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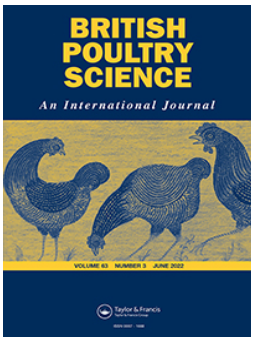

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Feeding dihydroquercetin in wheat-based diets to laying hens: impact on egg production and quality of fresh and stored eggs

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ABSTRACT

1. This study assessed the impact of dietary dihydroquercetin (DHQ) in wheat-based diets on egg production, composition and quality when fed to laying hens. A total of 80 Hy-Line Brown hens were allocated to 20 enriched layer cages, over two tiers, in groups of four birds.
2. Two wheat-based diets were used in the study. A basal diet, meeting the nutrient requirement of the hens, containing 11.56 MJ/kg AME and 172 g/kg crude protein, was mixed and split into two parts. One part was fed as prepared to the control group of birds. The second diet was made by adding 1.5 g DHQ per kg basal diet and fed to the treatment group of birds. This level was relatively high and extended the data on levels normally fed. The diets were fed in a meal form and did not contain any coccidiostat, antimicrobial growth promoters or other similar additives. Each diet was fed to hens in 10 replicate cages for 4 weeks, from 22 to 26 weeks of age, following randomisation.
3. Subsequently, eggs were investigated to determine the impact of dietary DHQ on the quality variables of fresh and 28-d stored eggs.
4. Overall, feeding 1.5 g/kg dietary DHQ for 4 weeks did not affect ($P > 0.05$) egg production or the quality of fresh and stored eggs. Any observed egg quality changes ($P < 0.05$) confirmed the expected effects of egg storage.

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Dihydroquercetin; taxifolin; egg quality; antioxidants; laying hens

Introduction

Consumers typically require that animals are fed safe, high-quality animal feed. Sustaining production performance has become even more challenging for the poultry industry, particularly due to high feed material cost and availability, driving research for alternatives. Therefore, much emphasis has been put on the need for more natural and sustainable animal feed ingredients (Mahfuz et al. 2021). The popularity of natural antioxidants to protect human and animal health and to increase the shelf life of products from animal origin has increased (Botsoglou et al. 2003; Weidmann 2012; Iskender et al. 2016). Flavonoids, being a major subgroup representing plant polyphenols, are considered natural antioxidants and have attracted attention for use in animal nutrition (Surai 2014; Yeung et al. 2021). Dihydroquercetin (DHQ), known as taxifolin, is a flavonoid extracted from plants including onions, milk thistle and various conifers (Weidmann 2012). Dihydroquercetin has been widely applied as an antioxidant for the surface treatment of fresh meat and fish and has been incorporated in animal diets to enhance productive performance and the antioxidant status of meat (Kamboh et al. 2015; Fomichev et al. 2016, 2020; Pirgozliev et al. 2019, 2020, 2021).

Studies investigating the use of quercetin, a chemical similar to DHQ, produced contradictory results on laying hen productive performance and the quality and shelf life of eggs. Liu et al. (2013) reported that feeding quercetin to laying hens improved performance and egg quality. However, Simitzis et al. (2018) did not find differences in layer performance or egg quality from birds fed quercetin, compared with an unsupplemented control. Feeding quercetin was, however, found to improve the oxidative stability of egg yolk. Limited studies have been carried out on the impact of dietary DHQ on laying hen productive performance and the quality and shelf life of eggs. Gorlov et al. (2019) studied the effect of dietary DHQ on the chemical composition of hatching eggs, but less attention has been paid to egg and shell quality variables.

The hen egg is an encapsulated source of macro and micro nutrients that meet the requirements to support embryonic development until hatching (Réhault-Godbert et al. 2019). The perfect balance and diversity in its nutrients along with its high availability and its affordable price makes the conventional hen egg a food product with high nutritional quality for consumers. Due to the continual rise in global egg consumption, along with the increasing use of natural antioxidants in poultry feed, it is important to understand the potential impact of these feed additives on the quality and shelf life of eggs.

