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Laying hens behave as omnivores with poultry meal included in their diet

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Abstract

This study was conducted to determine egg yield performance and quality, animal partiality to poultry meal, and consumer preferences for eggs produced by various feeding methods. A total of 72 Nick Brown laying hens, aged 22 weeks, were offered three feeding methods with 24 replicates per treatment and one hen per experimental unit. These methods consisted of i) vegetarian (no poultry meal), ii) omnivorous (5% poultry meal), and iii) a choice between vegetarian and omnivorous. Feed and water were provided ad libitum. The study lasted for 10 weeks. Feeding methods did not affect feed intake, feed conversion ratio, egg yield, and egg quality. However, they affected the malondialdehyde (MDA) value of eggs on the 42nd day of storage significantly (P <0.05). The highest MDA value was obtained from the eggs of 'omnivorous' hens. More hens (51.4%) in the choice group preferred omnivorous feed to 'vegetarian'. Panellists found organoleptic differences among sample eggs from hens subjected to various feeding methods. They reported that the eggs obtained from vegetarian hens were preferable. The conclusions were that i) no feeding method changed egg yield performance and quality, ii) omnivorous feeding shortened the shelf-life of eggs, iii) hens with a choice of feed did not reject the omnivorous diet, but increased their intake, and iv) the panellists disliked eggs from the omnivorous hens. Finally, these preferences should be considered in legislation for poultry feeding and animal husbandry.

Keywords: choice feeding, laying hen, organoleptic properties, poultry meal, shelf-life
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Introduction

Chickens are omnivorous animals. In nature and in extensive production systems, chickens eat small seeds, cereals, herbs and leaves, grubs, insects, and even small mammals. Chickens often dig to obtain adult insects and larvae and seed. When chickens are given the opportunity, they make their own diet according to their physiological and metabolic needs (Forbes, 1995; Sahin, 1999). In human-driven production systems, chickens need help in balancing their diets in terms of metabolic and physiological needs. Protein sources of vegetable and animal origin (e.g. by-products) are used to meet these needs.

Animal by-products are the parts of a slaughtered animal that are not consumed directly by humans. Most of this material is rendered to produce meat and bone meal, which is used in animal feed to improve protein levels (NRC,1994). The production of poultry for human consumption generates large amounts of by-products. In the European Union (EU) alone, every year six billion chickens, turkeys, and other food-producing birds are slaughtered for meat production, but about 25% of each of these animals is not used for direct human consumption (Campos *et al.*, 2020).

In the EU, Directive 1069/2009/EC for animal welfare-approved standards prohibited the use of animal by-products as feed ingredients in the aquaculture sector on 1 June 2013 (Jedrejek *et al.*, 2016). However, animal by-products and processed animal proteins, especially poultry meal, are still used in some countries. Poultry meal consists of the rendered clean parts of carcasses of slaughtered poultry such as necks, feet, undeveloped eggs, and intestines, but not feathers.

Studies on laying hens (Senkoylu *et al.*, 2005; Samli *et al.*, 2006; Hosseinzadeh *et al.*, 2010; Geshlog *et al.*, 2011), broilers (Jafari *et al.*, 2012; Silva *et al.*, 2014; Mahmood *et al.*, 2017; Ahmad *et al.*, 2017), and quail (Erturk & Celik, 2004; Mutucumarana *et al.*, 2010; Mutucumarana *et al.*, 2011) tested the use of these products. These studies were related to egg production and quality and the effects of poultry meal on egg

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shelf-life, and sensory properties were not investigated. In the literature, the diet preferences for protein sources of either animal or plant origin have not been studied in laying hens with diets that included poultry meal as an ingredient. Nor did these kinds of studies consider animal welfare and ethical issues. In this study, laying hens could decide between two diets that originated from plant and animal material to justify the use of poultry meal as a feed ingredient. Therefore, the aims were to determine i) egg yield performance and quality, ii) animal preferences for poultry meal, and ii) consumer preferences for eggs produced by these feeding methods.

Materials and Methods

The animal care protocol was approved by Kırşehir Ahi Evran University Ethical Review Committee (27.04.2016–68429034/26). Two diets, which were isocaloric and isonitrogenous, were prepared to meet the nutrient requirements of the Nick Brown hens that were used in this experiment (Table 1). One diet had components that were entirely of vegetable origin and the second included 5% poultry meal.

