TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2016) 40: 590-597

doi:10.3906/vet-1506-100

Effect of retinol and retinol esters on performance, egg quality, and blood and egg vitamin A levels in laying quails

Murat İLHAN, Tuba BÜLBÜL*
Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine,
Afyon Kocatepe University, Afyonkarahisar, Turkey

Received: 30.06.2015 • **Accepted/Published Online:** 17.03.2016 • **Final Version:** 02.11.2016

Abstract: This study aimed to determine the effects of retinol and retinol ester supplementation on performance, egg quality, serum and egg oxidative status, and vitamin A levels in laying quails. Three hundred quails were divided into 5 groups. The control group received a basal diet that contained vitamin premix without vitamin A or vitamin A precursors. The treatment groups received dietary supplementation with retinol and retinol esters [retinyl acetate (R-acetate), retinyl palmitate (R-palmitate), retinyl propionate (R-propionate), 3300 IU/kg]. Egg production and feed conversion ratio improved in all experimental groups. Serum β-carotene and malondialdehyde (MDA) levels decreased and serum retinol levels increased in all experimental groups. Yolk color in the 8th week decreased in the R-propionate group. Serum antioxidant activity levels increased in the R-palmitate and R-propionate groups. Yolk retinol levels increased in the R-palmitate and R-acetate groups. Yolk MDA level decreased in the retinol, R-acetate, and R-propionate groups on the 8th and 30th days and in the retinol and R-propionate groups on the 15th day of storage. In conclusion, the supplementation of laying quail diets with retinol and retinol esters affects performance and egg quality positively, while egg vitamin A and oxidative status differ depending on variations in dietary retinol and retinol ester supplementation.

Key words: Retinol, retinol ester, performance, egg quality, lipid oxidation, laying quail

1. Introduction

Vitamin A is one of the most important essential nutrients and is present in organisms as retinol, retinal, and retinoic acid. While retinoic acid does not convert to other forms, retinoids dissolve in fat and fat solvents and are resistant to high temperatures in nonoxidative conditions (1). Vitamin A is stored in the liver in poultry, depending on the amount of intake (2). The amount of retinol released into the bloodstream from a chicken's liver has been reported to differ depending on the vitamin A content of the diet (3). Retinoids play important roles in visual development, the protection of epithelial membranes, bone development, growth, embryonic development, and the improvement of productive performance due to their antioxidant effects against lipid peroxidation and their ability to strengthen the immune system (4–6).

The carotene and retinol requirements of birds fed under normal circumstances are met by using industrial byproducts and cereals, primarily corn gluten meal or corn gluten feed, which are used to supplement the diets (7). However, all retinoids present in these natural feed sources are not completely converted into vitamin A in poultry. Therefore, vitamin A is provided in the form of retinol, retinol esters, or carotenoids in the diet (1,3,7). The National Research Council (NRC) (8) recommends including a calculated amount of vitamin A precursors, which should ultimately yield 3300 IU/kg of feed, to meet the basic requirements of poultry. Studies of poultry have evaluated the effects of the use of various levels of retinol (9), retinyl acetate (R-acetate) (2,10,11), and retinyl palmitate (R-palmitate) (12) on laying performance and egg quality. However, no data are available concerning the use of retinyl propionate (R-propionate) in poultry.

In previous studies, researchers used individual precursors; no available study investigated the comparative efficacy of vitamin A precursors to determine which precursor is most effective. This study evaluated the effects of retinoids on laying performance, egg quality traits, serum oxidant-antioxidant balance, and β-carotene, retinol, and malondialdehyde (MDA) levels in the egg yolk by adding retinol and retinol esters to quail diets.

2. Materials and methods

2.1. Animals, diets, and experimental protocol

This study was carried out at the Animal Research Center of Afyon Kocatepe University in Turkey

* Correspondence: tbulbul@aku.edu.tr

after ethical committee approval was obtained (AKÜHADYEK-168-12). A total of 300 (200 female and 100 male) 8-week-old Japanese quails (*Coturnix coturnix japonica*) were randomly allocated into one control group and four experimental groups (60 birds per group). Each group was randomly divided into five subgroups (replicates) of 12 quails each. The birds were housed in laying cages (100 cm wide, 44 cm deep, 30 cm high in the front and 20 cm high in the rear; 110 cm^2 per quail) until the end of the study. Feed and water were provided ad libitum throughout the experiment. The photoperiod was set to a 16-h light : 8-h dark cycle during the experimental period. The research trial lasted for 10 weeks (2-week adaptation period on the basal diet and 8 weeks on the experimental diets). The feed ingredients were obtained from a commercial company (Tınaztepe Feed Factory, Afyonkarahisar, Turkey). The basal diet (20.14% crude protein, 12.19 MJ/kg metabolizable energy, 2.55% calcium, and 0.35% total phosphorus) was composed of corn, wheat flour, full-fat soybean, soybean meal, sunflower meal, and meat-bone meal. The nutrient contents of these ingredients were analyzed. The feed was formulated to meet the requirements of quails as recommended by the NRC (8), except for vitamin A precursor supplementation.

