Effects of replacement of corn with barley or barley flake in diets containing different levels of metabolizable energy on performance, egg quality and serum parameters of laying quails

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Abstract. *The current research was carried out to determine the effect of substituting barley or barley flake for corn in diets containing different levels of metabolizable energy on performance, egg quality, and serum parameters in laying quails. In the study, 120 female quails of the age of 22 weeks were randomly distributed to six treatment groups (with four replicates of five quails each) consisting of two metabolizable energy levels (2900 or 2800 kcal/kg) and three forms of cereals (corn, barley or barley flake). As a result of the research, with the reduction of diet metabolizable energy level to 2800 kcal/kg, while feed intake (P < 0.01), Haugh unit (P < 0.01), b* value of yolk (P < 0.05), and serum calcium (P < 0.05) and phosphorus (P < 0.05) concentrations increased significantly, eggshell thickness (P < 0.01) decreased importantly. Besides, the replacement of corn with barley or barley flake in the diets adversely affected egg production, feed intake, and Roche unit, a* and b* values of yolk (P < 0.01). Eggshell breaking strength, eggshell weight, and serum phosphorus concentration were statistically decreased in the groups which used barley or barley flake compared with those containing corn. As a result, lowering the metabolizable energy level in the diet increased feed intake, improved egg internal quality, and mineral metabolism, but adversely affected eggshell quality. Furthermore, the replacement of corn with barley negatively affected the performance, and egg quality, but flaking was effective on eliminating the negative effect of barley in eggshell breaking strength.*

Introduction

The use of barley in poultry feeding is substantially limited due to high cellulose, and beta-glucan, a soluble starch, content (Herstad and McNab, 1975). However, barley is used as an energy source in poultry feeding in those regions that are not proper for corn production (Jeroch and Danicke, 1995). Although the addition of beta-glucanase enzyme is a common practice to eliminate the factors limiting the use of barley in poultry diets (Lazaro et al., 2003), there is limited study examining the effects of heat treating on barley. One of these heat treatments is the flaking process, which consists of steaming the grains under high pressure and then pressing and drying. It was reported that the use of barley flake in the diets improves the retention of nutrients and the availability of diet energy in broilers (Gracia et al., 2003), while the use of 30% barley flake in the diet reduces feed intake in laying hens (Gürbüz and Özyürür, 2019). By flaking barley, it can be predicted that the antinutritional factors it contains will decrease and the digestibility of nutrients will increase. Based on these, the hypothesis of the current research is that the effects of barley flake will be better understood by comparing the use of corn and barley in diets with reduced metabolizable energy (ME) levels. For this purpose, this research was conducted to determine the effect of barley flake usage compared with corn and barley in diets containing different levels of ME on performance, egg quality, and serum parameters in laying quails.

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Material and methods

In the experiment, a total of 120 female Japanese quails at the age of 22 weeks were equally distributed to a total of six treatment groups consisting of 2 ME levels and 3 forms of cereals. Each treatment group was constituted of four subgroups with five quails each. During the ten-week experiment, the quails were fed with 6 treatment diets using corn, and barley or barley flake instead of 100% of corn as the cereal source in diets containing 2900 (control, (NRC, 1994)) and 2800 kcal/kg ME (Table 1). During the trial, a 16-hour lighting program was applied, and feed and water were given *ad-libitum* to the quails.

Determination of performance parameters

The mean body weight of the quails was determined using the values obtained by group weighing at the initial and final of the experiment, and body weight change (BWC) was calculated from these means. During the trial, the eggs were recorded daily and egg production (EP) was calculated as %. Feeds were given by weighing to the treatment groups and the feed intake (FI) was calculated as g/day/quail by subtracting the remaining feeds from the total feed at the end of the experiment. Egg weights (EW)