Under commercial conditions, Class A eggs are not permitted to be refrigerated at any stage throughout production and retail (Commission Regulation (EC) No 589 2008). However, eggs are often stored in a fridge by consumers after purchase. The same legislation requires that the date of minimum durability shall not be more than 28 d after laying. Information on changes to the physical characteristics of stored eggs is, however, inconsistent. Niemiec et al. (2001)

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reported that the dietary addition of primrose, linseed and rapeseed, with or without supplementation of 200 mg vitamin E/kg, did not affect egg weight after 20 d of storage at 12°C. However, Omri et al. (2019) found that feeding linseed, dried tomato paste and sweet pepper powder reduced the losses of egg albumen and the shell of eggs stored at 4°C for 30 d.

The majority of studies cited used maize-based diets, and so research on wheat-based diets containing phenolic compounds is limited. Therefore, the aim of this study was to assess the impact of DHQ in wheat-based diets on egg production, chemical composition and quality variables when fed to laying hens aged from 22 to 26 weeks of age. Subsequently, eggs from the study were investigated to determine the impact of dietary DHQ on the quality variables of 28-d stored eggs.

Materials and methods

The experiment was conducted at the National Institute of Poultry Husbandry (NIPH) and approved by the Research Ethics Committee of Harper Adams University (UK). This manuscript is reported in line with the ARRIVE guidelines (Kilkenny et al. 2010).

Dietary formulation

Two wheat-barley-soybean-based diets were used in the study. The basal diet was formulated to meet the nutrient requirement of the hens, containing 11.56 MJ/kg AME and 172 g/kg crude protein (Table 1). The basal diet was offered to the control group (C) of hens, whereas the experimental group was fed the basal diet supplemented with 1.5 g of Siberian Larch (*Larix sibirica*) extract (JSC NPF Flavit, IBI RAS, Pushchino city, Moscow region, Russian Federation 142 290). According to the supplier, this extract contained over 85% pure DHQ, with the remainder including other flavonoids, saponins and water (DHQ). The experimental diets were fed in a meal form for 4 weeks, between 22 and 26 weeks of age and did not contain any coccidiostat, antimicrobial growth promoters or other additives.

Table 1. Formulation of the experimental basal diet.

Ingredients (g/kg)	
Barley	100.0
Wheat	535.0
Soybean meal (48% CP)	175.0
Full fat soya	50.0
L Lysine	0.5
DL Methionine	1.5
Soya oil	20.0
Limestone	100.0
Monocalcium Phosphate	8.0
Salt	2.5
Sodium bicarbonate	1.5
Layer Vit-Min Premix[1]	1.0
Titanium Dioxide	5.0
Determined values	
Dry matter (g/kg)	905
Gross energy (MJ/kg)	14.63
Crude protein (g/kg)	167
Ether extract (g/kg)	48
Calcium (g/kg)	47.7
Total Phosphorus (g/kg)	5.5
Total carotenoids (μg/g)	0.627
Vitamin E (μg/g)	17.248
AME (MJ/kg)[2]	11.56

[1]The premix provided (units/kg diet) the following: [1]Premix (per kg feed): Vit A (retinyl acetate) 10.000 IE; Vit D3 (cholecalciferol) 2.000 IE; Vit E (dl-a-tocopherol) 25 mg; Vit K3 (menadione) 1,5 mg; Vit B1 (thiamine) 1,0 mg; Vit B2 (riboflavin) 3,5 mg; Vit B6 (pyridoxine-HCl) 1,0 mg; Vit B12 (cyanocobalamin) 15 μg; Niacin 30 mg; D-pantothenic acid 12 mg; Choline chloride 350 mg; folic acid 0,8 mg; Biotin 0,1 mg; Iron 50 mg; copper 10 mg; Manganese 60 mg; Zinc 54 mg; Iodine 0,7 mg; Selenium 0,1 mg. [2]The value for AME was calculated.

Experimental design

To maximise the chances of detecting responses to dietary DHQ, the experiment was undertaken during a time of high-energy requirement for egg production. A total of 80 Hy-Line Brown hens were housed four per cage in 20 enriched layer cages (Hellmann Poultry GmbH & Co. KG), over two tiers, between 22 and 26 weeks of age. The experiment was conducted using a randomised block design with 10 spatial blocks located over two tier levels (two blocks per tier, each block consisting of two cages given different experimental diets). The temperature was maintained at 21°C, and relative humidity was between 50% and 70%. The birds had *ad libitum* access to feed and water and were given 14 h of light each 24 h.