Table 1 Composition of experimental diets for laying hens that were entirely vegetarian, contained poultry meal, or involved a choice between these diets

Ingredient	Diets	
	Vegetarian	Omnivorous
Wheat	130.0	130.0
Corn	520.0	540.0
Poultry meal[1]		50.0
Soybean meal (48% crude protein)	205.0	140.0
Full-fat soybean	37.0	36.0
Vegetable oil	4.0	3.5
Dicalcium phosphate	16.8	13.2
Limestone	82.0	82.0
L-Threonine	0.2	0.3
Salt	2.5	2.5
Vitamin-mineral premix[2]	2.5	2.5
Calculated nutrient composition (g/kg)		
Dry matter	900	900
Crude protein	170	170
Calcium	36	36
Available phosphorus	4.1	4.1
Sodium	1.7	1.7
Methionine + cysteine	6.6	6.0
Lysine	7.5	7.5
Metabolizable energy (kcal/kg)	2800	2800
Analysed nutrient composition (g/kg)		
Dry matter	898.8	899.8
Crude protein	169.3	169.9
Ether extract	40.5	47.5
Crude fibre	49.2	48.4
Crude ash	80.3	74.4

[1]Dry matter: 950 g/kg, crude protein: 634 /.kg, ether extract: 115 g/kg, crude fibre: 22 g/kg, crude ash: 98 g/kg
[2]Retinol acetate: 4500 mcg, cholecalciferol: 50 mg, tocopheryl acetate: 40 mg, menadione: 5 mg, thiamine: 3 mg; riboflavin: 6 mg, pyridoxine: 5 mg, cobalamin: 0.03 mg, nicotinic acid: 30 mg, biotin: 0.1 mg, calcium d-pantothenate: 12 mg, folic acid: 1 mg, choline chloride: 400 mg, manganese: 80 mg, iron: 35 mg, zinc: 50 mg, copper: 5 mg, iodine: 2 mg, cobalt: 0.4 mg, selenium, 0.15 mg per kg of diet

Seventy-two 22-week-old Nick Brown layers were used in this study. Before the experiment, the bodyweights (BWs) of the hens, their egg production, and egg weights were recorded for two weeks. The hens were then divided into three groups with equal bodyweight and egg production. These groups were allocated to three feeding methods, namely i) vegetarian (no poultry meal), ii) omnivorous (5% poultry meal), and iii) a choice between these diets. Each group included 24 birds that were kept individually in cages (35 cm x 45 cm x 45 cm). Feed and water were provided ad libitum throughout the experiment. The experimental unit containing the cages was lit for 16 hours per day.

The change in bodyweight from the beginning to the end of the experiment was calculated. Egg production and feed intake were recorded daily and evaluated weekly. Feed conversion ratio (FCR) was calculated weekly as the ratio of feed intake (g) to egg mass (g).

To determine the feed preferences in the choice group, individual feeders were divided in half. The right side contained a vegetarian diet and the left an omnivorous one. To acclimatize the animals, for two days they were fed a vegetarian feed for 12 hours. Then for the next 12 hours, the omnivorous feeds were placed in the divided feeders. After the animals had been acclimatized to the positions of the vegetarian and omnivorous feeds, the diets were offered simultaneously in equal amounts.

Egg yield and damaged (cracked, unshelled) eggs were recorded daily. Egg quality analyses were performed weekly using 12 eggs collected from each group in the last consecutive three days of the week. These eggs were weighed individually, and their width and length were measured with a calliper to calculate their egg shape index as the ratio of width to length multiplied by 100. Eggshell breaking strength was assessed with a Brookfield CT3 texture analyser on eggs set with the blunt end down, with force applied at the sharp end.

The weights of egg albumen, yolk, and shell were recorded with the use of a domestic egg separator. Eggshell thickness was measured at three locations (sharp end, blunt end, and equator) with a 0.01 mm precision thickness gauge after the shell membranes had been removed and the mean value for each egg was calculated. The heights and widths of egg yolks were determined by micrometer. Egg yolk colour was estimated with a DSM yolk colour fan and measured with a Minolta CR 410 (Minolta Camera Co., Osaka, Japan) chroma meter set on the L* (lightness), a* (redness), and b* (yellowness) system. Haugh units (HU) were calculated from the egg weights and albumen heights using the HU formula [HU=100 log (H-1.7 $W^{0.37}$ + 7.57)].

