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Full Length Research Paper

Effects of powder and extract form of green tea and marigold, and α-tocopheryl acetate on performance, egg quality and egg yolk cholesterol levels of laying hens in late phase of production

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This study was conducted to investigate the effect of feeding two forms (powder and extract) of green tea and marigold as well as α-tocopheryl acetate on performance, egg quality, egg yolk and also serum cholesterol concentrations of laying hens. A total of 150 hens, aged 64 weeks were divided into six groups with five replicate cages containing five hens each for each treatment. Dietary treatments were as following: control, two diets including 0.5% extract of green tea (Gteaext) or marigold (Marext), two diets including 1.5% powder of green tea (Gteapow) and marigold (Marpow) and an α-tocopheryl acetate (α-TA) diet (200 mg/Kg). Feeding α-TA significantly ($p<0.05$) improved feed intake, egg production, yolk weight, and yolk index. Feeding Gteaext decreased feed consumption and feed conversion ratio. Compared to control, serum low-density lipoprotein (LDL) and cholesterol concentrations were reduced by diets containing Gteaext, Marext, Gteapow or α-TA, significantly ($p<0.05$). Also, feeding Gteaext reduced serum triglycerides and increased ratios of HDL to cholesterol and LDL in comparison to control. Egg yolk cholesterol was greater in hens on Marpow and control compared to other diets. In general, incorporating antioxidant components derived from herbal plants (green tea and marigold) in laying hens diets as alternative options for α-tocopheryl acetate can be effective based on cost and availability.

Key words: Natural antioxidants, yolk cholesterol, laying hens, egg quality, green tea extract, marigold extract.

INTRODUCTION

Farm animals in intensive farming systems are frequently exposed to stress (Wood et al., 2004). Deficiencies of natural protective substances or excess exposure to stimulators of reactive oxygen metabolites production (Miller et al., 1993) may increase oxidative damage to important biological macromolecules such as lipids, proteins and DNA, affecting their normal function and consequently leading to reduced performance or disease (Valko et al., 2007). Apart from polyunsaturated fatty acids (PUFA), other factors such as nutrition, environmental temperature and ethological stress can cause oxidative stress and increase the requirement for antioxidant supplementation (Wood et al., 2004).

Vitamin E is the most active natural antioxidant used in animal feed, to improve performance, to strengthen immunological status, and to increase the vitamin E content of food of animal origin and thus increase the vitamin E intake of consumers (McDowell, 1989; Sunder et al., 1997; Flachowsky, 2000). Vitamin E usually is supplemented in the synthetic (all-rac tocopherol) or semi-synthetic (α-tocopheryl acetate) form, which are less effective than the natural form (α-tocopherol) (Azzi

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Abbreviations: α-TA, α-tocopheryl acetate; **Gteaext,** green tea extract; **Gteapow,** green tea powder; **HDL,** high-density lipoprotein; **HMG-CoA,** 3-hydroxy-3-methylglutaryl coenzyme A; **LDL,** low-density lipoprotein; **Marext,** marigold extract; **Marpow,** marigold powder; **PUFA,** polyunsaturated fatty acids; **VLDL,** very low density lipoproteins.

Table 1. Composition and calculated analysis of the basal diet ingredients and analysis.

Ingredients	(%)
Yellow corn	57.70
Soybean meal (44% CP)	18.56
Barley	7.00
Wheat bran	1.00
Yellow grease	2.80
Oyster shell	4.70
Calcium carbonate	5.25
Dicalcium phosphate	1.39
Sodium chloride	0.26
Vitamin- premix*	0.25
Mineral premix**	0.25
Zeolite	0.10
Sodium bicarbonate	0.90
DL-methionine	0.15
Vitamin D_3	0.004
Calculated analysis	
Metabolizable energy (kcal/kg)	2750
Crude protein, %	13.50
Lysine, %	0.69
Methionine plus cystine, %	0.56
Calcium,%	4.00
Available phosphorus,%	0.35
Na, %	0.15

*Provided per kg of diet: vitamin A (as retinyl acetate), 8800 IU; vitamin D, 2500 IU; vitamin E (as α-tocopheryl acetate), 11 IU; vitamin K, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 4 mg; vitamin B_6, 2.4 mg; vitamin B_{12}, 0.015 mg; pantothenic acid, 10 mg; niacin, 34 mg; folic acid, 0.5 mg; biotin, 0.15 mg; choline chloride, 140 mg.**Provided per kg of diet: manganese, 80 mg; copper, 8 mg; iodine, 0.86 mg; selenium, 0.3 mg; zinc, 80 mg; iron, 75 mg.