Hen performance, egg production and determination of egg quality

The hens in each cage were bulk weighed at the beginning and the end of the four-week experimental period. Feed intake (FI) of each cage was recorded and calculated per hen per day. Egg numbers were recorded every day and egg weight was determined once per week, assuming that this is the average egg weight for the week. The number of dirty and cracked eggs, eggs with a double yolk, wrinkled eggs, eggs with a soft shell and hen mortality were also recorded daily. The feed conversion ratio for egg production (FCR) was calculated as:

$$Feed\ intake(g)/eggs\ laid(g)$$

Egg and egg shell-quality analyses were performed on a total of 20 eggs which had been collected, one egg from each cage, on the last day of the experiment (26 weeks old). The analyses of the eggs were completed after 1 d of storage at 15°C. Eggs were individually weighed, and albumen height (AH) and Haugh units (HU) were measured using Technical Services and Supplies (TSS) Egg Ware (Chessingham Park, Dunnington, York, YO19 5SE, England) as previously described (Pirgozliev et al. 2010; Whiting et al. 2019). Yolk colour was measured using a DSM YolkFan^{TM}. The yolk and white were then separated to determine the pH of each, using a FC2133 Foodcare pH and temperature electrode probe (Hanna Instruments Ltd, Leighton Buzzard, UK). Eggshells were washed and left to dry for 24 h in an air-forced oven at 40°C with the membrane in place. Once dried, eggshells were weighed and shell thickness was measured by averaging measurements taken at three locations on the equator using a TSS QCT shell thickness micrometre. Shell thickness index was calculated as described by Fox (1976). The surface area of the egg was calculated as 4.835 × Fresh egg weight 0.662 (Paganelli et al. 1974) and used to calculate the index in mg/cm^2 per day, dividing the dry eggshell weight by the egg weight.

At the end of the study, one more egg from each cage was obtained, and the impact of dietary treatment on shell colour was determined by Konica Minolta Chroma Metre CR – 400/410 (Minolta, Tokyo, Japan) as defined by the Commission Internationale de L'Eclairage (CIE) colour system. L* indicated lightness, while a* and b* represented chromaticity coordinates. Then, the contents of each egg were broken out, freeze-dried, ground using a mortar and pestle and the impact of dietary treatment on the colour of the internal contents was determined by Konica Minolta Chroma Metre.

Proximate analysis of experimental diets and eggs

At the end of the study, another egg was collected from each cage. The eggshell was removed, ground and used for mineral analysis. The yolk and albumen were freeze dried, ground and analysed for chemical composition. Dry matter (DM) of the feed samples, yolk and albumen was determined by drying samples in a forced draft oven at 105°C to a constant weight (AOAC 2006; method 934.01). Crude protein (6.25 × N) in samples was determined by the combustion method (AOAC, 2006; method 990.03) using a LECO FP-528 N (Leco Corp., St. Joseph, MI). Fat (as ether extract) in samples was extracted with diethyl ether by the ether extraction method (AOAC, 2005; method 945.16) using a Soxtec system (Foss Ltd., Warrington, UK). Ash content of the eggshells was determined by pre-ashing using a Bunsen burner and placing samples in a muffle furnace at 550°C for 6 h. Mineral concentrations in the diets and eggshells were determined by inductively coupled plasma emission spectrometry (Optima 4300 DV Dual View ICPOE spectrometer, Perkin-Elmer, Beaconsfield, UK), as described by Tanner et al. (2002). The concentration of total carotenoids and total vitamin E in the feed samples, yolk and albumen were determined, as previously described (Surai 2002; Karadas et al. 2014; Kljak et al. 2021).

Egg storage

At the end of the final week of the study (at 26 weeks of hen age), three eggs were collected from each cage and stored for 28 d at 15°C. Egg quality measurements were taken every 2 weeks (0, 2 and 4 weeks after storage). One egg from each cage was tested at each time period to determine the studied values. Measurements determined included, albumen height, HU, albumen and yolk pH and yolk colour values. The same egg was used to record egg weight over time.

Statistical analysis

Egg data were analysed using Genstat (18th edition) statistical software package (IACR Rothamsted, Hertfordshire, UK). Comparisons among the studied variables were performed by one-way ANOVA. Comparisons among the studied variables for the storage investigation were performed by a two-way ANOVA using a 2 × 3 factorial design (dietary DHQ × storage period). Data were checked for homogeneity and normality prior to ANOVA. Results were considered significant at $P < 0.05$. Data are expressed as means and their pooled standard errors (SEM).

Table 2. Effect of dietary dihydroquercetin (DHQ) on hen weight, feed intake and egg production over 4 weeks of feeding.