At the end of the experiment, 12 eggs, taken randomly from each group, were used to determine the cholesterol content of the egg yolk (Ismail *et al.*, 2013). Eggs were hard boiled to separate the yolk, and 0.1 g samples of yolks were weighed in a sterile 10 ml Falcon tube. Cooked yolk was extracted with 4 ml isopropanol, vortexed for two to three minutes, and centrifuged at 3000 rpm for 10 minutes. The cholesterol level (mg of cholesterol g^{-1} of egg yolk) was determined with an ultraviolet spectrophotometer (Shimadzu UV Mini 1240) and commercial kit (Diagnostic Systems D1C37-125). Cholesterol content was calculated (Boehringer, 1989) as:

$$\text{Cholesterol content in extract (ECV)}(\text{mg dl}^{-1}) = \frac{\text{Read value in sample}}{\text{Read value in standard}} \times \text{Concentration of standard}$$

$$\text{Egg yolk cholesterol mg/g} = \frac{4(\text{ECV}/100)}{\text{Sample values (g)}}$$

Malondialdehyde analysis is used in feed and food analysis to assess shelf-life. Higher MDA values mean a shorter shelf-life. The rancidity of the fats in the egg yolk is the most important factor in the shelf-life of the egg. Lipid oxidation was assessed based on the MDA formed during storage (Botsoglou *et al.*, 2005). Egg samples from each group at the middle and end of the experiment were stored at 4 °C to determine the value of MDA, a marker of beta-oxidation of lipids. Malondialdehyde levels were determined (Tarladgis *et al.*, 1960) at 0, 21 and 42 days. A yolk sample (2 g) was mixed with 12 mL TCA (ethanol dissolved in 3 ml 7.5% TCA, 0.1% EDTA, 0.1% propil galat). The mixture was homogenized with ULTRA-TURRAX (IKA-T18) for 15 - 20 seconds and filtered through Whatman filter paper. After filtration, a 3 mL supernatant was transferred to another tube and mixed with 3 mL 0.02 M of thiobarbituric acid. The final mixture was incubated for 40 minutes at 100 °C. After incubation, the mixture was allowed to cool under tap water and centrifuged at 2000 rpm for five minutes, then its absorbance spectrum was determined with a spectrophotometer (Shimadzu UV Mini 1240) at a wavelength of 530. The final value was expressed as mg MDA per kg sample (Kilic & Richards, 2003).

The sensory evaluations were performed by 10 trained panellists from TUBITAK Marmara Research Centre Food Institute according to ISO (2011) and ISO (2006) criteria. The eggs were boiled in an equal

amount of hot water for eight minutes, then cooled to 40 °C. The samples were randomly coded with a three-digit number and given to the panellists. A scale of 1 - 9 points was used for each sensory feature. Scales were evaluated according to the quality grading method (ISO, 2003). The scoring scale criteria are given in Table 2.

Table 2 Scoring scale used in sensory evaluation of eggs[1]

Sensory quality properties	Scoring scale
Smell: the sensation of smell experienced by the sample while next to the nose	1= Normal egg smell - 9 = Abnormal smell
Appearance, colour: colour of egg yolk	1 = Normal egg yolk - 9 = Abnormal colour
Taste: the sensation of taste when egg yolk is taken into mouth	1 = Normal egg taste - 9 = Abnormal taste
Impression after tasting: sensations left in the mouth after egg yolk is swallowed	1 = Normal egg taste - 9 = Unpleasant taste
General preference: overall acceptance of sensations for all organoleptic properties of sample	1 = Like extremely - 9 = Dislike extremely

[1] ISO (2003)

Data for egg yield, quality, shelf-life, and sensory properties were analysed with one-way ANOVA (Windows version of SPSS, release 15.00 (SPSS Inc, Chicago, Illinois, USA). Data for diet preference of laying hens were analysed with a t-test in the same software. In the percentage data, ArcSin transformation was applied before analysis. The means were ranked with Duncan's multiple range tests. The results of the statistical analysis were presented as mean values and standard error of the means (SEM). Level of significance was regarded as P <0.05.

