdasht				Sardari			
AME[1] (kcal kg^{-1})	2,720		2,580		2,720		2,580	
Grindazym[2]	0	0.03	0	0.03	0	0.03	0	0.03
Feed ingredients								
Wheat	72.73	72.73	73.34	73.34	72.17	72.17	72.71	72.71
Soybean meal	13.66	13.66	10.94	10.94	14.69	14.69	11.98	11.98
Soybean oil	1.74	1.74	0.50	0.50	1.74	1.74	0.50	0.50
Oyster shell	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
Lime stone	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69
Dicalcium phosphate	0.83	0.83	0.86	0.86	0.81	0.81	0.84	0.84
Common salt	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Sand	1.60	1.57	4.82	4.79	0.03	—	3.19	3.16
Mineral-vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HCl-Lys	0.03	0.03	0.13	0.13	0.01	0.01	0.11	0.11
DL-Met	0.10	0.10	0.12	0.12	0.10	0.10	0.12	0.12
Calculated analysis								
Crude protein	14.58	14.58	13.58	13.58	14.58	14.58	13.58	13.58
Ether extract	2.61	2.61	1.47	1.47	2.65	2.65	1.51	1.51
Crude fiber	3.36	3.36	3.19	3.19	2.94	2.94	2.77	2.77
Calcium	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Available phosphorous	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Lys	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Met	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Met + Cys	0.57	0.57	0.56	0.56	0.57	0.57	0.56	0.56

[1] AME: apparent metabolizable energy. [2] Grindazym® GP 15,000: 36,000 FXU g^{-1} endo-1, 4-β-xylanase (EC 3.2.1.8.), and 15,000 BGU g^{-1} endo-1, 4- β-glucanase (EC 3.2.1.4.) per g. [3] Mineral-vitamin 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; vitamin A, 18,000 IU; vitamin D3, 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.

lents, under the conditions of the assay. Diets were formulated to have a constant energy to protein ratio; however, the reduced-AME diets were supplemented with DL-methionine and L-lysine in a way that the ratio of total sulfur amino acids to lysine was similar to that in the normal-AME diets (Khajali *et al.,* 2008). All the experimental diets were given in mash form, and the birds had free access to diets and water. The two wheat cultivars used in the study came from the agriculture and natural resources center of Kermanshah province, in 2009. They were selected to have relatively similar starch and CP contents, and to obtain a large range in NSP content according to previously published data (Parsaie *et al.,* 2006). The nutrient analysis of the two wheat cultivars (Table 2) was carried out according to the standard methods of analysis (AOAC, 1995) in order to determine dry matter (DM, method 934.01), crude protein (CP, method 954.01), crude fiber (CF, method 962.09), ether extract (EE, method

Table 2. Nutrient analysis of the two wheat cultivars used in the study[1]

Item[2]	Marvdasht	Sardari
Dry matter (%)	90.85	90.04
Ash (%)	1.51	3.03
Ether extract-EE (%)	1.29	1.32
Crude fiber-CF (%)	3.33	2.63
Crude protein-CP (%)	11.75	11.06
Nitrogen free extract-NFE (%)	72.97	72.00
Starch (%)	69.90	69.17
Soluble NSP[3] (%)	1.30	1.85
Non-soluble NSP (%)	8.34	11.90
Total NSP (%)	9.60	13.75
AME_n (kcal kg^{-1})	3,149	3,091

[1] The composition is given as feed basis. [2] NFE = 100 – (% Humidity + % EE + % CF + % CP + % Ash); apparent metabolizable energy (AME_n) = 34.92 CP + 63.1 EE + 36.42 NFE (NRC, 1994). The other nutrients levels were analyzed following AOAC (1995). [3] NSP: non-starch polysaccharides.

920.39) and ash (method 942.05). A modified method of AOAC (1995, method 991.43) was used for soluble and insoluble NSPs analysis of the wheat cultivars. Briefly, 1 g of dried wheat sample (in duplicate) was subjected to sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase. Then insoluble NSPs were filtered and the residue was washed with warm distilled water. Combined solution of the filtrate and water washings were precipitated with 4 volumes of 95% ethanol for soluble NSPs determination. The precipitate was then filtered and dried. Both soluble and insoluble NSP residues were corrected for protein, ash and blank for the final calculation of soluble dietary fiber and insoluble dietary fiber values. The AME contents were estimated according to NRC (1994).