and Stocker, 2000). Besides the limited antioxidant efficacy of vitamin E in the case of high PUFA intake, there are reports of its pro-oxidative action when consumed in high amounts or in the absence of other antioxidants (Hasty et al., 2007). Because of the above-mentioned facts and the increased awareness by the consumers, which led to the ban on nutritive antibiotics in Europe, there is a demand for discovering new plant extracts (as natural antioxidants) that can delay lipid oxidation in lipid-containing food products and reduce yolk cholesterol content in eggs. Beneficial activity of such extract is related to the content of various secondary metabolites such as polyphenols, carotenoids, triterpenes and essential oils. Plant polyphenols capture free radicals, chelates metals ions and inhibit lipoxygenases, so they can be used as natural preservatives, inhibiting the onset of peroxidation of oils (Ramadan et al., 2003; Papuc et al., 2007, 2008). This improvement could be attributed to the content of essential oils that have active components with antimicrobial, antifungal and antioxidant activities; and accordingly could improve the bird's utilization of dietary nutrients (Williams and Losa, 2001). Green tea (*Camellia sinensis*) is rich in flavonoids and other polyphenols that have been shown to possess a wide range of biological and pharmaceutical benefits, including anti-carcinogenic, anti-oxidative, and hypolipidemic activities (Buschman, 1998). These beneficial effects are may be attributed to polyphenols such as epigallacathechin-3-gallate, which are known to possess powerful anti-oxidative and anti-carcinogenic properties (Trevisanato and Kim, 2000). The feeding of tea extracts to laying hens improved egg quality by increasing albumen thickness (Yamane et al., 1999; Biswas et al., 2001) and decreasing crude fat in egg yolk in a short-term experiment (Biswas et al., 2001).

Furthermore, feeding green tea powder to laying hens in a long-term study had favorable effects on egg quality traits such as thick albumen stability without adverse effect on laying performance (Biswas et al., 2000) and also lowered cholesterol and triglycerides content of eggs (Biswas and Wakita, 2000, Unganbayer et al., 2005; Koo and Noh, 2007; Yamane et al., 1999). There was growing interest, in the last decade, in the antioxidant proprieties of marigold (*Calendula officinalis* Linn.) because of its relatively higher content of polyphenol, essential oils, flavonoids and carotenoids content (C´etkovic´ et al., 2004; Kishimoto et al., 2005; Khalil et al., 2007). *In vitro* studies showed an effective radical scavenging capacity of several differently prepared extracts of marigold flowers (C´etkovic´ et al., 2003; Miliauskas et al., 2004), which encouraged researchers to use this natural antioxidant in farm experiments (Preethi et al., 2006, 2009). To our knowledge, there are no available data on the effect of marigold on performance and egg quality of laying hens. The objectives of this study were: (A) to determine the efficacy of green tea or marigold as a hypocholesterolemic agents in laying hens compared to α-tocopheryl acetate and (B) to study the effects of dietary green tea and marigold powder and extract on productive performance of laying hens in comparison to dietary α-tocopheryl acetate.

MATERIALS AND METHODS

Animal care and dietary treatments

A total of 150 Hy-Line W36 white hens aged 64 weeks was randomly assigned to 6 experimental treatments in a completely randomized design. Five birds were housed in each cage (45, 45 and 40 cm) in a windowed poultry house at a light regimen of 16 h light and 8 h dark. Each treatment had 5 replicates (5 birds per replicate). The experimental diets were formulated to meet nutrient requirement of laying hens according to NRC (1994) and the composition of the basal diet is shown in Table 1. The experimental treatments were a corn-soybean diet with no supplementation as negative control, diet containing 200 mg/kg DL-α-tocopheryl (α-TA) acetate as positive control, diets containing 1.5% Gteapow (green tea powder) or 1.5% marpow (marigold powder) and diets

containing 0.5% Gteaext (green tea extract) or 0.5% Marext (marigold extract). Feed and water were provided *ad-libitum*. The birds were given two weeks adaptation period before the trial began and the trial lasted 12 weeks.