The control group was fed the basal diet, while the experimental groups were fed the basal diet supplemented with retinol (Sigma R7632; 0.99 mg/kg), R-acetate (Sigma Aldrich R0300000; 1.135 mg/kg), R-palmitate (Sigma Aldrich 46959-U; 1.815 mg/kg), and R-propionate (Sigma Aldrich 87809; 1.181 mg/kg) at a dose of 3300 IU/kg for each supplement by dissolving them in corn oil (Table 1). The abovementioned quantities of each supplemented precursor (mg/kg) were calculated to yield 3300 IU of vitamin A/kg of feed. The vitamin precursors were first dissolved in a small amount of oil and then mixed into the remaining corn oil (2.40% corn oil in feed). The oil was then mixed into the feed along with the other vitamin premix. The nutrient composition of the basal diet, including dry matter, crude protein, crude fat, crude fiber, crude ash, calcium and phosphorus contents, starch, and sugar, was determined according to the Association of Official Agricultural Chemists. The metabolizable energy (ME) level of the basal diet was calculated according to Carpenter and Clegg (13) (Table 2).

2.2. Laying performance

The body weights of the quails were determined (sensitivity of 0.001 g) at the beginning and end of the study. The feed intake was recorded biweekly (14-day intervals) as the group average, and the feed conversion ratio (FCR) was calculated biweekly as the amount of feed consumed per kilogram of eggs. Eggs were collected daily, and egg production was calculated based on percent production for egg numbers. Egg weight was recorded every 14 days using all eggs.

2.3. Egg quality traits

In this study, 15 eggs from each group (3 eggs from each replicate) were collected to determine the internal and external quality parameters of the eggs on the 30th and 60th days. The eggs were examined for weight (g), length (mm), width (mm), egg shell thickness (mm), albumen index (%), yolk index (%), Haugh unit (HU), egg shape index, and yolk color index. Egg width, egg length, yolk width, albumen length, and albumen width were measured to the nearest 0.01 mm using digital calipers (Mitutoyo Digimatic Caliper, Japan). The albumen and yolk heights were measured to the nearest 0.01 mm using a digital micrometer. The egg shape, yolk, and albumen indexes were calculated from these measurements. The HU was calculated using the formula developed by Haugh. Measurements of the thickness of dried shells, including the membrane, were obtained from 2 sides in the equatorial region, as well as on the blunt and sharp ends, and were made to the nearest 0.01 mm using a digital micrometer. The egg yolk color score was determined by matching the yolk with one of the 15 bands of the 1961 Roch Improved Yolk Color Fan. The formulas used in the measurement of egg quality parameters were as follows:

Shape index (%) = [egg width (mm) / egg length (mm)] × 100,

Yolk index (%) = [yolk height (mm) / yolk width (mm)] × 100,

Albumen index (%) = albumen height (mm) / [(average albumen length (mm) + width (mm) / 2) × 100],

HU = 100 log [albumen height (mm) + 7.57 – 1.7 × egg $weight^{0.37}$ (g)].

2.4. Collection and storage of serum and egg samples

At the end of the experiment (8th week of the experiment, 18 weeks old), a total of 10 birds from each group (2 birds

Table 1. Dietary treatment protocol.

Control	Retinol	R-acetate	R-palmitate	R-propionate
Basal diet without vitamin A precursors and with vitamin premix (no retinol or any other additives)	Basal diet plus retinol (3300 IU/kg of feed)	Basal diet plus R-acetate (3300 IU/kg of feed)	Basal diet plus R-palmitate (3300 IU/kg of feed)	Basal diet plus R-propionate (3300 IU/kg of feed)

Table 2. Ingredients and chemical composition of the basal diet.