Results and Discussion

There were no significant differences among the groups in BW on the last day of the experiment. Omnivorous chickens tended to increase BW. Feeding methods did not affect feed intake, laying rate, egg weight, egg mass and FCR (P >0.05). The effects of feeding methods on production performance of laying hens are shown in Table 3

Table 3 Effects of feeding methods on production performance of laying hens over a 10-week period

Parameters	Feeding methods			SE	P-value
	Omnivorous	Vegetarian	Choice		
Initial bodyweight, g	1555.6	1556.8	1557.5	12.04	0.998
Final bodyweight, g	1573.0	1560.3	1557.7	17.01	0.927
Bodyweight change, g	17.4	3.5	0.2	17.55	0.916
Feed intake, g/day	109.2	106.5	108.0	5.94	0.449
Laying rate, %	96.8	93.6	93.7	0.70	0.104
Egg weight, g	54.6	54.6	54.2	0.33	0.781
Egg mass, g	53.3	51.3	51.2	0.43	0.092
Feed conversion ratio	2.07	2.11	2.14	0.04	0.499
Diet preference	51.4[a]	48.6[b]		0.99	0.047

[a,b,c] Within a row, means with a common superscript did not differ at probability P =0.05

Hens preferred the omnivorous diet to the vegetarian diet (P <0.05) (Figure 1) Figure 1 shows that the animals increased the omnivorous choice gradually each week. This gradual preference reflected the overall diet preference in becoming significant (Table 3).

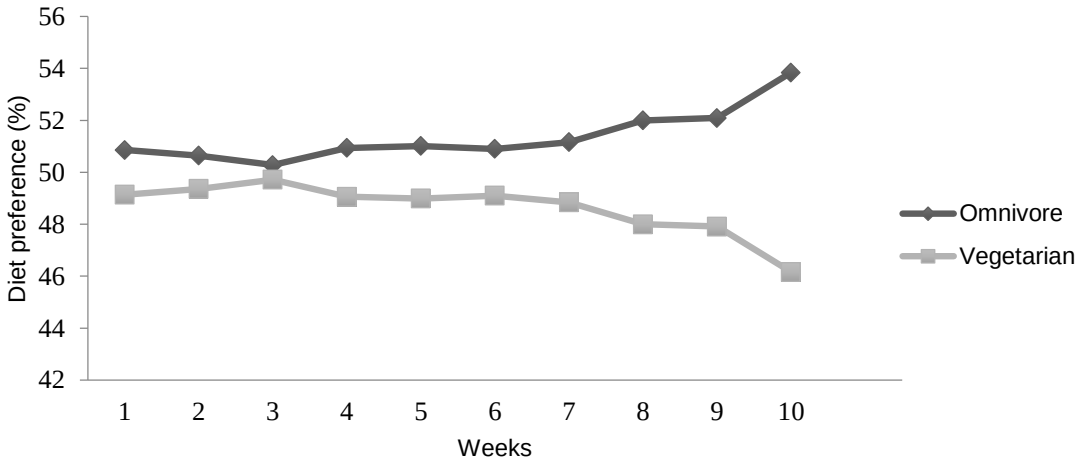

Figure 1 Preference of laying hens for the omnivorous diet or the vegetarian diet, %

None of the egg quality parameters and cholesterol content measures were significantly different (P >0.05). The effects of feeding method on egg quality parameters of laying hens are summarized in Table 4.

Table 4 Effects of feeding method on egg quality parameters of laying hens for 10 weeks

Parameters	Feeding methods			SE	P-value
	Omnivorous	Vegetarian	Choice		
Egg weight, g	55.9	55.5	55.8	0.11	0.394
Eggshell strength, N	50.1	48.2	48.8	0.69	0.515
Albumen pH	8.5	8.6	8.6	0.02	0.393
Yolk lightness, L*	73.8	74.0	74.2	0.11	0.354
Yolk redness, a*	20.7	20.1	20.2	0.14	0.298
Yolk yellowness, b*	32.9	32.9	32.6	0.17	0.242
Eggshell thickness, mm	0.40	0.39	0.39	0.01	0.086
Yolk colour, DSM yolk fan	11.9	11.8	11.9	0.04	0.551
Egg shape index	78.8	78.7	79.1	0.16	0.305
Albumen index	13.4	12.8	12.5	0.14	0.064
Yolk index	50.2	50.0	51.1	0.21	0.361
Haugh units	96.6	95.0	95.1	0.41	0.235
Eggshell weight, g	6.3	6.4	6.4	0.04	0.189
Cholesterol, mg/g	13.2	13.0	13.1	0.12	0.110

In the fifth and tenth weeks of the experiment, the MDA values of the eggs stored for 21 days were not affected statistically by feeding methods (P >0.05). However, when the eggs were stored for 42 days, the feeding method affected the MDA values significantly (P <0.01). At the end of the experiment, the highest MDA value (0.16 mg/kg) was obtained from the eggs of omnivorous hens after 42 days storage, followed by the eggs of choice (0.14 mg MDA/kg) and vegetarian diets (0.12 mg MDA/kg) animals. Malondialdehyde values of egg yolks are shown in Table 5.