Performance production and egg quality traits

Production performance of the laying hens was measured from 65 to 72 wk of age. Daily egg production per replicate cage was recorded, and at the end of the experiment, the total number of eggs laid per bird was calculated. Similarly, eggs laid per replicate cage were weighed daily and at the end of the experiment, the average egg weight per bird was calculated. Undesired eggs such as soft-shelled, cracked, and broken were also recorded daily. The generated data (number of eggs and egg weight) were used to calculate egg mass per bird (egg number in replicate × average egg weight). Feed intake and estimated AME intake were measured on a weekly basis. Data on feed intake and egg mass were used to calculate FCR (feed intake/egg mass, g g^{-1}). Body weights were recorded at the beginning and the end of the experiment to determine body weight changes.

For measuring the egg quality characteristics, 3-d eggs from each replicate were collected at the end of the experimental period (wk-7) and weighed. Eggshell weight, eggshell thickness, egg specific gravity, albumen height, yolk index and yolk color were measured on 10 eggs from each treatment (2 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 & Bradford, 1979). The eggshell thickness was measured using a FHK eggshell thickness gauge (Fujihira Co. Ltd., Tokyo, Japan). Haugh units were calculated as an indicator of interior egg quality. Albumen height was documented at three different sites by using a spherometer, and Haugh units (Eisen *et al.,* 1962) were calculated as follows: $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$ where H = albumin height (mm) and W = egg weight (g). Yolks were separated using an egg separator and 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 at score 1 to a dark orange at score 15 (Vuilleumier, 1969).

Blood parameters

Blood samples were collected from the wing vein of six randomly selected birds per treatment (one hen per replicate) at d-35 of the experiment. The blood samples for differential counts of white blood cells (leukocytes profile) were collected into bottles pretreated with heparin, as anti-coagulant. After providing the blood smear and staining by May-Grünwald-Giemsa stain, differential counting of white blood cells (leukocyte profile) was done using light microscope (Gross & Siegel, 1983) and heterophil to lymphocyte (H/L) ratio was calculated. Blood samples for serum metabolites were collected into sample bottles containing no anti-coagulant and then centrifuged (15 min, 3,000 rpm). The sera were removed and stored at –20°C until further analysis. Serum glucose, triglycerides, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) were analyzed using the diagnostic kit (Pars Azmun, Tehran, Iran), and enzymatic methods. Serum cortisol was analyzed with the RIA kit (Pars Azmun, Tehran, Iran) and serum T3 and T4 were measured by an ELISA kit (Pishtaz Teb, Tehran, Iran).

Statistical analysis

Data were subjected to ANOVA in a completely randomized design with 2 × 2 × 2 factorial arrangements of treatments using GLM procedure of SAS (v.9.1, SAS Inst. Inc., Cary, NC, USA). All statements of significance are based on a probability of < 0.05. The mean values were compared by least significant difference (LSD) test. The following model was considered for analysis:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + \\ + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijkl},$$

where Y_{ijkl} is the characteristic that was measured; μ is the overall mean; A_i is main effect of the wheat cultivar; B_j is the main effect of AME; C_k is the main effect of enzyme; $(AB)_{ij}$ is the effect of the interaction between wheat cultivar and AME; $(AC)_{ik}$ is the effect of the interaction between wheat cultivar and enzyme; $(BC)_{jk}$ is the effect of the interaction between AME and enzyme; $(ABC)_{ijk}$ is the three-way interaction of the wheat cultivar, AME, and enzyme; and ε_{ijkl} is the random error term. Where the interaction effect was significant, the effects of the main factors were not discussed.

Results

Performance production

The effects of dietary treatments on production performance of laying hens during the 7-wk period of the study are presented in Table 3. The results indicated that a decrease in the energy content of the diet had no effect on feed intake ($p > 0.05$), but resulting in a decrease in AME intake ($p < 0.05$). The AME reduction reduced egg production and egg mass ($p < 0.05$). However, the AME concentration of the diet did not affect egg weight. Moreover, the birds receiving a diet with a low AME content had lower body weight and higher FCR as compared with birds receiving a diet with a normal AME content ($p < 0.05$). Enzyme addition had no significant effect on production performance and there was no interaction between AME and enzyme on the measured performance criteria. An interaction was detected between wheat cultivar and enzyme on feed intake and AME intake ($p < 0.05$). Dietary enzyme addition caused an increase in feed intake and AME intake of the birds fed the diet with Sardari cultivar ($p < 0.05$) but had no significant effect on the feed intake or AME intake of the birds receiving Marvdasht cultivar. Nevertheless, birds receiving a diet with Marvdasht cultivar had higher feed intake and