Ingredients	%
Yellow corn (8.5%)	49.70
Wheat flour (13.5%)	5.35
Full fat soybean (35.5%)	5.20
Soybean meal (48%)	21.50
Sunflower meal (37%)	6.10
Meat and bone meal (38%)	2.00
Corn oil	2.40
Limestone	5.70
Dicalcium phosphate	1.00
Salt	0.25
$NaHCO_3$	0.20
L-Lysine	0.15
DL-Methionine	0.10
Vitamin premix[1]	0.25
Mineral premix[2]	0.10
Chemical composition (analyzed) (%)	
Dry matter	91.85
Crude ash	8.65
Crude protein	20.14
Crude fat	6.74
Crude fiber	4.63
Nitrogen free extract	51.69
Calcium	2.55
Total phosphorus	0.35
Metabolizable energy[3], MJ/kg	12.19

[1]The vitamin premix contained the following (per kg of diet): 2.4 mg of cholecalciferol, 0.075 mg of α-tocopherol acetate, 5 mg of thiamine mononitrate, 10 mg of vitamin riboflavin, 5 mg of pyridoxine, 0.3 mg of cyanocobalamin, 0.05 mg of niacin, 30 mg of vitamin K_3 from menadione dimethylpyrimidinol bisulfite, 0.1 mg of D Biotin, 15 mg of pantothenic acid (calcium pantothenate), and 1.5 mg of folic acid.

[2]The mineral premix contained the following (per kg of diet): 80 mg of $MnSO_4$, 60 mg of $FeSO_4$, 60 mg of ZnO, 5 mg of $CuSO_4$, 0.5 mg of $CoCO_3$, 2 mg of $Ca(IO_3)_2$, and 0.150 mg of Na_2SeO_3.

[3]The metabolizable energy content of the diets was estimated according to the equation of Carpenter and Clegg (Leeson and Summers, 2001): ME = 53 + 38 [(crude protein %) + (2.25 × ether extract %) + (1.1 × starch %) + (1.05 × sugar %)].

from each replicate) were slaughtered, and blood samples were kept in opaque heparin-free tubes at 4 °C for 24 h. Immediately after incubation, serum samples were obtained and placed in a centrifuge for 15 min at 1800 × *g*. The serum samples were placed in opaque Eppendorf tubes and stored at –18 °C to determine the serum β-carotene, retinol, MDA, and antioxidant activity (AOA) levels.

In the 4th and 8th weeks of the experiment, a total of 10 yolks (2 from each replicate) were weighed, placed in opaque glass tubes, and stored at –18 °C to determine yolk β-carotene and retinol levels.

At the end of the experiment, 40 eggs from each group (8 from each replicate) were stored in the refrigerator at 4 °C. On days 1, 8, 15, and 30 of storage, 10 yolks were weighed, placed into opaque glass tubes, and kept at –18 °C to determine MDA levels.

2.5. Serum and yolk retinol and β-carotene levels

The yolks were separated and weighed. The yolks were then homogenized at 20,000 rpm for 1 min in phosphate buffer, pH 7, using a homogenizer (IKA T18 basic, IKA-Werke GmbH & Co., Staufen, Germany). For the extraction of the homogenized samples, 1 mL of 95% ethanol containing 20 µg of butylated hydroxytoluene (BHT, Sigma Aldrich) and 3 mL of hexane were added to 1 mL of homogenized yolk. The mixture was stirred for 10 min by manual inversion and then centrifuged at 2000 rpm for 10 min (14). After centrifugation, a quarter of the hexane phase was placed on an enzyme-linked immunosorbent assay (ELISA) plate, and β-carotene and retinol were determined based on the absorbance at 453 nm and 325 nm, respectively, using an ELISA reader (MWGt Lambda Scan 200, BioTek Instruments, Winooski, VT, USA) (15). The serum was also extracted and measured using the same process.

2.6. Serum and yolk MDA levels

The serum MDA level was determined using a double-boiling method to measure MDA from free radicals, as reported by Draper and Hadley (16). This method is based on the principle of making spectrophotometric measurements at a wavelength of 532 nm after MDA reacts with thiobarbituric acid (TBA). According to this method, 0.5 mL of serum was mixed with 2.5 mL of 10% trichloroacetic acid (TCA) in a clean screw-cap test tube and heated to 95 °C for 15 min. The mixture was then cooled and centrifuged at 5000 rpm for 10 min. One milliliter of the obtained supernatant was collected and mixed with 0.5 mL of 0.67% TBA. The mixture was boiled for 15 min and cooled immediately. Afterwards, the absorbance value against water was determined at 532 nm using the ELISA reader. The resulting values were multiplied by the dilution factor.

Yolk MDA levels on days 1, 8, 15, and 30 of storage were measured using an ELISA modification of the spectrophotometric method, as noted by Kanner and Rosenthal (17). Samples weighing 0.2 g were derived from sample yolks for thiobarbituric acid reactive substances (TBARS) analysis and were placed into 10-mL test tubes with 1.8 mL of 3.86% perchloric acid per tube. This

homogenized mixture was filtered through filter paper, and 0.5 mL of the filtrate was stirred with 1 mL of 20 mL of TBA solution and left in a boiling water bath for 30 min. The absorbance was read at 532 nm in a spectrophotometer.

