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Does the Type of Cereal Grain in the Diet Influence Egg Quantity and Quality Measures of Laying Hens Without the Use of Carbohydrases?

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Abstract

An experiment was performed to evaluate the influence of the type of dietary cereal grains, in the absence of carbohydrases, on egg quantity and quality measures of laying hens. A number of 72 laying hens (Hy-Line W80) were randomly assigned to three treatment groups (corn, wheat, and barley diets). Hens (81 weeks of age) were beginning their second laying cycle after a non-fasted molting practice. The duration of the experiment covered 81 to 90 weeks of age. Results indicated that egg production and egg mass were superior in hens that received corn and wheat when compared to hens fed with barley. Such a difference was significant ($P < 0.05$) in weeks 8 and 9. Egg weight was not affected by the type of cereal grain. Feeding barley caused a poor feed conversion ratio (FCR) as opposed to feeding corn or wheat, which significantly differed in weeks 4, 8, and 9. The egg yolk color index was significantly ($P < 0.05$) greater in hens on the corn diet than those on the wheat or barley diets. Feeding hens with barley worsened body weight uniformity in comparison with feeding corn or wheat. Hens on the wheat diet exhibited a greater concentration of hemoglobin relative to the corn or the barley diet ($P < 0.05$). The plasma content of total cholesterol was higher in hens fed with corn than hens consumed wheat or barley ($P < 0.05$). The type of cereal grain did not significantly affect eggshell thickness. In conclusion, the type of cereal grain compromised productive performance without any significant effect on eggshell quality measures when no carbohydrase enzyme was supplemented.

Introduction

Poultry diets are commonly composed of corn grain as the main cereal in most parts of the world. Recent trends in consuming corn for the biofuel industry have had an impact on the availability and cost of corn. The production of wheat and barley seems like a potential strategy in terms of economic and sustainability considerations since it requires water and fertilizer to a lesser extent (Grote *et al*., 2021).

Wheat and barley are more fibrous and they have a lower metabolizable energy (ME) content compared to corn. Substituting wheat and barley for corn has been reported to affect the performance of broiler chickens. Broiler chickens require high-energy diets ranging from 3000 to 3200 kcal ME/kg. In contrast, laying hens do not need such high-energy diets, and their dietary ME contents typically fall between 2750 to 2900 kcal/kg. Additionally, feeding laying hens with high-energy diets can lead to the development of fatty liver hemorrhagic syndrome (Rozenboim *et al*., 2016). Taken together, wheat and barley could be used in laying hen diets without compromising their productive performance and health status.

Liebert *et al.* (2005) substituted wheat for corn without the use of carbohydrases in laying hens (Lohmann Brown) from 22 to 61 wk of age. These researchers reported no significant difference. Lázaro *et al.* (2003) indicated that the substitution of wheat for corn did not affect egg production and egg quality of laying hens (Lohmann White) from 20 to 44 wk of age. Safaa *et al.* (2009) evaluated the productive

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performance and egg quality of laying hens (Lohmann Brown) from 20 to 48 wk of age in response to corn or wheat diets. They found no significant difference when corn was replaced by wheat. Roberts *et al.* (2007) revealed that the inclusion of high-fiber feedstuffs in diets of laying hens (Hy-Line W-36) had no significant effect on egg production, but it could reduce ammonia emission from manure, which was considered a positive impact on the environment. In addition, feeding high-fiber diets to laying hens is associated with a lower incidence of feather pecking and cannibalism, which is an additional benefit from the animal welfare point of view (Desbruslais *et al.*, 2021).

Limited research is available on the use of barley in laying hen diets. Mathlouthi *et al.* (2002) reported a comparable egg mass in laying hens (ISA Brown) fed with wheat alone or in combination with barley. Hamilton and Proudfoot (1993) reported that barley could be used in laying hen diets up to 60% without any significant impact on productive performance. Most research has evaluated the response of laying hens to the dietary type of cereal grain in different stages of the first production cycle. Numerous research suggested that chicken's gut gets adapted to a high-fiber diet over time (Salih *et al.*, 1991; Jacob and Pescatore, 2012; Desbruslais *et al.*, 2021). Therefore, the present study aimed to evaluate the response of laying hens to different types of cereal grain in the second production cycle in the absence of carbohydrases.