Table 3. Effect of dietary wheat cultivar, apparent metabolizable energy (AME) level and enzyme (Grindazym) supplementation on production performance of laying hens (65 to 72 wk of age)

	Egg production (%)	Egg weight (g)	Egg mass (g hen^{-1} day^{-1})	Feed intake[1] (g hen^{-1} day^{-1})	AME intake (kcal hen^{-1} day^{-1})	Feed conversion (g feed g^{-1} egg)	Energy efficiency (kcal AME g^{-1} egg)	Body weight change (g)
Wheat cultivar								
Sardari	73.39	62.96	41.19^{b}	114.69	303.89	2.46	6.51	99.22
Marvdasht	77.16	63.80	50.23^{a}	117.77	312.11	2.37	6.27	96.27
AME (kcal kg^{-1})								
2,580	71.39^{b}	63.25	46.05^{b}	116.34	300.15^{b}	2.54^{a}	6.57	30.67^{b}
2,720	79.16^{a}	63.50	51.37^{a}	116.12	315.85^{a}	2.28^{b}	6.20	164.83^{a}
Enzyme (g kg^{-1})								
0.0	73.91	63.22	47.71	115.81	306.88	2.45	6.51	96.81
0.3	76.65	63.52	49.71	116.65	309.13	2.37	6.26	98.68
SEM[2]	0.856	0.265	0.847	0.493	1.817	0.035	0.098	11.495
Sources of variation[3]								
Wheat cultivar (W)	NS	NS	0.04	< 0.01	< 0.01	NS	NS	NS
AME	< 0.01	NS	<0.01	NS	<0.01	< 0.01	NS	< 0.01
Enzyme (E)	NS	NS	NS	NS	NS	NS	NS	NS
W × AME	NS	NS	NS	NS	NS	NS	NS	NS
W × E	NS	NS	NS	0.02	0.02	NS	NS	NS
AME × E	NS	NS	NS	NS	NS	NS	NS	NS
W × AME × E	NS	NS	NS	NS	NS	NS	NS	NS

[1] As feed basis. [2] SEM: standard error of means. [3] *p* values. [a,b] Means (n = 5) within column with different superscripts are significantly different ($p < 0.05$), LSD test were applied to compare means. NS: not significant.

AME intake ($p < 0.05$) and exhibited higher egg mass than that of those receiving a diet with Sardari cultivar ($p < 0.05$).

Egg quality traits

Egg quality traits of the experimental groups are summarized in Table 4. The birds receiving a diet with Marvdasht cultivar had better yolk color compared to those receiving a diet with Sardari cultivar ($p<0.05$). Moreover, birds receiving a diet with Marvdasht cultivar produced lower undesired eggs ($p<0.05$). No significant effect of dietary treatments was found on egg index, yolk index, Haugh unit, eggshell weight and eggshell thickness.

Blood parameters

As shown in Table 5, among the serum biochemical parameters (glucose, triglycerides, total cholesterol, HDL and LDL) only serum level of glucose was affected by two-way interaction between AME level and enzyme ($p < 0.05$). Enzyme supplementation increased serum level of glucose when birds receiving a diet with low AME level ($p < 0.05$) but had no significant effect on serum glucose concentration when birds receiving a diet with normal AME level. No significant effect of dietary treatments was found on serum levels of T3, T4 and cortisol hormones (Table 5). Similarly, differential counts of white blood cells did not differ among birds receiving different dietary treatments (Table 6).

Discussion

The results of the present study indicated that a decrease in the energy content of the diet from 2,720 to 2,580 kcal kg^{-1} had no significant effect on feed intake, but resulting in a net decrease in AME intake of 5%. This was unexpected as others (Stilborn & Waldroup, 1990; De Lange & Birkett, 2005) have re-

Table 4. Effect of dietary wheat cultivar, apparent metabolizable energy (AME) level and enzyme (Grindazym) supplementation on egg quality characteristics of laying hens (65 to 72 wk of age)