2.7. Serum AOA level

Antioxidant activity was determined colorimetrically in the serum using the modified method described by Koracevic et al. (18). The standard solution of the Fe-EDTA complex reacts with hydrogen peroxide due to the Fenton reaction, allowing the formation of hydroxyl radicals. These reactive oxygen radicals disrupt benzoate due to TBARS release. Supplementation with antioxidants leads to the suppression of TBARS production. This reaction is measured spectrophotometrically; the suppression of color development is determined as the AOA.

An Fe-EDTA blended control group was prepared for each sample, and H_2O_2 was added. For every single series of the analysis, negative control groups (K_1 and K_0) were formed. Standards containing 1 mmol/L uric acid were used for the measurement. The standards were incubated in a water bath at 100 °C for 10 min. The standards were then cooled in a water bath, and the absorbance was determined at 540 nm in an ELISA reader.

2.8. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the GLM procedures of SPSS 10.00 (SPSS Inc., Chicago, IL, USA). Tukey's test was applied to identify significant differences between groups. All replicates were the experimental units for all analyses. All data were expressed as the mean ± standard error (SE). $P < 0.05$ was considered to be the limit for statistical significance.

3. Results

The effects of retinol and retinol ester supplementation on laying performance and egg quality traits in quails are shown in Tables 3 and 4. The body weights, feed intake, and egg weight were not changed by the dietary treatments ($P > 0.05$).

Egg production increased ($P < 0.001$) in all of the retinol and retinol ester-supplemented groups; the FCR was improved ($P < 0.01$) in the same group (Table 3). The egg yolk color index in the 8th week decreased ($P < 0.001$) in the R-propionate group when compared to the control group and the other experimental groups (Table 4). During the 4th and 8th weeks of the experiment, no changes ($P > 0.05$) in the other egg quality traits were observed (Table 4).

Serum β-carotene ($P < 0.001$) and MDA ($P < 0.01$) levels decreased in the retinol and retinol ester groups, whereas the serum retinol level increased ($P < 0.001$) in the same groups (Table 5). In addition to the lower serum β-carotene level in the R-propionate group in comparison to the R-palmitate group, the increase in retinol levels was much more notable in the R-propionate group than in the other retinol and retinol ester groups ($P < 0.001$). The serum AOA increased ($P < 0.001$) in the R-palmitate and R-propionate groups in comparison to the control, retinol, and R-acetate groups (Table 5).

The yolk retinol levels increased ($P < 0.001$) in the R-acetate and R-palmitate groups in the 4th week and in the retinol, R-acetate, and R-palmitate groups in the 8th week. The yolk β-carotene level exhibited no differences among the groups during either period (Table 6).

The yolk MDA level (Table 7) did not show changes between the groups on the first day after storage ($P > 0.05$). However, the yolk MDA level decreased in the retinol, R-acetate, and R-propionate groups on the 8th ($P < 0.001$) and 30th ($P < 0.05$) days and in the retinol and the R-propionate groups on the 15th day ($P < 0.05$) of storage.

4. Discussion

Although vitamin A has been reported to play an important role in poultry growth performance (19), our results showed that the average body weight values of female and male quails before and after the study did not differ significantly (Table 3). The absence of a body weight change after supplementation with retinol and retinol esters in this study indicates that the vitamin level in the

Table 3. Effect of retinol and retinol esters on the performance of laying quails.

Item	Control	Retinol	R-acetate	R-palmitate	R-propionate	P-value
Initial body weight, g	181.48 ± 2.22	181.28 ± 1.52	181.27 ± 1.10	181.92 ± 1.32	181.94 ± 1.23	0.992
Final body weight, g	191.97 ± 2.12	196.26 ± 1.90	195.66 ± 1.54	194.35 ± 1.79	194.50 ± 1.54	0.520
Egg production, %	75.33 ± 0.93[b]	86.53 ± 3.28[a]	86.10 ± 1.03[a]	82.20 ± 1.51[a]	88.52 ± 1.41[a]	0.001
Feed intake, g/day per group	30.30 ± 0.24	31.05 ± 0.39	30.75 ± 0.33	29.65 ± 0.20	30.75 ± 0.44	0.067
FCR, kg feed/kg egg	3.08 ± 0.04[a]	2.83 ± 0.10[b]	2.77 ± 0.02[b]	2.76 ± 0.05[b]	2.68 ± 0.04[b]	0.002
Egg weight, g	13.03 ± 0.12	12.70 ± 0.13	12.84 ± 0.07	13.06 ± 0.08	12.94 ± 0.18	0.290

[a, b]: Within a row, values that do not share a common superscript letter are significantly different.