Materials and Methods

The animal care procedures followed in the experiment were approved by the ethics committee of Shahrekord University, Shahrekord, Iran (SKU-1403ec).

Birds and dietary treatments

A number of 72 laying hens (Hy-line W80 at age 81 wk) were randomly selected from a commercial flock (n= 2000) that had already passed through a non-fasted molting practice. Initial weights of selected hens were averaged 1090 g ± 25. Hens were randomly allocated to three dietary treatments with six replicates. Each replicate was a wire cage with dimensions of 45 × 45 × 45 cm. Each cage was supplied with an automatic nipple drinker and a feeder. The experimental trial lasted from 81 to 90 weeks of age, during which a 16L:8D lighting schedule was applied at a temperature of 19–21°C and a relative humidity of 30-35%. Hens were allowed free access to feed and water.

Table 1. Feed composition and chemical analysis of dietary treatments for Hy-Line W-80 laying hens.

	Corn diet	Wheat diet	Barley diet
Corn	62.96	-	-
Soybean mean (44%CP)	21.31	14.5	17.9
Wheat	-	68.77	-
Barley	-	-	60.75
Nacl	0.25	0.2	0.2
$NaHCO_3$	0.22	0.2	0.3
$CaCO_3$	10.21	10.1	10.2
Soy oil	2.89	3.5	8.6
Dicalcium phosphate	1.25	1.33	1.06
L-Lysine	0.08	0.235	0.119
DL-methionine	0.205	0.286	0.234
L-threonine	0.030	0.123	0.076
Potassium carbonate	0.060	0.221	0.026
Vitamin supplement*	0.25	0.25	0.25
Trace mineral supplement**	0.25	0.25	0.25
Phytase ***	0.035	0.035	0.035
Chemical composition			
Metabolizable energy (kcal/kg)	2,880	2,880	2,880
Crude protein (%)	14.35	14.35	14.35
SID Methionine (%)	0.428	0.45	0.42
SID Met+Cys (%)	0.64	0.64	0.64
SID Lysine (%)	0.703	0.703	0.703
SID Threonine (%)	0.492	0.492	0.492
Crude fiber (%)	3.13	3.42	4.95
Dietary electrolyte balance (meq/kg)	195	195	195

*Vitamins premix provided per kg of diet: vitamin A, 20000 IU; vitamin D3, 8250 IU; vitamin E, 10 IU; vitamin K_3, 6.5 mg; vitamin B_1, 2.2 mg; vitamin B_2, 4 mg; vitamin B_3, 8 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.015 mg; folic acid, 0.56 mg; choline chloride, 200 mg. **Trace mineral premix provided per kg of diet: manganese, 275 mg; iron, 150 mg; zinc, 250 mg; copper, 25 mg; iodine, 3.5 mg; and selenium, 0.75 mg. ***Phytase (phytanik; Jivanik, Isfahan, Iran) 1,000 FTU- (Matrix Value: AvP: 0.161%).

Three dietary treatments were formulated based on corn, wheat, or barley as the sole source of cereal grain. Dietary treatments had similar contents of metabolizable energy, crude protein, amino acids, calcium, available phosphorous, and dietary electrolyte balance. Nutrient specifications were aligned with the strain's recommendations (Table 1). All experimental diets were supplemented with phytase (1,000 FTU Phytanik, Jivanik, Isfahan, Iran) as the sole source of exogenous enzyme at 0.35 g/kg.

Production parameters

The number and weight of all eggs per cage were recorded on a daily basis. These data were used to calculate egg production (EP), egg weight (EW), and egg mass (EM). Feed conversion ratio (FCR) was calculated, taking into account the amount of feed consumed. Egg weight was measured with an electronic scale accurate to 0.01 g.

Egg quality traits

Egg index (the ratio of egg weight to egg length, multiplied by 100), eggshell thickness (FHK, Tokyo, Japan), eggshell breaking strength (FHK, Tokyo, Japan), yolk color (DSM-Firmenich, Heerlen, Netherlands), albumen height (the tripod micrometer, Mitutoyo, Japan), and the Haugh unit were determined on 90th week from 10 eggs per treatment.