Table 1. Experimental diets and calculated nutrient contents

Ingredients, %	2800 kcal/kg ME		2900 kcal/kg ME	
	Corn	Barley/barley flake	Corn	Barley/barley flake
Corn	60.35	–	58.00	–
Barley or barley flake	–	58.45	–	56.20
Soybean meal	31.00	28.00	31.50	28.60
Soybean oil	1.10	6.05	2.95	7.71
Limestone	5.60	5.66	5.60	5.64
Dicalcium phosphate	1.18	1.06	1.18	1.07
Salt	0.35	0.35	0.35	0.35
Premix[1]	0.25	0.25	0.25	0.25
DL methionine	0.17	0.18	0.17	0.18
Total	100.00	100.00	100.00	100.00
Chemical composition, %				
ME, kcal/kg	2801	2800	2901	2901
Crude protein	20.00	20.00	20.03	20.02
Calcium	2.50	2.51	2.50	2.50
Available phosphorus	0.35	0.35	0.35	0.35
Lysine	1.05	1.05	1.06	1.05
Methionine	0.45	0.45	0.45	0.45
Methionine + cysteine	0.82	0.84	0.82	0.83

[1]**Premix** is supplied per kg of diet: manganese 80 mg, iron 60 mg, copper 5 mg, iodine 1 mg, selenium 0.15 mg, vitamin A 8.800 IU, vitamin D_3 2.200 IU, vitamin E 11 mg, nicotine acid 44 mg, Cal-D-Pan 8.8 mg, riboflavin 4.4 mg, thiamine 2.5 mg, vitamin B_{12} 6.6 mg, folic acid 1 mg, biotin 0.11 mg, choline 220 mg.

were determined as g by weighing all eggs collected in the last three days of the experiment. Egg mass (EM) was calculated as g/day/quail with the formula (EP x EW)/100. Feed conversion ratio (FCR) was calculated as g feed/g egg with the FI/EM formula.

Determination of egg external quality

During the experiment, broken, cracked and damaged eggs were recorded and calculated as % of the total eggs. After determining the weights of all the eggs collected in the last three days of the experiment in air and water, their specific weights were calculated in g/cm^3 with the formula EW/(EW-EW in water). Eggshell breaking strength was measured by applying supported systematic pressure to the blunt of the eggs (Egg Force Reader, Orka Food Technology, Israel). After the eggs were broken on a clean glass table, egg residues in the eggshell were cleaned, then the eggshells were dried for three days at room temperature, and eggshell weights were calculated as % of the egg weights. Eggshell thickness was calculated by taking the average of the values obtained by measuring from three points of the egg (equator, blunt and pointed parts) using a micrometer (Mitutoyo, 0.01 mm, Japan).

Determination of egg internal quality

Once again, the albumen height of these eggs was measured with a height gauge and the Haugh unit was calculated by the formula 100 x log (albumen height + 7.57–1.7 x EW$^{0.37}$) (Haugh, 1934). Egg yolk colour and L *, a * or b * values were measured with Roche colour scale and colorimeter, respectively (Minolta Chromameter CR 400 (Minolta Co., Osaka, Japan) (Romero et al., 2002).

Serum biochemical parameters

At the end of the trial (week 10), 3 mL of blood was taken from one random quail (4 quails from each treatment group) in similar body weights to determine serum parameters. Blood samples were centrifuged at 3000 rpm for 5 minutes. The obtained serum was stored at –20°C until analyzed, and glucose, cholesterol, HDL, creatinine, total protein, albumin, globulin, calcium, and phosphorus concentrations were determined in an auto-analyser using commercial kits (DDS® Spectrophotometric Kits, Diasis Diagnostic Systems Co., İstanbul Turkey).

Statistical analysis

For the experiment was carried out with 4 subgroups in six treatment groups consisting of two different levels of diet ME (2900 kcal/kg and 2800) and 3 different cereal forms, the trial results were analyzed by ANOVA using the General Linear Model (GLM) procedure in Minitab (Minitab, 2000), and differences between the group means were determined by Duncan's multiple range tests (Duncan, 1955).

Results and discussion

Performance parameters

Effect of replacement of corn with barley or barley flake in diets containing different levels of ME on the performance of laying quails is shown in Table 2.