	C	DHQ	SEM	P
Hen start weight (kg)	1.854	1.786	0.0309	0.157
Hen end weight (kg)	1.959	1.897	0.0597	0.478
Weight gain hen period (kg)	0.105	0.111	0.0343	0.872
FI (g/b/d)	117.8	118.5	3.06	0.879
Egg mass (g/b/d)	54.78	53.09	1.136	0.320
Egg weight (g)	58.65	57.19	1.139	0.388
FCR egg production (kg:kg)	2.160	2.250	0.0955	0.521
Egg production (%)	93.5	92.8	1.55	0.763

C, control; SEM, pooled standard error of means; Data are means of 10 replicate cages with 4 birds per cage; P value describes significance between treatments determined by ANOVA; Results statistically significant $P < 0.05$.

Results

Effect of dietary DHQ on egg production, egg quality, proximate, carotenoid, vitamin E and mineral analysis of eggs at study end point

The results of the impact of DHQ on egg quality, proximate, carotenoid, vitamin E and mineral analysis are presented in Tables 2, 3 and 4, respectively. There were no statistically significant ($P > 0.05$) differences in the weight of the hens and the rest of the studied variables throughout the study (Table 2). The overall means of FI, daily egg mass, egg weight, FCR and egg production were 118.2 g, 53.94 g, 57.92 g, 2.205 g:g and 93.2%, respectively.

Egg quality was similar between different diets and there were no differences ($P > 0.05$) between the studied variables (Table 3). The colour of the yolk was the most variable measurement for DSM YolkFan® and for the a* chromaticity measurement.

Table 3. Effect of dihydroquercetin (DHQ) on egg and eggshell quality variables when fed to laying hens for 4 weeks.

	C	DHQ	SEM	P
Albumen height (mm)	8.44	8.38	0.408	0.919
Haugh unit	91.7	91.9	2.15	0.954
Albumen pH	8.43	8.43	0.060	0.991
Yolk pH	6.28	6.22	0.052	0.403
Eggshell thickness (mm)	0.333	0.354	0.0093	0.149
Eggshell weight (g)	5.20	5.37	0.157	0.475
Surface area (cm^2)	71.9	70.0	1.11	0.266
Shell thickness index mg/cm^2	72.3	76.7	1.88	0.131
DSM YolkFan™	2.30	2.20	0.299	0.811
Yolk colour:				
L	82.6	81.6	2.09	0.723
a*	0.43	0.66	0.864	0.801
b*	17.49	16.24	1.263	0.349
Eggshell colour:				
L	57.86	60.30	1.235	0.200
a*	20.50	18.62	0.845	0.156
b*	32.50	30.57	0.764	0.111
Deformities %				
% cracked	1.47	1.66	0.489	0.792
% double yolk	3.08	1.81	0.732	0.252
% soft shell	0.88	0.43	0.309	0.334
% wrinkled	0.14	0.00	0.100	0.343

C, control; SEM, pooled standard error of means; Data are means of 10 replicate cages with 4 birds per cage; Colour system defined as L* indicates lightness, while a* and b* are chromaticity coordinates; P value describes significance between treatments determined by ANOVA; Results are statistically significant when $P < 0.05$.

Table 4. Proximate analysis, total carotenoid and vitamin E of the yolk and albumen and mineral analysis of the eggshell after feeding dihydroquercetin (DHQ) to laying hens for 4 weeks.

	C	DHQ	SEM	P
Yolk and albumen:				
Dry matter (g/kg)	315.5	315.0	3.74	0.930
Crude protein (g/kg)	374.8	373.1	6.37	0.853
Crude fat (g/kg)	211.6	210.9	7.19	0.948
Total carotenoids (µg/g)	2.96	2.58	26.9	0.288
Vitamin E (µg/g)	42.5	44.3	18.1	0.629
Eggshell:				
Calcium (g/kg)	82.5	83.5	2.09	0.750
Total Phosphorus (g/kg)	4.7	4.8	0.11	0.389
Ash (g/kg)	101.5	102.4	1.93	0.746

C, control; SEM, pooled standard error of means; Data are means of 10 replicate cages with 4 birds per cage; P value describes significance between treatments determined by ANOVA; Results are statistically significant when P < 0.05.

Table 5. Effect of length of storage on egg quality variables of laying hens fed dietary dihydroquercetin (DHQ) for 4 weeks.