Body weight

The body weight of individual hens was recorded every two weeks throughout the trial by means of a hanging scale accurate to 10 g. The uniformity of body weight was calculated by measuring the coefficient of variation (CV) for each treatment group.

Blood parameters

Ten laying hens per treatment were randomly picked for blood collection at the end of the trial (Week 90). After weighing, blood samples were collected in tubes containing anticoagulants and centrifuged for 15 minutes at 2000 g. The resulting plasma samples were used for the determination of cholesterol (Total, LDL-c, and HDL-c), alanine transaminase (ALT), aspartate transaminase (AST) and glucose (GLU) with the aid of commercial kits according to the manufacturer's guidelines (Pars Azmoon Kits; Pars Azmoon, Tehran, Iran). An aliquot of blood was sampled for the determination of hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration (MCHC), as described by Ware (2020). An additional droplet of blood was spread on a laboratory glass slide and stained using the May-Grunwald and Giemsa procedur

e. A minimum of 100 leukocytes were counted to obtain granular (heterophils) and nongranular (lymphocytes), and the ratio of heterophils to lymphocytes (H/L) was then calculated. All chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

Statistical analysis

The data were analyzed in a completely randomized design using the General Linear Model (GLM) procedure of SAS software (SAS, 2015). Mean comparison was conducted by the Duncan's multiple-range test.

Results

Table 2 displays the effect of dietary cereal type on the productive performance of laying hens throughout the experimental trial. The type of dietary cereal grain had a significant (P < 0.05) effect on productive measures in the beginning (week 1) and the end of the trial (weeks 8 and 9) so that hens fed with the corn diet had greater egg production and egg mass compared to hens fed with the barley diet. There was no significant difference among treatments with respect to egg weight. Hens on the barley diet constantly had a poorer FCR, which was significantly (P < 0.05) different from the corn and wheat groups at the end of the trial (weeks 8 and 9).

Table 3 indicates body weight and its CV among different cereal grains. There were no significant differences between treatments in terms of body weight. Body weight of hens fed with barley showed a higher CV relative to that of the corn or wheat groups.

Table 4 presents the results related to egg quality traits of laying hens fed with different types of cereal grains. No significant difference was found between treatments with respect to egg dimensions (length, width, and their ratio measured as the egg index). Eggshell parameters, including thickness, strength against breaking force, egg density, and eggshell percentage, were not significantly different between the treatment groups. Egg internal quality measures, including albumen height and percentage, the Haugh unit, and yolk diameter and percentage, were not significantly different between treatments. However, the yolk color index was significantly reduced when corn was replaced by wheat or barley (P < 0.05).

Table 5 presents blood and plasma variables in hens received different types of cereal grains. Hens fed with wheat had greater hemoglobin and mean corpuscular hemoglobin concentrations than those received corn or barley. However, the hematocrit value remained unchanged between dietary treatments. The number of heterophils and lymphocytes and their ratio showed no significant difference among treatment groups. The activity of ALT and AST enzymes, an indicator of liver function, was not affected by the type of cereal.

Nevertheless, total cholesterol content in plasma was higher in the corn group than in the wheat or barley groups ($P < 0.05$), with no significant changes in HDL-c and LDL-c contents.

Table 2. Effect of dietary cereal type on productive performance of laying hens throughout the trial