As the main factor, diet ME levels did not affect performance parameters except for FI ($P > 0.05$). Feed intake significantly increased with the reduction of the diet ME level ($P < 0.01$). Similar to the current study, Elangovan et al. (2004) reported that FI augmented by lowering the diet ME level from 2900 kcal/kg to 2700 kcal/kg and 2500 kcal/kg. Contrarily, Lotfi et al. (2018) reported that with the reduction of dietary ME to 2750 kcal/kg, the FI of laying quails decreased compared with 2964 kcal/kg and 3050 kcal/kg ME. Similar results were stated by Freitas et al. (2005) and Barreto et al. (2007) for ME levels of 2585–3050 kcal/kg. In the present study, EP and EW were not affected by diet ME levels. It is clear that quails fed with a diet containing low ME increased feed intake in order to maintain EP and EW. On the other hand, in researches where it was demonstrated that FI diminished with the decrease in dietary ME level, EP (Lotfi et al., 2018) and/or EW (Freitas et al., 2005; Barreto et al., 2007) parameters were also negatively affected by the diet ME levels. This situation is important to explain the differences among studies. Moreover, it is claimed that the treatment diets and the ME levels of these diets, as well as the genetic structures of the birds, affect these differences.

While the effects of the cereal form on diets of laying quails were important on EP ($P < 0.05$) and FI ($P < 0.01$), this effect was not observed on other performance parameters ($P > 0.05$). With the substituting barley or barley flake for corn in the diet, EP and FI considerably decreased and there was no difference between barley or barley flake in terms of these parameters. These mentioned results disagree with Jamroz et al. (2001), Lazaro et al. (2003), Herbert et al. (2011) and Kılıç and Olgun (2021) who indicated that the EP and FI were not affected by the replacement of corn by barley. The crude fiber content of barley is higher than that of corn; hence it is known that feeding birds with barley-based diets causes them to not consume enough feed due to their digestive system capacity (Jeroch and Danicke, 1995). In the current study, the decrease in FI of quails fed barley or barley flake-based diets can be due to the high

Table 2. Effect of replacement of corn with barley or barley flake in diets containing different levels of ME on the performance in laying quails

ME, kcal/kg	Cereal form	BWC, g	EP, %	EW, g	EM, g/d/quail	FI, g/d/quail	FCR, g feed/g egg
2900		2.71	87.55	13.69	11.98	32.10[B]	2.69
2800		–2.12	87.53	13.72	12.00	33.86[A]	2.83
SEM		3.024	1.460	0.221	0.247	0.582	0.066
	Corn	–1.81	91.11[a]	13.37	12.17	35.41[A]	2.92
	Barley	0.81	85.13[b]	14.01	11.92	31.86[B]	2.68
	Barley flake	1.88	86.37[b]	13.74	11.88	31.68[B]	2.69
	SEM	3.739	1.544	0.673	0.049	0.464	0.075
2900	Corn	0.00	89.91	13.61	12.24	33.82	2.77
2900	Barley	3.25	83.93	14.03	11.76	31.25	2.67
2900	Barley flake	4.89	88.79	13.42	11.93	31.22	2.63
2800	Corn	–3.63	92.31	13.12	12.10	36.99	3.07
2800	Barley	–1.62	86.33	13.99	12.07	32.46	2.69
2800	Barley flake	–1.12	83.96	14.06	11.83	32.12	2.74
SEM		5.176	2.100	0.759	0.440	0.479	0.097
Probabilities, $P \leqslant$							
ME		0.330	0.994	0.903	0.950	<0.001	0.114
Cereal form		0.816	0.030	0.260	0.793	<0.001	0.427
Interactions		0.980	0.184	0.345	0.867	0.070	0.427

BWC: Body weight change, EP: Egg production, EW: Egg weight, EM: Egg mass, FI: Feed intake, FCR: Feed conversion ratio. SEM: Standard error means.
[A, B]Within a column, values not sharing a common letter are statistically different; $P < 0.01$.
[a, b]Within a column, values not sharing a common letter are statistically different; $P < 0.05$.

fiber content of barley. At the same time, in the status of the low FI, the nutrients required for EP can be deficient. . The flaking process increases the viscosity of barley in the digestive tract of broilers (Gracia et al., 2003). This can be the probable reason why barley flake is incapable of eliminating this negativity in FI and EP. The interaction groups formed by the dietary ME levels and cereal forms did not statistically affect the performance of the laying quails ($P > 0.05$).

Egg external quality

Effect of substituting barley or barley flake for corn in diets containing different levels of ME on egg external quality of laying quails demonstrated is in Table 3.