Table 4. Effect of retinol and retinol esters on the egg quality traits of laying quails.

Item	Control	Retinol	R-acetate	R-palmitate	R-propionate	P-value
Shape index, %						
4th week	77.31 ± 0.75	77.39 ± 0.87	77.81 ± 1.06	78.05 ± 0.36	79.01 ± 0.93	0.611
8th week	78.89 ± 0.95	78.90 ± 2.04	76.69 ± 1.14	78.15 ± 0.63	75.97 ± 1.27	0.436
Egg shell thickness, μm						
4th week	197.0 ± 4.0	203.0 ± 4.8	206.8 ± 4.4	203.4 ± 4.3	210.3 ± 4.4	0.296
8th week	187.2 ± 5.3	189.3 ± 5.3	177.0 ± 3.0	190.1 ± 2.5	182.1 ± 2.04	0.187
Yolk index, %						
4th week	51.98 ± 0.75	49.96 ± 0.72	49.12 ± 1.54	48.73 ± 1.17	50.24 ± 1.01	0.260
8th week	53.81 ± 3.40	50.03 ± 0.84	50.67 ± 1.51	50.34 ± 1.28	48.51 ± 0.84	0.446
Albumen index, %						
4th week	12.02 ± 0.99	11.76 ± 0.46	12.01 ± 0.30	11.68 ± 0.56	13.08 ± 0.98	0.653
8th week	11.34 ± 0.85	10.68 ± 0.44	9.75 ± 0.76	10.04 ± 0.93	10.27 ± 0.65	0.603
Haugh unit						
4th week	75.54 ± 2.92	69.74 ± 1.80	72.68 ± 1.56	73.24 ± 2.35	71.39 ± 2.11	0.432
8th week	80.77 ± 5.24	85.91 ± 2.82	76.27 ± 5.84	77.24 ± 4.54	85.39 ± 6.94	0.571
Yolk color index						
4th week	9.66 ± 0.25	9.66 ± 0.21	8.80 ± 0.24	9.00 ± 0.23	8.73 ± 0.22	0.070
8th week	9.28 ± 0.47^{a}	8.64 ± 0.40^{a}	9.04 ± 0.20^{a}	8.91 ± 0.19^{a}	7.00 ± 0.33^{b}	0.001

[a,b]: Within a row, values that do not share a common superscript letter are significantly different.

Table 5. Effect of retinol and retinol esters on the serum β-carotene (μg/L), retinol (mg/L), MDA (nmol/L), and AOA (mmol/L) levels of laying quails.

Item	Control	Retinol	R-acetate	R-palmitate	R-propionate	P-value
β-Carotene	64.56 ± 8.22^{a}	26.92 ± 1.36^{bc}	25.30 ± 3.80^{bc}	34.10 ± 3.20^{b}	21.10 ± 1.76^{c}	0.001
Retinol	3.20 ± 0.39^{c}	4.66 ± 0.195^{b}	5.26 ± 0.53^{b}	4.78 ± 0.26^{b}	6.76 ± 0.29^{a}	0.001
MDA	5.69 ± 0.59^{a}	3.82 ± 0.34^{b}	3.92 ± 0.31^{b}	4.36 ± 0.34^{b}	3.82 ± 0.25^{b}	0.008
AOA	5.94 ± 0.25^{c}	6.36 ± 0.18^{c}	6.25 ± 0.26^{c}	7.00 ± 0.20^{b}	7.72 ± 0.10^{a}	0.001

[a,b,c]: Within a row, values that do not share a common superscript letter are significantly different.

raw feed materials that constituted the basal diet was adequate for the body weight.

In this study, retinol and retinol ester supplementation of the diet increased egg production. However, the egg weight was not affected by retinol and retinol ester supplementation of the diets (Table 3). Vitamin A has been reported to be essential for egg production; egg production has been shown to decrease in laying hens fed diets deficient in vitamin A (11). Some studies have shown that when retinol ester supplementation contains vitamin A at the required level, egg production remains the same in hens (2,20), and that R-acetate supplementation above the required level increases egg production (2,11).

In the current study, feed intake was not affected during the 8-week period of study. Moreover, the FCR was affected positively in all groups supplemented with retinol and retinol esters (Table 3). This effect was associated with the fact that the feed intake did not change but egg production increased in those groups. It has been reported that vitamin A at the required level for laying hens (2,20) or above the required level for laying quails (21) and R-acetate supplementation for laying hens do not change (2,10,11) or adversely affect (20) the FCR.