Cereal type	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Total mean
Egg production (%):										
Corn	8.7 ^{a}	53.2 ^{a}	61.9	74.7	88.1	85.9	86.7 ^{ab}	87.7 ^{a}	88.9 ^{a}	70.4
Wheat	6.3 ^{a}	46.1 ^{b}	61.3	75.3	86.7	86.4	89.7 ^{a}	88.5 ^{a}	87.0 ^{a}	69.6
Barley	3.2 ^{b}	51.6 ^{ab}	57.4	66.3	82.8	85.1	83.5 ^{b}	78.7 ^{b}	78.7 ^{b}	65.5
SEM	1.38	1.78	3.65	2.96	2.18	2.01	1.87	1.96	1.85	1.79
Egg weight (g):										
Corn	53.3	58.0	63.0	65.7	64.7	63.3	64.7	65.0	65.0	62.5
Wheat	53.1	59.5	61.3	63.3	63.0	65.2	63.6	64.3	66.0	62.1
Barley	55.1	59.0	62.0	65.0	63.2	65.0	64.5	64.6	63.7	62.3
SEM	1.85	2.79	1.41	1.22	1.89	1.21	1.67	2.88	2.77	1.93
Egg mass (g/b/d):										
Corn	4.7 ^{a}	30.6	38.7	49.0 ^{a}	57.0 ^{a}	54.3	57.0	57.3 ^{a}	57.3 ^{a}	44.5
Wheat	3.6 ^{ab}	27.3	38.0	47.3 ^{ab}	55.5 ^{ab}	56.3	56.0	57.0 ^{a}	58.3 ^{a}	43.8
Barley	1.7 ^{b}	30.3	35.4	42.3 ^{b}	52.6 ^{b}	56.0	54.2	51.3 ^{b}	51.3 ^{b}	41.7
SEM	0.67	1.76	2.71	1.74	0.56	1.39	1.41	1.32	1.29	1.42
Feed conversion ratio:										
Corn	-	3.35	2.71	2.19 ^{ab}	1.81	1.91	1.85	1.81 ^{b}	1.81 ^{b}	2.19
Wheat	-	3.72	2.74	2.11 ^{b}	1.88	1.81	1.81	1.81 ^{b}	1.77 ^{b}	2.21
Barley	-	3.41	2.91	2.42 ^{a}	1.89	1.86	1.91	2.03 ^{a}	2.06 ^{a}	2.30
SEM	-	0.212	0.204	0.091	0.085	0.076	0.049	0.051	0.038	0.075

$^{a–b}$ Means with different superscripts within each column have a significant difference ($P < 0.05$)

Table 3. Effect of dietary cereal type on body weight and uniformity of laying hens throughout the trial

Cereal grain	Week 1	Week 3	Week 5	Week 7	Week 9
Body weight (g/hen):					
Corn	1100	1497 (397)	1560 (63)	1600 (40)	1610 (10)
Wheat	1094	1570 (476)	1608 (38)	1671 (63)	1690 (19)
Barley	1089	1580 (491)	1640 (60)	1661 (21)	1665 (4)
SEM	17.9	29.8	31.6	37.8	35.6
Coefficient of variation (CV):					
Corn	0.3	4.7	2.0	2.8	2.6
Wheat	0.7	4.8	1.9	1.9	1.7
Barley	0.8	7.0	4.8	6.0	5.9
SEM	0.27	1.93	1.75	1.87	2.46

Data in parenthesis show body weight gain (g)

Table 4. Effect of dietary cereal type on the quality of egg shell and egg contents of laying hens measured at the end of trial (Week 9)

	Corn	Wheat	Barley	*SEM*	*P*-value
Egg length (mm)	58.5	58.6	58.3	2.81	0.908
Egg width (mm)	45.4	45.6	44.6	1.03	0.080
Egg shape index	77.6	77.8	76.4	5.86	0.322
Eggshell Thickness (mm)	0.33	0.36	0.33	0.003	0.208
Eggshell breaking strength (Kg/cm^2)	3.0	3.4	3.3	0.043	0.119
Albumen height (mm)	7.97	7.94	7.36	1.027	0.241
Haugh Unit	87.0	87.0	84.2	4.14	0.458
Yolk color index	6.6^{a}	1.6^{b}	1.7^{b}	0.44	0.001
Yolk percentage	28.4	29.5	29.4	5.23	0.364
Albumen percentage	59.4	57.3	57.3	7.2	0.106
Eggshell percentage	12.4	13.2	13.2	1.26	0.121
Yolk diameter (mm)	40	43	42	2.67	0.101
Egg density (g/cm^3)	1.08	1.10	1.08	0.001	0.283

Table 5. Effect of dietary cereal type on blood and plasma indices of laying hens measured at the end of trial (Week 9)