The cracked eggs, eggshell breaking strength, eggshell weight, and specific weight were not affected by the dietary ME levels as the main factor, but the eggshell thickness significantly decreased with the reduction of diet energy from 2900 kcal/kg to 2800 kcal/kg ($P < 0.01$). Congruently with the current research, Elangovan et al. (2004) and Lotfi et al. (2018) reported that the eggshell thickness was negatively affected by the reduction of diet energy (2700 kcal/kg and 2750 kcal/kg ME). However, Hurtado-Nery et al. (2015) and Agboola et al. (2016) stated that dietary ME level (2750–3200 kcal/kg) did not affect quails. Also, similar results were obtained in laying hens (Junqueira et al., 2006; Wu et al., 2008).

While the cereal form as the main factor considerably affected the eggshell breaking strength ($P < 0.05$) and eggshell weight ($P < 0.01$) among the egg external quality traits, this effect was found to be unimportant in the other eggshell quality parameters ($P > 0.05$). The eggshell breaking strength decreased significantly in the group which used barley as a cereal form in the diet compared with the groups that used corn and barley flake. The eggshell weight reduced with the use of barley in the diet compared with the group containing corn, but the eggshell weight of the group using barley flake was similar to the other groups. In certain studies related to the subject, Jamroz et al. (2001) and Perez-Bonilla et al. (2011) indicated that the use of barley in laying hen diets (66% and 45%, respectively) did not affect eggshell breaking strength and eggshell weight. Beta-glucan in barley can reduce the bioavailability of some minerals such as calcium, which are important in eggshell formation, by increasing viscosity (Cardoso et al., 2014) in the digestive tract. The fact that groups fed with barley-based diets showed lower eggshell breaking and eggshell weight than groups fed with

Table 3. Effect of replacement of corn with barley or barley flake in diets containing different levels of ME on the egg external quality in laying quails

ME, kcal/kg	Cereal form	Cracked egg, %	Eggshell breaking strength, kg	Eggshell weight, % of EW	Eggshell thickness, µm	Specific weight, g/cm^3
2900		0.51	1.37	8.16	195.3^{A}	1.071
2800		1.54	1.47	8.14	188.3^{B}	1.072
SEM		0.621	0.048	0.155	1.24	0.0012
	Corn	0.56	1.47^{a}	8.39^{A}	189.9	1.073
	Barley	0.51	1.29^{b}	7.79^{B}	194.6	1.069
	Barley flake	2.00	1.50^{a}	8.27^{AB}	190.8	1.072
	SEM	0.673	0.049	0.162	1.91	0.0014
2900	Corn	0.43	1.34	8.14^{AB}	194.5	1.072
2900	Barley	0.72	1.27	7.63^{B}	198.5	1.068
2900	Barley flake	0.38	1.49	8.70^{A}	192.8	1.073
2800	Corn	0.70	1.59	8.65^{A}	185.3	1.074
2800	Barley	0.30	1.31	7.95^{B}	190.8	1.071
2800	Barley flake	3.64	1.52	7.84^{B}	188.8	1.071
SEM		0.759	0.065	0.178	1.74	0.0020
Probabilities, $P \leqslant$						
ME		0.356	0.076	0.935	<0.001	0.852
Cereal form		0.461	0.012	0.010	0.068	0.254
Interactions		0.363	0.231	0.003	0.425	0.473

SEM: Standard error means.
$^{A, B}$Within a column, values not sharing a common letter are statistically different; $P < 0.01$.
$^{a, b}$Within a column, values not sharing a common letter are statistically different; $P < 0.05$.

corn-based diets can be due to this. Gürbüz and Özyürür (2019) stated that the use of barley flake in the diet did not affect eggshell breaking strength and eggshell weight. The heat and pressure applied during the flaking process could have possibly advanced the availability of calcium required for eggshell formation. This situation can be the reason for the effectiveness of barley flake in reducing/eliminating its negative effect on eggshell quality.

Interactions of dietary ME levels and the cereal forms only affected eggshell weight ($P < 0.01$) but did not affect other parameters ($P > 0.05$). The highest eggshell weight was observed in the group that used barley flake with 2900 kcal/kg ME in the diet, and the differences between this group and the groups that used barley with 2900 kcal/kg ME and barley flake with 2800 kcal/kg ME were significant.

Egg internal quality

Effect of replacement of corn with barley or barley flake in diets containing different levels of ME on the performance of laying quails is shown in Table 4.