Items	Undesired eggs[1] (%)	Shell weight (g)	Shell thickness (mm × 10^{-2})	Specific gravity	Egg shape index	Haugh unit	Yolk index	Yolk color (Roche)
Wheat cultivar								
Sardari	1.37[a]	5.86	38.15	1.075	76.03	87.17	45.54	1.00[b]
Marvdasht	0.77[b]	5.78	37.75	1.072	74.47	87.38	44.60	1.20[a]
AME (kcal kg^{-1})								
2,580	1.21	5.72	37.68	1.074	75.15	86.26	45.62	1.15
2,720	0.98	2.92	38.30	1.073	75.37	88.29	44.52	1.05
Enzyme (g kg^{-1})								
0.0	1.9	5.87	38.28	1.073	75.06	86.52	45.16	1.10
0.3	0.84	5.78	37.61	1.074	75.43	87.95	44.99	1.10
SEM[2]	0.133	0.090	0.440	0.001	0.279	0.565	0.346	0.042
Sources of variation[3]								
Wheat cultivar (W)	0.05	NS	NS	NS	NS	NS	NS	0.04
AME	NS	NS	NS	NS	NS	NS	NS	NS
Enzyme (E)	NS	NS	NS	NS	NS	NS	NS	NS
W × AME	NS	NS	NS	NS	NS	NS	NS	NS
W × E	NS	NS	NS	NS	NS	NS	NS	NS
AME × E	NS	NS	NS	NS	NS	NS	NS	NS
W × AME × E	NS	NS	NS	NS	NS	NS	NS	NS

[1] The percentage of all cracked, broken and shellness egg of total laid eggs. [2] SEM: standard error of means. [3] *p* values. [a, b] Means (n = 15) within column with different superscripts are significantly different ($p < 0.05$), LSD test. NS: not significant.

Table 5. Effect of dietary wheat cultivar, apparent metabolizable energy (AME) level and enzyme (Grindazym) supplementation on selected serum hormone and metabolite concentrations of laying hens (70 wk of age)

Items	T3 (ng mL^{-1})	T4 (µg dL^{-1})	Cortisol (µg dL^{-1})	Glucose (mg dL^{-1})	Triglycerides (mg dL^{-1})	Total cholesterol (mg dL^{-1})	LDL (mg dL^{-1})	HDL (mg dL^{-1})
Wheat cultivar								
Sardari	0.42	0.16	1.93	287.47	1,864.00	200.47	68.57	47.68
Marvdasht	0.35	0.16	1.73	260.50	1,971.00	195.22	67.27	49.16
AME (kcal kg^{-1})								
2,580	0.39	0.13	1.78	278.57	1,977.57	203.26	69.63	49.52
2,720	0.38	0.18	1.91	260.39	1,852.83	192.27	66.16	47.22
Enzyme (g kg^{-1})								
0.0	0.40	0.21	1.81	269.84	1,709.89	188.00	63.63	45.36
0.3	0.36	0.11	1.85	269.61	2,135.39	208.39	72.50	51.61
SEM[1]	0.008	0.008	0.048	7.237	129.605	9.436	2.253	2.177
Sources of variation[2]								
Wheat cultivar (W)	NS	NS	NS	NS	NS	NS	NS	NS
AME	NS	NS	NS	NS	NS	NS	NS	NS
Enzyme (E)	NS	NS	NS	NS	NS	NS	NS	NS
W × ME	NS	NS	NS	NS	NS	NS	NS	NS
W × E	NS	NS	NS	NS	NS	NS	NS	NS
AME× E	NS	NS	NS	0.02	NS	NS	NS	NS
W × AME × E	NS	NS	NS	NS	NS	NS	NS	NS

[1] SEM: standard error of means. [2] *p* values. NS: not significant.

ported that laying hens will increase feed intake when fed low AME diets. However, similar findings have been cited (Jalal *et al.,* 2007). Likewise, Jalal *et al.* (2006) reported no significant effect of dietary AME level (2,800, 2,850 and 2,900 kcal kg^{-1} of diet) on feed intake in laying hens from 20 to 35 wk of age. On the other hand, they found no effect of dietary AME level on the egg production and egg mass, whereas in the present study, the AME reduction significantly reduced egg production and egg mass in the overall experimental period. Similar results have been reported by Mathlouthi *et al.* (2002) comparing diets with 2,650 and 2,750 kcal of AME kg^{-1}. In contrast, Harms *et al.* (2000), in Single Comb White Leghorn (SCWL) hens fed diets varying from 2,500 to 3,100 kcal of AME kg^{-1}, did not detect any significant difference in egg production with changes in the energy content of the diet. Moreover, in the present study the birds receiving a diet with a low AME content had significantly lower body weight as compared with birds receiving a diet with a normal AME content.