	Corn	Wheat	Barley	*SEM*	*P*-value
Hemoglobin (mg/dL)	6.4 [b]	8.9 [a]	6.7 [b]	0.89	0.013
Hematocrit (%)	33.1	32.7	30.5	4.72	0.548
Mean corpuscular hemoglobin concentration (g/dL)	19 [b]	27 [a]	22 [b]	0.051	0.011
Heterophils (%)	26.3	31.6	23.2	4.32	0.548
Lymphocytes (%)	67.3	63.7	74.2	12.44	0.252
Heterophils / Lymphocytes	0.40	0.54	0.33	0.079	0.403
Alanine Transaminase (U/L)	44.6	46.2	41.2	12.8	0.089
Aspartate transaminase (U/L)	124.5	149.7	146.9	32.1	0.435
Total cholesterol (mg/dL)	284[a]	261[b]	246[b]	22.3	0.014
LDL-c (mg/dL)	18.3	18.1	23.5	2.89	0.352
HDL-c (mg/dL)	41.3	43.5	36.4	3.73	0.081

Discussion

Cereal grains constitute 60 to 70% of laying hen diets and contribute to about 70% of dietary metabolizable energy and 30-40% of dietary protein intake. Therefore, the type of cereal grain in laying hen diets has an enormous impact on the economy of egg production.

In the early weeks of the second production cycle, hens fed with corn experienced a superior egg production rate over the barley (week 1) and wheat (week 2) groups. In the upcoming weeks (weeks 3 through 6), no significant difference was observed among dietary treatments. However, toward the end of the trial (weeks 8 and 9), the egg production rate of hens fed with barley was significantly lower than that of hens received corn and wheat, and consequently, the barley group had a poorer FCR. Nevertheless, the average egg production rate in the entire trial was not significantly differed among dietary treatments. This finding suggests that laying hens may stay on wheat-based diets without compromising productive performance whereas they hardly sustain their productive performance on barley-based diets. It is noteworthy that the concentration of NSP in today's wheat cultivars has been reduced over the past decades by plant breeding strategies (Bedford *et al*., 2024). In this regard, Cowieson *et al*. (2015) reported that the total concentration of arabinose + xylose in the 1990s and 2010s cultivars of wheat was 81 and 59 g/kg, respectively. This is likely the reason that the response of hens to the wheat-based diet was comparable to that of the corn control. Kim *et al.* (2022) have recently reported that chickens offered the wheat-based diet showed an age-related improvement in soluble NSP utilization, as reflected in improved soluble NSP digestibility. Therefore, insignificant egg production performance between the corn and wheat diet observed in the present trial for laying hens in the second production cycle could be anticipated. Hendrix Genetics recently recommended including insoluble fiber in the diet of laying hens to obtain a good feather cover and improved livability and intestinal health. Livability is positively linked to dietary fiber intake as it makes birds feel full, which in turn results in eliminating their need for feather ingestion (pecking and cannibalism). Dietary fiber also helps to prevent fatty livers (Leentfaar, 2020).

The explanation for poor performance in the barley-based diet could be attributed to a higher crude fiber content in barley when compared to corn and wheat (6.1, 2.6 and 3.2% CF, respectively, by analysis). Although the experimental diets were isoenergetic and isonitrogenous, a higher CF content means a higher energy burden for nutrient assimilation, which subsequently results in poorer performance. Likewise, a large portion of CF of barley is composed of water-soluble non-starch polysaccharides (WSNSPs), which results in viscous digesta and hinders nutrient assimilation (Nguyen *et al.,* 2022). Additionally, the gastrointestinal tract of laying hens hosts a diverse microbial community that is essential for nutrient assimilation, and the presence of WSNSPs causes dysbiosis, which is associated with poor productive performance (Zhang *et al.,* 2024). The present findings suggest that cereal processing or exogenous carbohydrases should be considered when barley is included in laying hen diets. However, hens showed comparable productive performance in both corn and wheat-based diets.

Egg weight was not affected by the type of cereal grain. This observation is consistent with previous reports (Pérez-Bonilla *et al.,* 2011). Moreover, Safaa *et al.* (2009) did not observe any significant effect on egg weight when corn was substituted by an equal amount of wheat in laying hen diets. Similar results have been reported by Lázaro *et al.* (2003) in laying hens from 20 to 44wk of age.