As the main factor, the dietary ME levels did not affect the Roche unit, L* and a* values statistically ($P > 0.05$), but the Haugh unit ($P < 0.01$) and b* value ($P < 0.05$) were considerably affected. The Haugh unit advanced significantly with the reduction of the diet energy level from 2900 kcal/kg to 2800 kcal/kg ME. Whereas these results agree with the reports of Elangovan et al. (2004) and Perez-Bonilla et al. (2011), they disagree with Lotfi et al. (2018) and Gunawardana et al. (2009) who stated that the diet ME level did not affect the Haugh unit. Increasing the amount of protein and amino acids consumed improves the Haugh unit (Wu et al., 2005). In the current study, an advance in Haugh units could have occurred as the low diet ME level increased the amount of protein and amino acids consumed with risen FI. The b* value significantly improved with the reduction of a dietary energy level from 2900 kcal/kg to 2800 kcal/kg ME. In the literature, there is no research examining the effect of diet ME level on the b* value of yolk in previous years. In the present study, the increase in the yolk b* value can be explained by the fact that corn used in the diet containing low ME is more than that one containing high ME.

The effect of substituting barley or barley flake for corn in diets on the Roche unit, a* and b* values was significant ($P < 0.01$), but this effect was unimportant in the Haugh unit and L* value ($P > 0.05$). With the replacement of corn by barley or barley flake in

Table 4. Effect of replacement of corn with barley or barley flake in diets containing different levels of ME on the egg internal quality in laying quails

ME, kcal/kg	Cereal form	Haugh unit	Roche unit	L*	a*	b*
2900		62.88^{B}	2.75	52.74	–1.707	17.41^{b}
2800		71.35^{A}	3.05	52.18	–1.692	19.02^{a}
SEM		1.307	0.5212	0.3673	0.2812	1.503
	Corn	68.32	5.28^{A}	51.77	–0.428^{A}	24.94^{A}
	Barley	66.98	1.82^{B}	52.46	–2.303^{B}	15.36^{B}
	Barley flake	66.04	1.61^{B}	53.15	–2.368^{B}	14.35^{B}
	SEM	2.274	0.1743	0.4273	0.0955	0.626
2900	Corn	64.33	5.41	52.46	–0.353	23.45
2900	Barley	63.27	1.51	52.56	–2.318	14.31
2900	Barley flake	61.05	1.35	53.19	–2.450	14.47
2800	Corn	72.31	5.14	51.07	–0.503	26.42
2800	Barley	70.70	2.14	52.37	–2.288	16.41
2800	Barley flake	71.04	1.88	53.11	–2.285	14.23
SEM		2.160	0.2163	0.5907	0.1328	0.694
Probabilities, $P \leqslant$						
ME		0.001	0.125	0.287	0.898	0.026
Cereal form		0.657	<0.001	0.109	<0.001	<0.001
Interactions		0.862	0.118	0.510	0.547	0.154

SEM: Standard error means.
$^{A, B}$Within a column, values not sharing a common letter are statistically different; $P < 0.01$.
$^{a, b}$Within a column, values not sharing a common letter are statistically different; $P < 0.05$.

the diets of laying quails, the Roche unit, a* and b* value decreased considerably ($P < 0.01$). Nonetheless, similar results were obtained in the barley and barley flake groups in terms of these parameters. The results of research conducted by Perez-Bonilla et al. (2011) on this subject showed that the use of 45% barley in the diet reduced the yolk colour. Similarly, Herbert et al. (2011) reported that the yolk colour decreased with the use of 57.7% barley in laying hen diets. Moreover, research results stated by Kılıç and Olgun (2021) are in harmony with the current results.

Serum biochemical constituents

Effect of substituting barley or barley flake for corn in diets containing different levels of ME on serum biochemical constituents of laying quails is demonstrated in Table 5.

Serum glucose, cholesterol, HDL, creatinine, total protein, albumin, and globulin concentrations were not affected in the treatment groups ($P > 0.05$).

Serum calcium and phosphorus concentrations were significantly raised with the reduction of dietary ME as the main factor ($P < 0.05$). According to Table 2, decreasing the diet ME level increased FI. That is, FI was increased by the low dietary ME level that could lead to improvement in serum calcium and phosphorus concentrations. Besides, less vegetable oil was used in the low ME diet compared with the control diet. Increasing the oil level in the diet ascends the saponification between fatty acids and calcium, and so decreases the availability of calcium (Atteh et al., 1989; Tancharoenrat et al., 2014). In connection with the information above mentioned, the low amount of oil in the diet possibly has increased the availability of calcium and phosphorus.