The AME concentration of the diet did not affect egg weight, which is consistent with the results reported by Çiftci *et al.* (2003b), Pérez-Bonilla *et al.* (2012) and Li *et al.* (2013). On the other hand, Bouvarel *et al.* (2010) analyzed data from 11 experiments conducted for the last 20 years and reported that egg weight increased 0.96 g per each 10 kcal of extra energy intake per day. The reasons for the inconsistencies in relation to the effects of an increase in AME content of the diet on egg weight are not apparent but might depend on the fat and the linoleic acid content of the diets. When the AME concentration of the diet increases, there is usually a concomitant increase in both fat and linoleic acid contents. If the linoleic acid content of the control diet is below hen requirements, an increase in AME will result in higher intake of this nutrient and increase in egg weight (Grobas *et al.,* 2001). In the current study, the level of dietary added fat increased from 0.50 to 1.74% as the energy content of the diet increased. Grobas *et al.* (1999, 2001) reported that an increase in fat content of the diet resulted in increase in egg weight. However, they suggested that laying hens require no more than 1.15% linoleic acid in the diet to maximize egg weight and that when this minimal amount of linoleic acid was met, an increase in

Table 6. Effect of dietary wheat cultivar, apparent metabolizable energy (AME) level and enzyme (Grindazym) supplementation on differential counts of white blood cells and heterophil to lymphocyte (H/L) ratio in laying hens (70 wk of age)

Items	Heterophil	Lymphocyte	Monocyte	Eosinophil	Basophil	H/L ratio
Wheat cultivar						
Sardari	37.60	59.40	0.80	0.75	1.40	0.67
Marvdasht	34.26	64.22	0.33	0.50	1.05	0.54
AME (kcal kg^{-1})						
2,580	37.40	59.95	0.70	0.75	1.35	0.66
2,720	34.22	63.61	0.44	0.50	1.11	0.56
Enzyme (g kg^{-1})						
0.0	36.26	61.68	0.57	0.42	1.05	0.63
0.3	35.52	61.68	0.57	1.00	1.42	0.59
SEM[1]	1.268	9.43	1.177	0.081	0.020	0.023
Sources of variation[2]						
Wheat cultivar (W)	NS	NS	NS	NS	NS	NS
AME	NS	NS	NS	NS	NS	NS
Enzyme (E)	NS	NS	NS	NS	NS	NS
W × AME	NS	NS	NS	NS	NS	NS
W × E	NS	NS	NS	NS	NS	NS
AME × E	NS	NS	NS	NS	NS	NS
W × AME × E	NS	NS	NS	NS	NS	NS

[1] SEM: standard error of means. [2] *p* values. NS: not significant.

supplemental fat resulted in further increases in egg weight, irrespective of its linoleic acid content.

The AME reduction significantly increased FCR, in agreement with most previous reports (Grobas *et al.,* 1999; Pérez-Bonilla *et al.,* 2012; Li *et al.,* 2013). In contrast, Keshavarz (1998) reported no difference in FCR in SCWL hens from 18 to 66 wk of age fed diets with 2,820 or 3,040 kcal of AME kg^{-1}. Pérez-Bonilla *et al.* (2012) noted that hens given the high-energy diet may have lower feed intake but have higher energy intake than hens fed the normal or low-energy diets, but the excess of energy was used for increases in body weight rather than for improvements in egg mass production. Consequently, the efficiency of converting feed energy to egg mass was hindered when the very high-energy diet was used.

Enzyme addition had no significant effect on laying hens' performance and there was no interaction between AME and enzyme on the measured performance criteria. However, Mathlouthi *et al.* (2002) found that xylanase addition in laying hens fed with low-AME wheat-based diets was equivalent to an increase of at least 100 kcal of AME kg^{-1}. They also reported significant improvement of egg production, egg mass and FCR as a result of dietary xylanase supplementation. The experimental period could be considered among the factors responsible for differences obtained in the present study. Mathlouthi *et al.* (2003) reported no significant improvement in egg production, egg weight or egg mass when a commercial enzyme preparation containing xylanase and β-glucanase were added to a corn-soybean meal diet fed to 45-wk-old laying hens for 9 wk (from 45 to 54 wk of age). They concluded that enzyme supplementation might be beneficial during production peak because laying hens need high levels of nutrients to maintain body growth and high egg production. To support this view, Mirzaie *et al.* (2012) found that xylanase supplementation increased egg production and egg mass and improved FCR per kg of eggs throughout the experimental period (25 to 47 wk of age), with the benefits being more pronounced during the first stage of the laying period (25 to 33 wk of age).