Table 5 Effects of feeding methods on malondialdehyde values (mg/kg) in eggs collected in the fifth and tenth weeks

Week	Storage time, d	Feeding methods			SE	P-value
		Omnivorous	Vegetarian	Choice		
5	0	0.11	0.10	0.12	0.003	0.125
	21	0.12	0.12	0.13	0.013	0.349
	42	0.14[a]	0.13[ab]	0.12[b]	0.003	0.038
10	0	0.11	0.08	0.11	0.002	0.387
	21	0.11	0.10	0.11	0.004	0.431
	42	0.16[a]	0.12[c]	0.14[b]	0.003	0.021

[a,b] Within a row, means with a common superscript did not differ at probability *P* =0.05

The panellists gave similar reactions to the taste of eggs during and after tasting (*P* >0.05). However, their responses to smell, colour and overall preference differed (*P* <0.01). The most acceptable eggs were obtained from vegetarian hens, whereas the eggs that were most disliked were obtained from omnivorous hens. The sensory results of the panellists are given in Table 6.

Table 6 Effects of feeding method on sensory properties of eggs

Sensory properties	Feeding methods			SE	P-value
	Omnivorous	Vegetarian	Choice		
Smell	2.7[b]	1.1[c]	4.4[a]	0.26	0.000
Colour	2.4[b]	1.1[c]	4.1[a]	0.23	0.000
Taste	2.1	1.9	2.6	0.13	0.121
Impression after taste	1.5	1.9	1.8	0.12	0.460
Overall preference	2.7[a]	1.4[b]	2.3[ab]	0.12	0.000

[a,b,c] Within a row, means with a common superscript did not differ at probability *P* =0.05

Feeding method did not affect egg production and quality parameters, except for MDA value. These insignificant effects on egg production and quality parameters could be attributed to their chemical composition in macro nutrient level since both diets were isocaloric and isonitrogenous. In addition, the experimental diet did not affect the metabolism of hens at the administered level, thus did not cause changes in their physiological responses. This was evident in the unchanged bodyweight at the beginning and end of the study. Similarly, other authors showed that dietary inclusion of poultry meal in various doses did not affect egg production and quality parameters (Raja *et al.*, 2001; Erturk & Çelik, 2004; Senkoylu *et al.*, 2005; Samli *et al.*, 2006; Hosseinzadeh *et al.*, 2012; Mahmud *et al.*, 2015). For example, Hosseinzadeh *et al.* (2012) supplemented a layer diet with 2.5%, 5%, and 7.5% poultry meal, and found no effects on egg production, egg mass, shape index, eggshell thickness, eggshell weight and Haugh unit. Feeding method did not affect feed intake and FCR (Hosseinzadeh *et al.*, 2012).

The nutritional similarities in the diets did not encourage or discourage the animals. They consumed feeds according to their nutritional requirements. This was evidenced by choice-fed animals since they did not change their daily feed intake compared with omnivorous and vegetarian animals. Consequently, choice-fed hens preferred the omnivorous diet without changing total feed intake. Contrary to the current findings, Samli *et al.* (2006) and Geshlog *et al.* (2011) reported that egg production, feed intake and egg mass were affected adversely by increased inclusion of poultry meal, but not FCR. In the present experiment, hens did not decrease feed intake or reject feeds in the choice system. This means that the current inclusion rate was at an acceptable level of organoleptic properties of poultry meal for hens. However, Samli *et al.* (2006) found a decrease in feed intake for inclusion rates of 5% and 10% poultry meal in a layer diet. This discrepancy

might be because of the origin and processing of their poultry. The nutrient composition (955 g DM, 630 g CP, 118 g EE, and 207 g ash per kg) of their poultry meal was different from that of current study.