The external and some internal quality traits of the eggs were not affected by retinol and retinol ester supplementation of the diet (Table 4). Although the color

Table 6. Effect of retinol and retinol esters on the egg yolk β-carotene (μg/g) and retinol (μg/g) levels of laying quails.

Item	Control	Retinol	R-acetate	R-palmitate	R-propionate	P-value
β-Carotene						
4th week	0.64 ± 0.07	0.73 ± 0.04	0.61 ± 0.05	0.75 ± 0.05	0.68 ± 0.03	0.073
8th week	0.6 3 ± 0.06	0.70 ± 0.03	0.67 ± 0.03	0.75 ± 0.03	0.67 ± 0.04	0.317
Retinol						
4th week	5.76 ± 0.88^{c}	6.84 ± 0.34^{bc}	7.61 ± 0.66^{b}	9.57 ± 0.03^{a}	5.93 ± 0.24^{c}	0.001
8th week	5.51 ± 0.43^{c}	7.49 ± 0.22^{ab}	8.05 ± 0.45^{a}	8.34 ± 0.44^{a}	6.41 ± 0.25^{bc}	0.001

^{a,b,c}: Within a row, values that do not share a common superscript letter are significantly different.

Table 7. Effect of retinol and retinol esters on the egg yolk MDA levels (mg MDA/kg sample) on the 1st, 8th, 15th, and 30th days of storage.

	Control	Retinol	R-acetate	R-palmitate	R-propionate	P-value
1st day	1.75 ± 0.25	1.09 ± 0.27	1.25 ± 0.11	1.64 ± 0.19	1.56 ± 0.17	0.158
8th day	3.47 ± 0.15^{a}	2.46 ± 0.16^{c}	2.86 ± 0.16^{bc}	3.18 ± 0.22^{ab}	2.39 ± 0.22^{c}	0.001
15th day	4.22 ± 0.31^{a}	3.18 ± 0.16^{b}	3.76 ± 0.68^{ab}	3.95 ± 0.14^{ab}	3.17 ± 0.33^{b}	0.048
30th day	5.28 ± 0.36^{a}	4.11 ± 0.25^{b}	4.31 ± 0.26^{b}	4.52 ± 0.23^{ab}	4.37 ± 0.22^{b}	0.036

^{a,b,c}: Within a row, values that do not share a common superscript letter are significantly different.

of the yolk did not indicate a change caused by retinol and retinol ester supplementation in the 4th week of the experiment, yolk color decreased in the 8th week after supplementation with R-propionate (Table 4). The color of the yolk depends on the storage of carotenoids in the yolk (22). The observed fading of the yolk color in the R-propionate group in this study may be associated with a substantially lower plasma carotene level.

In this study, serum β-carotene levels appeared to decrease in all groups with retinol and retinol ester supplementation; serum retinol levels increased in the same groups (Table 5). Serum retinol levels have been reported to be related to the retinol level in the diet, and plasma retinol levels are a determinant of the vitamin A level in the diet (3). In our study, the fact that the plasma retinol level increased in the experimental groups might be related to vitamin A in the diet. On the other hand, in the group supplemented with R-propionate, the serum retinol level was higher, although retinol or retinol ester was supplemented at the same level. Although several factors affect the absorption of vitamins, the only effective variable in this study was the variation in the fatty acids forming the retinyl ester. Dietary retinol is eluted to retinol and fatty acids via hydrolysis by retinyl ester hydrolases; however, the retinol is reesterified in the intestinal cells and is stored in the liver and other tissues in the form of palmitate and acetate ester. The retinol is released from the liver into the bloodstream via another hydrolysis reaction (14,23,24). In this context, retinol and propionate ester are thought to be released into the circulation without being stored in the liver and other tissues.

Egg yolk is a significant source of vitamin A, depending on the level of vitamin A in the mixed feeds of the poultry (10). Vitamin A is stored in the egg primarily as retinol and a small amount of R-acetate (25). In this study, the retinol content of the egg yolk was determined to increase in the groups with R-palmitate and R-acetate supplementation by 66.2% and 32.1%, respectively, in the first month, and 51.4% and 46.2%, respectively, in the second month (Table 6). Some studies also reported an increase in the retinol content in the egg yolk with retinol (26) and R-acetate supplementation of hen diets (10,11,23). Depending on the forms (12) and the levels (23) of vitamin A in the diet, the liver plays an important role as a regulator of this vitamin's metabolism. In the same context, the high retinol content of the egg yolks of the groups fed diets supplemented with R-palmitate and R-acetate indicates that among the forms of vitamin A, R-palmitate and R-acetate are more efficient in transferring from the liver to the egg yolk than the other forms of vitamin A.