An interesting interaction was detected between wheat cultivar and enzyme on feed intake and AME intake. Dietary enzyme addition caused an increase in feed intake and AME intake of the birds fed the diet with Sardari cultivar but had no significant effect on the feed intake or AME intake of the birds receiving

Marvdasht cultivar. The greater response of Sardari cultivar to enzyme may be partly related to the NSP content of it (13.90 *vs.* 9.60% for Sardari and Marvdasht cultivars, respectively). According to the range provided by Parsaie *et al.* (2006), poor quality wheats tend to have greater responses to enzyme. Nevertheless, birds receiving a diet with Marvdasht cultivar had significantly higher feed intake and AME intake and exhibited significantly higher egg mass than that of those receiving a diet with Sardari cultivar. Pirgozliev *et al.* (2010) examined the effect of dietary xylanase (400, 800, 1,200, or 1,600 FXU kg^{-1} diet) on the availability of nutrients for laying hens when fed on wheat-rye-soy-based diets and reported that the AME and nitrogen metabolizability coefficients of xylanase-supplemented diets were greater than the control diet. In addition, they reported that xylanase supplementation significantly improved the coefficients of metabolizability of indispensable, dispensable and total amino acids.

The wheat cultivar significantly affected yolk color and percentage of undesired eggs. The birds receiving a diet with Marvdasht cultivar had better yolk color compared to those receiving a diet with Sardari cultivar. The higher soluble carotenoid content of the Marvdasht cultivar might be involved, which were not analyzed in the present study.

No significant effect of dietary treatments was found on egg index, yolk index, Haugh unit, eggshell weight and eggshell thickness. Typically, xylanase addition to layer feed appears to have little effects on egg quality traits. Mirzaie *et al.* (2012) found no effect of dietary xylanase on any of the egg quality traits, except eggshell thickness at 47 wk of age that was improved by xylanase supplementation in laying hens fed diets containing 23 to 69% wheat. Roberts & Choct (2006) reported that enzyme supplementation improved eggshell breaking strength in hens fed wheat-based diets. In contrast, Rafuse *et al.* (2004) observed that diets based on wheat with a mixture of xylanase and protease did not affect albumen height, or weight of yolk, eggshell and albumen when measured 3 wk (32 wk of age) after the hens began consuming the experimental diets. Likewise, Çiftci *et al.* (2003a) reported no effect on eggshell thickness when 30% corn was substituted by wheat in diets for SCWL hens from 27 to 43 wk of age.

The information available on the effects of AME level of wheat-based diets on egg-quality traits is scarce. Çiftci *et al.* (2003b) reported no effect on egg shape index, breaking strength and shell thickness when Hisex Brown laying hens fed wheat-based diets containing two levels of AME (2,680 and 2,790 kcal kg^{-1}). Similar results were reported by other researchers (Jalal *et al.,* 2007; Novak *et al.,* 2008; Pérez-Bonilla *et al.,* 2012; Li *et al.,* 2013) who evaluated the effect of different dietary AME levels on egg quality traits in laying hens fed corn-based diets.

Thyroid hormones play an important role on the regulation of general metabolism, growth and tissue differentiation as well as gene expression (Ooi *et al.,* 2004). According to Collin *et al.* (2003), enzyme addition can directly or indirectly promote the activity of deiodinase in liver and kidney tissues, and, thus, promoting the transformation of T4 into T3. However, in the present study, dietary treatments did not have any significant influence on the serum concentration of T4. Similarly, serum levels of triglycerides, total cholesterol, HDL, LDL, and cortisol hormone as well as differential counts of white blood cells did not differ among birds receiving different dietary treatments. However, serum level of glucose was affected by two-way interaction between AME level and enzyme. Enzyme supplementation increased serum level of glucose when birds receiving a diet with low AME level but had no significant effect on serum glucose concentration when birds receiving a diet with normal AME level. Enzyme addition can increase intestinal starch digestibility (Choct *et al.,* 1999) and sugar absorption; however, blood glucose concentration depends upon many factors such as blood levels of insulin and glucagon.

In conclusion, the results of the present study suggest that enzyme supplementation may have a positive effect on the feed intake of laying hens when fed on wheat-based diets; however, its effect is cultivar dependent and does not necessarily mean that enzyme supplementation always benefit production. Further, the results do not support the idea that laying hens tend to increases feed intake when fed low-AME diets.

Acknowledgments

Funding of this work by Razi University (Kermanshah, Iran) is kindly appreciated.

References

AOAC, 1995. Official methods of analysis of AOAC International, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Bedford MR, Schultz H, 1998. Exogenous enzymes for pigs and poultry. Nutr Res Rev 11: 91-114.

Bouvarel I, Nys Y, Panheleux M, Lescoat P, 2010. Comment l'alimentation des poules influence la qualité des oeufs. INRA Prod Anim 23: 167-182.