The most important factor in the shelf-life of the egg is the breakdown of lipids. MDA values are determined in eggs to estimate lipid peroxidation, which is a measure of deterioration (Guclu *et al.*, 2008). Eggs produced by omnivorous hens displayed a higher amount of MDA when these eggs were stored for 42 days, probably because of the fatty acid composition of poultry meal. Because of the double bonds in their structure, unsaturated fatty acids are less stable than saturated fatty acids and are easily oxidized (Poureslami *et al.*, 2010). Unsaturated fatty acids are high in poultry meat (Al-Khalaifah *et al.*, 2020; Jayasena *et al.*, 2013). Additionally, the fatty acids in poultry meal in the present diet were more prone to degradation. Higher MDA values in egg samples were obtained from omnivorous hens. This means that these eggs had a shorter shelf-life than eggs from vegetarian animals. Many studies attempted to increase the shelf-life of eggs with plant extracts (Kara *et al.*, 2016; Batista *et al.*, 2017; Simitsiz *et al.*, 2018; Yu *et al.*, 2018; Goliomytis *et al.*, 2019; Pires *et al.*, 2020; Aydin & Bolukbası, 2020), and other preparations (Ogunwole *et al.*, 2015; Long *et al.*, 2016; Konca *et al.*, 2019; Shang *et al.*, 2020). When poultry meal is used for this purpose, it should be combined with a suitable feed additive.

In the choice group, the birds ate the two diets more or less equally. However, with time they tended to increase their consumption of the omnivorous diet (Figure 1). Their preference for poultry meal may be attributed to the similarities between their body composition and poultry meal (Sahin, 1999). The selection between a diet that included poultry meal and one that did not previously has not been studied in laying hens. Only Bhuiyan *et al.* (2012) offered broiler chicks protein sources of vegetable and animal origin simultaneously. These chicks preferred an animal protein source during the development and finishing periods. This finding supported the results of diet preferences in the current study. The ability of chickens to select a balanced diet if offered a choice was demonstrated by a number of researchers (Leeson & Summers, 1978; Forbes & Covasa, 1995; Forbes, 1995; Pousga *et al.*, 2005). It was postulated that the underlying mechanism in diet selection of birds is that their biological needs for production are determined genetically.

In the present experiment, the authors tried to determine the preferences of panellists for eggs produced with various feeding methods. Panellists gave the best grades for smell, taste, colour, and overall evaluation to eggs produced by the vegetarian diet. In the general evaluation, the eggs produced by the omnivorous diet were not acceptable to the panellists, perhaps because of the penetration of the original aroma of poultry meal. Raw poultry meat has a blood-like taste with little aroma. During processing, volatile aromatic compounds react with non-volatile compounds in muscle and fatty tissues, resulting in a new aroma of poultry meal (Jayesena *et al.*, 2013). However, apparently no study has investigated the penetration of aroma compounds in eggs, although there have been studies on the effects of dietary ingredients (Toyes-Vargas *et al.*, 2018; Semwogerere *et al.*, 2019; Dalle Zotte *et al.*, 2019) and additives (Buckiuniene *et al.*, 2018; Konca *et al.*, 2019) on the sensory properties of eggs. These ingredients were usually of plant origin and not animal.

When feed and eggs were evaluated for organoleptic properties, preference tests by animals and the panellists showed that the inclusion of poultry meal did not present a problem for animals, whereas the eggs produced by the omnivorous diet were problematic for panellists. This inconsistency should interest legislators and poultry producers.

The fatty acid composition of poultry meal was expected to cause an increase in the cholesterol content of eggs, but this did not happen. The cholesterol contents of eggs were similar in all groups. Again, there was no study in the literature on the effect of poultry meal on the cholesterol content of eggs. Only Geshlog *et al.* (2011) reported that dietary poultry meal did not affect the serum cholesterol level of laying hens.

Conclusion

Dietary poultry meal did not affect egg performance and quality, irrespective of feeding method. The tendencies of increases in egg production and bodyweight in omnivorous groups may be because the chemical composition of poultry meal is similar to their own body composition. On the other hand, these increases may be because of the lower need for metabolic processing of nutrients in poultry meal compared with vegetable ingredients. However, the diet containing poultry meal shortened the shelf-life of the eggs. Hens offered a choice did not reject the omnivorous feed and increased consumption over time. The panellists preferred the eggs from the vegetarian diet. These animal and consumer preferences should be considered when deciding to use poultry meal in feeding laying hens.

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Authors' Contributions

HÇ was responsible for the experimental design, preparation and execution of the project. AŞ supervised HÇ and participated in project planning, its implementation, analysis of the data, and interpretation of the results. HÇ wrote the initial manuscript. AŞ edited and corrected the manuscript before submission.

Conflict of Interest Declaration

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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