	Egg weight (g)	Albumen pH	Albumen height (mm)	Haugh Units	Yolk pH	Yolk colour (DSM YolkFan)
DHQ						
-	57.55	8.83	5.74	72.87	6.15	2.00
+	55.30	8.86	5.60	72.41	6.17	1.93
SEM	1.086	0.033	0.145	1.030	0.029	0.125
Storage (d)						
0	57.85	8.43	8.41	91.78	6.25	2.25
14	56.09	9.03	4.66	66.45	5.98	1.45
28	55.33	9.09	3.95	59.69	6.25	2.20
SEM	0.570	0.040	0.178	1.262	0.036	0.153
DHQ × Storage (d)						
− 0	58.99	8.43	8.44	91.69[a]	6.28	2.30
− 14	56.81	9.01	4.49	64.32[b]	5.94	1.40
− 28	56.86	9.06	4.30	62.59[b]	6.22	2.30
+ 0	56.72	8.43	8.38	91.87[a]	6.22	2.20
+ 14	55.38	9.05	4.82	68.57[c]	6.03	1.50
+ 28	53.80	9.11	3.59	56.78[d]	6.27	2.10
SEM	1.363	0.056	0.251	1.785	0.051	0.216
P-values						
DHQ	0.181	0.504	0.478	0.754	0.528	0.707
Storage	0.015	<0.001	<0.001	<0.001	<0.001	<0.001
DHQ × Storage	0.587	0.892	0.124	0.025	0.289	0.780

C, control; SEM, pooled standard error of means; Data are means of 10 replicate cages with 4 birds per cage; P value describes significance between treatments determined by 2 × 3 factorial ANOVA; Results are statistically significant when P < 0.05.

The egg chemical composition, carotenoids and vitamin E content did not differ (P > 0.05) between treatments (Table 4). The crude fat was the most variable nutrient, although mean levels were low compared to dry matter and crude protein.

Effect of dietary DHQ and length of storage on egg quality variables

Feeding DHQ did not change (P > 0.05) any of the studied variables (Table 5). Over the storage period, AH and HU decreased, although albumen and yolk pH increased with time (P < 0.001). Egg weight decreased with the duration of storage (P = 0.015). Yolk colour intensity decreased during storage (P < 0.001). There was a DHQ × storage time interaction (P = 0.025) which suggested that, under prolonged storage, feeding DHQ may reduce the HU.

Discussion

The main purpose of the study was to evaluate the effects of dietary supplementation with DHQ on egg production and quality of fresh and stored eggs. Some antioxidant properties of eggs were measured. All results were within the expected range for production and egg quality for Hy-Line Brown laying hens at this age when fed wheat-soy layer diets.

Research by Liu et al. (2014), Jahanian et al. (2015) and Chen et al. (2018) found an increase in the egg production and egg mass of layers fed phenolic components (quercetin, dry leaf extract from purple coneflower or eucalyptus, respectively). Liu et al. (2014) reported that birds fed quercetin from 39 to 47 weeks of age had increased eggshell thickness, eggshell strength and feed efficiency. In addition to increased egg production, Wang et al. (2018) reported improved feed efficiency and higher egg albumen height and HU, when feeding tea polyphenols to laying hens. When feeding resveratrol, Feng et al. (2017) observed improved feed efficiency and egg quality variables, but no changes in production. Simitzis et al. (2018) did not find changes in egg production and quality when fed quercetin supplemented diets for 4 weeks. In contradiction to a previous report (Liu et al. 2013), Liu et al. (2014) did not find any responses for laying rate, eggshell thickness or strength when feeding graded levels of quercetin to layers from 28 to 36 weeks of age. It is possible that the lack of response to the DHQ in the present study (in terms of performance) may have been due to the ability of the laying hen to use body reserves to maintain egg production over moderate periods of time (Morris 1969). In agreement, Özek et al. (2011) reported no differences in egg production during 16 weeks of lay (52–68 weeks of age) when layers were fed phenolic components (carvacrol and thymol), which suggested that the length of the feeding period may not necessarily be the reason for the lack of response. Indeed, Çimrin (2019) reported lower egg production and egg weight when feeding phenolic components (carvacrol and thymol) to laying hens for 8 weeks (48–56 weeks of age).