Carré B, Idi A, Maisonnier S, Melcion JP, Oury FX, Gómez J, Pluchard P, 2002. Relationships between digestibilities of food components and characteristics of wheats (*Triticum aestivum*) introduced as the only cereal source in a broiler chicken diet. Br Poult Sci 43: 404-415.

Choct M, Hughes RJ, 1999. Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. Aust J Agric Res 50: 689-702.

Choct M, Hughes RJ, Bedford MR, 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. Br Poult Sci 40: 419-422.

Choct M, Kocher A, Waters DLE, Pettersson D, Ross G, 2004. A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. Br J Nutr 92: 53-61.

Çiftci I, Yenice E, Eleroglu H, 2003a. Use of triticale alone and in combination with wheat or maize: effects of diet type and enzyme supplementation on hen performance, egg quality, organ weights, intestinal viscosity, and digestive system characteristics. Anim Feed Sci Technol 105: 149-161.

Çiftci I, Yenice, E, Gökçeyrek D, Öztürk E, 2003b. Effects of energy level and enzyme supplementation in wheat-base layer diets on hen performance and egg quality. Acta Agric Scand A Anim Sci 53: 113-119.

Collin A, Malheiros RD, Moraes V, Van As P, Darras VM, Taouis M, Decuypere E, Buyse J, 2003. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. Br J Nutr 90: 261-269.

De Lange CFM, Birkett SH, 2005. Characterization of useful energy content in swine and poultry feed ingredients. Can J Anim Sci 85: 269-280.

Eisen EJ, Bohren BB, McKean HE, 1962. The Haugh unit as a measure of egg albumen quality. Poult Sci 41: 1461-1468.

Federation of Animal Science Societies Writing Committee, 2010. Guide for the care and use of agricultural animals in research and teaching, 3rd ed. Champaign-Urbana, IL, USA.

Ghobadi Z, Karimi A, 2012. Effect of feed processing and enzyme supplementation of wheat-based diets on performance of broiler chicks. J Appl Anim Res 40: 260-266.

Grobas S, Méndez J, De Blas C, Mateos GG, 1999. Laying hen productivity as affected by energy, supplemental fat, and linoleic acid concentration of the diet. Poult Sci 78: 1542-1551.

Grobas S, Méndez J, Lázaro R, De Blas C, Mateos GG, 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poult Sci 80: 1171-1179.

Gross WB, Siegel HS, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis 27: 972-978.

Gutiérrez-Álamo A, Pérez de Ayala P, Verstegen MWA, Den Hartog LA, Villamide MJ, 2008a. Variability in wheat: factors affecting its nutritional value. World's Poult Sci J 64: 20-39.

Gutiérrez-Álamo A, Verstegen MWA, Den Hartog LA, Pérez de Ayala P, Villamide MJ, 2008b. Effect of wheat cultivar and enzyme addition to broiler chicken diets on nutrient digestibility, performance, and apparent metabolizable energy content. Poult Sci 87: 759-767.

Harms RH, Russell GB, Sloan DR, 2000. Performance of four strains of commercial layers with major changes in dietary energy. J Appl Poult Res 9: 535-541.

Holder DP, Bradford MV, 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs and percent shell. Poult Sci 58: 250-251.

Jalal MA, Scheideler SE, Marx D, 2006. Effect of bird cage space and dietary metabolizable energy level on production parameters in laying hens. Poult Sci 85: 306-311.

Jalal MA, Scheideler SE, Pierson EM, 2007. Strain response of laying hens to varying dietary energy levels with and without Avizyme supplementation. J Appl Poult Res 16: 289-295.

Keshavarz K, 1998. The effect of light regimen, floor space, and energy and protein levels during the growing period on body weight and early egg size. Poult Sci 77: 1266-1279.

Khajali F, Khoshouie EA, Dehkordi SK, Hematian M, 2008. Production performance and egg quality of Hy-line W36 laying hens fed reduced-protein diets at a constant total sulfur amino acid: lysine ratio. J Appl Poult Res 17: 390-397.

Kim JC, Mullan BP, Simmins PH, Pluske JR, 2003. Variation in the chemical composition of wheats grown in Western Australia as influenced by variety, growing region, season and post-harvest storage. Aust J Agric Res 54: 541-550.

Li F, Zhang LM, Wu XH, Li CY, Yang XJ, Dong Y, Lemme A, Han JC, Yao JH, 2013. Effects of metabolizable energy and balanced protein on egg production, quality, and components of Lohmann Brown laying hens. J Appl Poult Res 22: 36-46.