Retinol and retinol esters were determined to decrease the level of serum MDA, which is an indicator of lipid peroxidation (Table 5). Retinol and carotenes react directly with free radicals and reduce MDA formation, leading to

lipid peroxidation (27). In a study assessing the impact of vitamin A supplementation on serum MDA levels, vitamin A (15,000 IU/kg retinol supplementation) was determined to reduce serum MDA levels in broilers under heat stress (28). In our study, in contradiction to the results reported by Kucuk et al. (28), this vitamin, which is more effective under stress conditions, had a greater positive effect on lipid peroxidation in the animals that were not exposed to environmental stress.

In the present study, serum AOA increased with R-palmitate and R-propionate supplementation in the diet, with the greatest increase in the R-propionate group (Table 5). Decreasing MDA and increasing AOA values indicate that R-palmitate and R-propionate affect AOA more positively. For the antioxidant system, β-carotene and vitamin A are of vital importance, and β-carotene stimulates AOA independently from vitamin A (24). The observed increase in AOA in the abovementioned groups and the observed decrease in MDA in the serum and retinol groups, which were supplemented with vitamin A, may have occurred because the retinol level increased more as a result of a decrease in serum carotene due to R-propionate. Moreover, supplementation of vitamin A, which has an antioxidant effect on quail diets, prevents lipid oxidation in the egg, and the severity of the antioxidative effect may vary depending on the form of vitamin A used.

In the present study, retinol, R-acetate, and R-propionate exerted different effects on the egg yolk MDA level, depending on the duration for which the eggs were kept at 4 °C. While there was no difference between the groups on the first day of storage, a decrease in comparison to the control group was noticed with storage time on different days in different groups (Table 7). The composition of dietary nutrients or feed additives has been reported to affect the egg yolk MDA concentration in many studies (29,30). Similarly, it has also been noted that the retinol content in the egg yolk prevents lipid oxidation; vitamin A, which can be found in an adequate amount in the egg yolk, must be added into diets to activate this protective effect, and vitamin A acts as an antioxidant by passing directly into the yolk (23). Thus, in the 8th week of this study, egg retinol levels exhibited a significant increase in the retinol, R-acetate, and R-propionate groups and a slight but nonsignificant increase in the R-palmitate group. The presence of low egg MDA levels in these groups, which are rich in retinol (the potent antioxidant properties of which are known), indicates that this decrease results from the egg retinol level.

In conclusion, retinol and retinol ester supplementation of laying quail diets exerts a positive effect on productive performance and egg quality traits and a preventive effect against lipid oxidation in serum and eggs. Supplementation of the diet with different retinol esters creates different effects on serum and egg vitamin A concentrations. It is more appropriate to supplement the diet with R-palmitate and R-acetate to increase the egg vitamin A levels. Moreover, a study designed with a longer study period might reveal a more pronounced effect.

Acknowledgments

This study was supported by the Scientific Research Project Committee of Afyon Kocatepe University in Afyonkarahisar, Turkey (Project No.: 13.SAG.BIL.08). This article was summarized from the MSc thesis of the same title.

References

1. McDowell P. Vitamins in Animal Nutrition: Vitamin A. San Diego, CA, USA: Academic Press, Inc.; 1998.
2. Lin H, Wang LF, Song JL, Xie YM, Yang QM. Effect of dietary supplemental levels of vitamin A on the egg production and immune responses of heat-stressed laying hens. Poultry Sci 2002; 81: 458-465.
3. West CE, Sijtsma SR, Peters HP, Rombout JH, Van Der Zijpp AJ. Production of chickens with marginal vitamin A deficiency. Br J Nutr 1992; 68: 283-291.
4. Ayaşan T, Karakozak E. Use of β-carotene in animal nutrition and its effects. Kafkas Univ Vet Fak Derg 2010; 16: 697-705.
5. Sevimli A, Yağcı A, Bülbül A. Matrix metalloproteinase, vitamin A and methylprednisolone effects on experimentally induced amyloid arthropathy. Biotech Histochem 2013; 88: 336-344.
6. Sevimli A, Bülbül T, Bülbül A, Yağcı A. Chicken amyloid arthropathy: serum amyloid A, interleukin-1β, interleukin-6, tumour necrosis factor -α and nitric oxide profile in acute phase (12th hour). Pol J Vet Sci 2013; 16: 241-247.
7. Blair R. Nutrition and Feeding of Organic Poultry. Wallingford, UK: CAB International; 2008.
8. NRC. Nutrient Requirements of Poultry. 9th ed. Washington, DC, USA: National Academy Press; 1994.
9. Fu Z, Kato H, Sugahara K, Kubo T. Retinoic acid accelerates the development of reproductive organs and egg production in Japanese quail (*Coturnix coturnix japonica*). Biol Reprod 2000; 63: 1795-1800.
10. Mendonca CX, Almeida CRM, Watanabe C. Effect of dietary vitamin A on egg yolk retinol and tocopherol levels. J Appl Poult Res 2002; 11: 373-378.
11. Mori AV, Mendonca CX, Almeida CRM, Pita MCG. Supplementing hen diets with vitamins A and E affects egg yolk retinol and α-tocopherol levels. J Appl Poult Res 2003; 12: 106-114.
12. Ramalho HMM, Dias Da Silva KH, Alves Dos Santos VV, Dos Santos Cavalcanti J, Dimenstein R. Effect of retinylpalmitate supplementation on egg yolk retinol and cholesterol concentrations in quail. Brit Poultry Sci 2008; 49: 475-481.