Feeding approximately 70 mg supplementary DHQ to layer parent flocks, Gorlov et al. (2019) established a positive effect on bird blood variables, including increased red blood cells, haemoglobin concentration and haematocrit. Further analysis of the chemical composition found that DHQ increased DM, CP, Ca, P and some amino acids in the hatching eggs, but no quality analyses were performed (Gorlov et al. 2019). Zoidis et al. (2021) found that supplementary quercetin increases Ca, Fe, Mg and Ni in eggshell when fed to layers from 70 to 74 weeks of age. Simitzis et al. (2018) reported that feeding dietary quercetin at 400 mg per kg of feed for 4 weeks increased feed intake and eggshell weight, although no changes were observed when feeding 200 and 800 mg quercetin per kg diet. This supported the view that feeding a dietary component for 4 weeks is enough to influence the chemical composition and quality of an egg (O'Sullivan et al. 2020), although there were no changes in egg chemical composition in the present four-week study.

However, the relatively different responses to dietary phenolic compounds in the literature regarding egg production, quality and chemical composition should be interpreted carefully. Phenolic compounds are a group of small molecules, characterised by their structures having at least one phenol unit. Based on their chemical structures, phenolic compounds can be divided into different subgroups, such as phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes and curcuminoids (Gan et al. 2019), which possess different biological activity and modes of action. This may partially explain the observed differences among the reports. Previous researchers have incorporated varying amounts of phenolic compounds with different purities into diets when designing and performing poultry experiments, which may be a reason for the variable responses. In addition, using various strains of birds at different ages may further complicate the outcomes.

As expected, albumen height decreases with time of storage, while albumen pH increases (Silversides and Budgell 2004). The reduced egg weight at the end of 4-week storage was caused by water exchange between the yolk and the egg white, as well as from the loss of water and carbon dioxide through the eggshell pores (Niemiec et al. 2001; Réhault-Godbert et al. 2019). Barbosa et al. (2011) reported that the storage of eggs for 28 d at room temperature (26.5°C) decreased yolk colour. In addition, Omri et al. (2019) found a positive correlation between yolk colouration of stored eggs and carotenoids content. Egg yolks are known for their high fat content and are susceptible to lipid oxidation. A report by Simitzis et al. (2018) showed that enrichment of the diet with different levels of quercetin, even for only 28 d, improved oxidative stability, determination by using the malondialdehyde (MDA) assay, of both fresh and stored eggs (for up to 90 d) in a dose-dependent manner. Nimalaratne et al. (2015), however, reported no change in the oxygen radical scavenging capacity values, as well as the contents of free amino acid, carotenoid and MDA in egg yolk during 6 weeks of storage under refrigeration. This suggested that a relatively short storage period, dietary vitamin E supplementation and a controlled environment, *i.e.* 15°C, contributed to the lack of interaction between dietary DHQ and the length of storage on the egg quality variables in the current study.

In addition, diets were supplemented with synthetic vitamin E (slightly exceeding NRC, 1994 recommendation of 12 IU/kg) which provided enough for hens to deposit antioxidants from diets into their egg yolks that could protect the lipids during egg storage. This may further explain why DHQ addition in the laying hen diets did not affect the quality of stored eggs.

Research by Botsoglou et al. (1998) and Botsoglou et al. (2005) demonstrated that laying hen diets containing 25–50 g/kg vitamin E (α-tocopherol acetate) provided sufficient antioxidant protection in the feed, although in enriched diets containing high levels of polyunsaturated fatty acids (PUFA) or conditions that increase birds stress (*e.g.* high ambient temperature), supplementation with phenolic compounds may be required. Although soya components may increase overall dietary fatty acid content, the present study was conducted under normal ambient temperature and formulated to contain 25 mg/kg vitamin E. Novel feed ingredients, such as insect meal (recently approved for feeding in the EU) and algae, are anticipated to increase PUFA in future poultry diets, thus more research with supplemental antioxidants is required to ensure readiness for the next generation of feed formulations.

In conclusion, the results of this study show that feeding 1.5 g/kg dietary DHQ for 4 weeks did not change egg production or the quality of fresh and stored eggs. The observed changes in egg quality confirmed the expected effects of storage time. Further research on feeding DHQ to laying hens at the expense of vitamin E is warranted.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

I.M.W. and V.P. conceived the idea, and V.P., S.P.R. and S.O.-D. designed the study. I.M.W., S.C.M. and V.P. conducted the experiment and collected the samples. I.M.W. and V.P. completed the analyses on energy and nutrient availability and egg quality variables. K.K. performed all antioxidant analysis. I.M.W., V.P., S.C.M. and A.G.A. analysed the data and in collaboration with all of the rest wrote the article.

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