Mathlouthi N, Larbier M, Mohamed MA, Lessire M, 2002. Performance of laying hens fed wheat, wheat-barley or wheat-barley-wheat bran based diets supplemented with xylanase. Can J Anim Sci 82: 193-199.

Mathlouthi N, Mohamed AMA, Larbier M, 2003. Effect of enzyme preparation containing xylanase and β-glucanase on performance of laying hens fed wheat/barley-or maize/soybean meal-based diets. Br Poult Sci 44: 60-66.

Mirzaie S, Zaghari M, Aminzadeh S, Shivazad M, Mateos GG, 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. Poult Sci 91: 413-425.

Mollah Y, Bryden WL, Wallis IR, Balnave D, Annison EF, 1983. Studies on low metabolisable energy wheats for

poultry using conventional and rapid assay procedures and the effects of processing. Br Poult Sci 24: 81-89.

Novak CL, Yakout HM, Remus J, 2008. Response to varying dietary energy and protein with or without enzyme supplementation on leghorn performance and economics. 2. Laying period. J Appl Poult Res 17: 17-33.

NRC, 1994. Nutrient requirements of poultry, 9th rev ed. Natl Acad Press, Washington, DC, USA.

Ooi GT, Tawadros N, Escalona RM, 2004. Pituitary cell lines and their endocrine applications. Mol Cell Endocrinol 228: 1-21.

Pan CF, Igbasan FA, Guenter W, Marquardt RR, 1998. The effects of enzyme and inorganic phosphorus supplements in wheat and rye based diets on laying hen performance, energy, and phosphorus availability. Poult Sci 77: 83-89.

Parsaie S, Shariatmadari F, Zamiri MJ, Khajeh K, 2006. Evaluation of starch, soluble and insoluble non-starch polysaccharides and metabolizable energy of 15 cultivars of Iranian wheat. J Agric Soc Sci 2: 260-263.

Pérez-Bonilla A, Novoa S, García J, Mohiti-Asli M, Frikha M, Mateos GG, 2012. Effects of energy concentration of the diet on productive performance and egg quality of brown egg-laying hens differing in initial body weight. Poult Sci 91: 3156-3166.

Pirgozliev V, Bedford MR, Acamovic T, 2010. Effect of dietary xylanase on energy, amino acid and mineral metabolism, and egg production and quality in laying hens. Br Poult Sci 51: 639-647.

Rafuse JL, Silversides FG, Bedford MR, Simmins PH, 2004. Effect of wheat cultivar and enzyme supplementation on nutrient availability and performance of laying hens. Can J Anim Sci 84: 397-402.

Rafuse JL, Silversides FG, Bedford MR, Simmins PH, 2005. Effect of cultivar and enzyme supplementation on nutrient availability and performance of broilers fed Maritime Canadian wheat. Can J Anim Sci 85: 493-499.

Roberts JR, Choct M, 2006. Effects of commercial enzyme preparations on egg and eggshell quality in laying hens. Br Poult Sci 47: 501-510.

Steenfeldt S, 2001. The dietary effect of different wheat cultivars for broiler chickens. Br Poult Sci 42: 595-609.

Stilborn HL, Waldroup PW, 1990. An evaluation of low-energy feedstuffs in diets for laying hens. Anim Feed Sci Technol 27: 327-339.

Svihus B, Gullord M, 2002. Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. Anim Feed Sci Technol 102: 71-92.

Swennen Q, Janssens GPJ, Millet S, Vansant G, Decuypere E, Buyse J, 2005. Effect of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: endocrine functioning and intermediary metabolism. Poult Sci 84: 1051-1057.

Vuilleumier JP, 1969. The "Roche yolk colour fan" – An instrument for measuring yolk colour. Poult Sci 48: 767-779.

Wang ZR, Qiao SY, Lu WQ, Li DF, 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult Sci 84: 875-881.

Xiao-Ying D, Chu-Fen Y, Sheng-Qiu T, Qing-Yan J, Xiao-Ting Z, 2010. Effect and mechanism of glutamine on productive performance and egg quality of laying hens. Asian-Aust J Anim Sci 23: 1049-1056.

Zhang GG, Yang ZB, Zhang QQ, Yang WR, Jiang SZ, 2012. A multienzyme preparation enhances the utilization of nutrients and energy from pure corn and wheat diets in broilers. J Appl Poult Res 21: 216-225.

Zou J, Zheng P, Zhang K, Ding X, Bai S, 2013. Effects of exogenous enzymes and dietary energy on performance and digestive physiology of broilers. J Anim Sci Biotechnol 4: 14-22.