13. Leeson S, Summers JD. Nutrition of the Chicken. Guelph, Canada: University Books; 2001.

14. Frolik CA, Olson JA. Extraction, separation, and chemical analysis of retinoids. In: Sporn MB, Roberts AB, Goodman DS, editors. The Retinoids. Vol. 1. Orlando, FL, USA: Academic Press, Inc.; 1984. pp. 181-233.

15. Suzuki JP, Katoh NA. A simple and cheap method for measuring serum vitamin in cattle using only spectrophotometer. Jpn J Vet Sci 1990; 52: 1281-1283.

16. Draper H, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421-430.

17. Kanner J, Rosenthli I. An assessment of lipid oxidation in foods. Pure Appl Chem 1992; 64: 1959-1964.

18. Koracevic D, Koracevic G, Djordjevic V, Andrejevic V, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001; 54: 356-361.

19. Uni Z, Zaiger G, Gal-Garber O, Pines M, Rozenboim I, Reifen R. Vitamin A deficiency interferes with proliferation and maturation of cells in the chicken small intestine. Brit Poultry Sci 2000; 41: 410-415.

20. Coşkun B, Inal F, Celik I, Erganiş O, Tiftik AM, Kurtoğlu F, Kuyucuoğlu Y, Ok U. Effects of dietary levels of vitamin A on the egg yield and immune response of laying hens. Poultry Sci 1998; 77: 542-546.

21. Marques RH, Gravena RA, Da Silva JDT, Roccon J, Picarelli J, Hada FH, De Queiroz SA, Moraes MB. Effect of supplementation of diets for quails with vitamins A, D and E on performance of the birds and quality and enrichment of eggs. R Bras Zootec 2011; 40: 1222-1232.

22. Goodwin TW. Biochemistry of the Carotenoids. 2nd ed. New York, NY, USA: Chapman and Hall; 1980.

23. Surai PF, Ionov IA, Kuklenko TV, Kostjuk IA, Macpherson A, Speake BK, Noble RC, Sparks NHC. Effect of supplementing the hen's diet with vitamin A on the accumulation of vitamins A and E, ascorbic acid and carotenoids in the egg yolk and in the embryonic liver. Brit Poultry Sci 1998; 39: 257-263.

24. Murray RK, Granner DK, Mayes PA, Rodwell VW. Structure and function of the lipid-soluble vitamins. In: Mayes PA, editor. Harper's Biochemistry. 23rd ed. East Norwalk, CT, USA: Appleton & Lange; 1993. pp. 589-601.

25. Joshi PS, Murthy SN, Murthy SK, Ganguly J. Vitamin A economy of the developing embryo and of the freshly hatched chick. Biochem J 1973; 136: 757-761.

26. Hill EW, Scott ML, Norris LC, Henser GF. Reinvestigation of the vitamin A and D requirements of laying and breeding hens and their progeny. Poultry Sci 1961; 40: 1245-1247.

27. Sies H, Stahl W, Sundquist AR. Antioxidant functions of vitamins, vitamins E and C, β-carotene and other carotenoids. Ann NY Acad Sci 1992; 669: 7-20.

28. Kucuk O, Sahin N, Sahin K. Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. Biol Trace Elem Res 2003; 94: 225-235.

29. Zhang GF, Yang ZB, Wang Y, Yang WR, Jiang SZ, Gai GS. Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. Poultry Sci 2009; 88: 2159-2166.

30. Yesilbag D, Gezen SS, Biricik H, Bulbul T. Effect of a rosemary and oregano volatile oil mixture on performance, lipid oxidation of meat and haematological parameters in Pharaoh quails. Brit Poultry Sci 2012; 53: 89-97.