Means and the CVs of body weight are useful criteria to predict productive performance and the size and uniformity of eggs in layer flocks. Results of the present experiment showed no significant difference in terms of body weight and body weight gain among treatments. It is evident that birds gained the vast majority of weight during the first two weeks postmolt (week 1 through week 3). The proportion of weight gained during this period to total weight gain in the experiment was 78, 79, and 85% for the corn, wheat, and barley groups, respectively. It is also evident from our data that weight gain reached a plateau in week 9, which coincided with the peak egg production. A drawback of feeding barley to hens is the deterioration of body weight uniformity over time, as reflected in a higher CV.

Eggshell quality is crucial in the food safety of eggs and egg products because poor eggshell quality is associated with an increased risk of bacterial contamination from farm to fork (Cheng and Ning, 2023). Wengerska *et al*. (2023) indicated that any eggshell defect significantly influenced the characteristics of specific gravity, eggshell permeability, albumen height and pH, as well as yolk pH during storage. The findings of the present study revealed that the type of cereal grain had no significant effect on eggshell quality measures and egg internal quality traits. The only exception was egg yolk pigmentation, which was significantly higher in corn than other cereals. Safaa *et al.* (2009) reported significant differences in egg yolk color when wheat was substituted for corn. These findings suggest a lower pigment content in wheat compared to corn, which resulted in decreased yolk pigmentation (Zheng *et al.,* 2020). In line with the present finding, Jamroz *et al*. (2001) and Lazaro *et al*. (2003) reported comparable egg quality traits in hens fed wheat or barley diets. Perez-Bonilla *et al*. (2011) reported no significant effect of cereal type on egg quality measures in laying hens from 22 to 54 wk of age.

The type of cereals in the diet significantly influenced hemoglobin and the mean corpuscular hemoglobin concentrations (MCHC) in a way that the wheat group had a higher concentration of hemoglobin and MCHC than the corn and barley groups. It is noteworthy that zinc, copper, iron and vitamin B_9 contents in wheat are higher than in corn, and this may have a role in higher hemoglobin and MCHC levels. Copper is an important component of many enzymes which are critical to the maturation of hematopoietic cells. Zinc is recognized as an essential mineral in erythropoiesis by playing a key role in the activity of alfa-aminolevulinic acid dehydratase, the enzyme responsible for heme biosynthesis (Kim *et al.*, 2023).

Total cholesterol in plasma was affected by the type of cereal grain used in hen’s diet, so feeding wheat or barley was associated with a lower level of plasma cholesterol. This could be linked to a higher content of crude fiber in these grains. High fiber diets have been shown to reduce blood and yolk cholesterol levels in laying hens (Brown *et al.*, 1999; Desbruslais *et al*., 2021).

Zaefarian *et al.* (2015) reviewed starch digestibility in broiler chickens fed different types of cereal grains. They concluded that starch digestibility in cereals for chickens is relatively high, irrespective of the different structures of starch granules among cereals. Laying hens are seemingly more capable of digesting starch and fiber than broiler chickens. Walugembe *et al.* (2014) indicated that laying hens performed better on high-fiber diets than broiler chickens. As chicken’s gut gets adapted to high fiber diets over time (Salih *et al.,* 1991; Jacob and Pescatore, 2012; Desbruslais *et al.,* 2021), it was anticipated that laying hens in the second production cycle could maintain productive performance and egg quality measures on diets composed of wheat or barley without the need of supplementing NSP-degrading enzymes. Nevertheless, feeding laying hens with barley failed to sustain egg production rate, egg mass, and FCR. Meanwhile, replacing corn with barley was linked to the deterioration of body weight uniformity.

Conclusion

It can be concluded that the type of dietary cereal grain has an impact on the productive performance of laying hens in the second production cycle. Substitution of barley for corn or wheat was associated with poor production performance, especially during the peak production stage (7 to 9 weeks after the onset of the laying cycle). The wheat-based diet without the use of carbohydrase enzymes in aged laying hens did not have an adverse effect on production performance.

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