The replacement of corn with barley or barley flake in the diet considerably affected the serum phosphorus concentration of the laying quails ($P < 0.05$), but the other parameters were not statistically affected ($P > 0.05$). Serum phosphorus concentrations of the group with barley in the diet were significantly lower than the groups that consisted of corn and barley flake. Water-soluble non-starch polysaccharides such as beta-glucan and xylan increase intestinal viscosity and negatively affect the bioavailability of minerals in birds (Van der Klis et al., 1993; Kiarie et al., 2014).

Table 5. Effect of replacement of corn with barley or barley flake in diets containing different levels of ME on serum biochemical constituents in laying quails

ME, kcal/kg	Cereal form	Glucose, mg/dL	Cholesterol, mg/dL	HDL, mg/dL	Creatinine, mg/dL	Total protein, g/dL	Albumin, g/dL	Globulin, g/dL	Calcium, mg/dL	Phosphorus, mg/dL
2900		336	153	49.78	0.348	4.53	1.44	3.09	22.00^{b}	4.93^{b}
2800		329	150	45.94	0.343	4.46	1.43	3.03	24.76^{a}	5.93^{a}
SEM		5.1	7.7	2.599	0.0080	0.165	0.052	0.126	0.982	0.374
	Corn	327	161	44.63	0.330	4.60	1.40	3.20	23.49	6.28^{a}
	Barley	340	138	52.78	0.361	4.28	1.39	2.89	23.08	4.44^{b}
	Barley flake	331	160	46.18	0.344	4.61	1.51	3.10	23.58	5.59^{a}
	SEM	5.9	9.5	2.974	0.0089	0.198	0.060	0.151	1.291	0.408
2900	Corn	331	161	50.20	0.345	4.95	1.48	3.48	25.28^{AB}	5.93
2900	Barley	347	138	52.03	0.365	4.00	1.28	2.73	18.58^{C}	3.63
2900	Barley flake	331	160	47.10	0.333	4.65	1.58	3.08	22.15^{BC}	5.25
2800	Corn	322	161	39.05	0.315	4.25	1.33	2.93	21.70^{BC}	6.63
2800	Barley	334	156	53.53	0.358	4.55	1.50	3.05	27.58^{A}	5.25
2800	Barley flake	331	133	45.25	0.355	4.58	1.45	3.13	25.00^{AB}	5.93
SEM		8.5	12.4	3.935	0.0113	0.257	0.075	0.198	1.065	0.530
Probabilities, $P \leqslant$										
ME		0.354	0.813	0.286	0.619	0.733	0.801	0.732	0.009	0.039
Cereal form		0.329	0.502	0.157	0.058	0.374	0.253	0.321	0.898	0.012
Interactions		0.766	0.276	0.331	0.122	0.089	0.054	0.121	<0.001	0.626

SEM: Standard error means.

$^{A, B, C}$Within a column, values not sharing a common letter are statistically different; $P < 0.01$.

$^{a, b}$Within a column, values not sharing a common letter are statistically different; $P < 0.05$.

In the research using barley flake, it is indicated that barley flake increases viscosity and also improves nutrient retention (Gracia et al., 2003). Thus, the flaking process might have improved the availability of phosphorus. Possibly this effect on phosphorus availability can be due to the phytic acid disruption during the flaking process, but this needs to be investigated in detail.

The effect of interactions between dietary ME levels and the cereal forms on serum calcium concentration in laying quails was statistically important ($P < 0.01$), but this effect was not observed in other serum parameters ($P > 0.05$). The serum calcium concentration in the interaction group containing 2800 kcal/kg ME with barley in the diet was found significantly higher than in the groups containing 2900 kcal/kg ME with barley and barley flake and 2800 kcal/kg ME with corn.

Conclusions

As a result, reduction of the ME level in laying quail diets increased feed intake, improved the internal quality of an egg, as well as serum calcium and phosphorus concentrations, but the eggshell quality was deteriorated. Moreover, the total replacement of corn with barley or barley flake negatively affected the performance, eggshell quality, and yolk colour traits, while flaking of barley was efficient in preventing the negative effect of barley on the eggshell breaking strength; this effect was not observed in other parameters.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict in interest.

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