Production variables and egg quality characteristics

Production performances (egg production, egg mass, feed intake, and feed conversion ratio) were measured from 64 to 75 weeks of age. Daily egg production per replicate was recorded, and at the end of each experimental week, the total number of eggs laid per bird per week was calculated. Eggs laid per replicate were weighed daily and at the end of each experimental week, the average weight for that particular week was calculated. The data generated (number of eggs and egg weight) were used to calculate egg mass per bird per week (weekly egg number in replicate x average egg weight). Feed consumption was recorded at the end of each two weeks of the experimental period. Data on feed intake and egg mass were used to calculate feed conversion ratio (feed intake/egg mass; g/g).

For measuring the egg quality characteristics, 3-day eggs from each replicate were collected from the end of each 4 weeks period and weighed. Shell weight, shell thickness, shell strength, albumen height and yolk color were measured on 15 eggs from each treatment (3 eggs per cage) every 28 days. The eggshell thickness was measured using FHK eggshell thickness gauge and the eggshell strength was measured using FHK eggshell force gauge (FHK, Fujihira Industry Co. Ltd., Tokyo, Japan). Haugh units were calculated as an indicator of interior egg quality. Albumen height was documented at 3 different sites by using a spherometer, and Haugh units (Haugh, 1937) were calculated by the formula $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$. Yolks were separated using an egg separator and weighed. Albumen weight was calculated by subtracting the yolk and shell weight from the total egg weight. The yolk index was determined as the ratio of the yolk height to the yolk width and yolk color was compared to the Roche yolk color fan, which ranges from a pale yellow at score 1 to a dark orange at score 15 (Vuilleumier, 1969).

Measurement of serum biochemical variables

At the end of week 6 and 12, 10 birds (2 birds per each replicate) from each treatment were randomly chosen for blood sampling. Two millilitre blood samples were collected from the vein on the brachial wing. Blood samples were centrifuged at 3000 x g for 10 min and serum was collected and stored at –20°C for later analysis. Serum glucose, triglyceride, cholesterol, HDL and LDL concentrations were measured using a biochemical analyzer (Autolab, PM 4000, Autoanalyzaer Medical System, Rome, Italy). The data generated (cholesterol, HDL and LDL) were used to calculate HDL to LDL and HDL to cholesterol ratio.

Measurement of total lipids and cholesterol in yolk

At the end of the experimental period, four egg yolk samples from each treatment were separated. Yolk composites from each treatment were prepared by separating the yolk material from the viteline membrane and blending gently by hand to prevent incorporation of air bubbles into the sample. Total lipids were extracted with chloroform: methanol (2:1 vol/vol) from 1 g of yolk, according to the procedure of Folch et al. (1957) as modified by Washburn and Nix (1974). Yolk cholesterol and triglycerides were measured by the colorimetric method (ERBA CHEM-5, Beijing Biochemical Instrument Company, Beijing, China).

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 1999). The following model was used: $Y_{ijk} = \mu + \tau_i + t_j + \varepsilon_{ijk}$, where Y_{ijk} is the variable measured for the j^{th} replicate in the i^{th} treatment, μ is the overall mean, τ_i is the effect as a result of the i^{th} treatment, t_j is the effect as a result of the j^{th} period and ε_{ijk} is random error associated with the ijk^{th} recording. Mean differences were separated via the PDIFF option. A significance level of $p<0.05$ was used for all comparisons.

RESULTS AND DISCUSSION

Productivity performance

The effects of dietary treatments on hen performance parameters are given in Table 2. Supplementation of diets with green tea, marigold and α-tocopheryl acetate had no significant effects on feed intake when compared to the control, whereas inclusion of 200 mg/kg vitamin E resulted in greater feed intake than those fed green tea extract ($p<0.05$). In agreement with our result, Kojima et al. (2008) reported no significant changes in feed intake when layer diet was supplemented with 1% green tea powder. In contrast to this result, Unganbayar et al. (2005) stated that the feed intake in the layers fed with diets containing 1.5% green tea was significantly greater than those fed control diets. Inclusion of α-tocopheryl acetate in the diet significantly ($p<0.05$) improved egg production compared with the control. Compared to other treatments, α-tocopheryl acetate supplementation increased egg production relative to control significantly ($p<0.05$). There were approximately 6 and 7.5% increase in the egg production from laying hens fed diets containing extracts of marigold and green tea compared with the control group, respectively, but these increases were insignificant. Diets supplemented with green tea or marigold powder had no significant effects on egg production.

Individual egg weight was not affected by the dietary treatments. The addition of α-tocopheryl acetate significantly ($p<0.05$) increased weekly egg mass compared to control and Marpow diets. In some conditions, plasma egg yolk precursor proteins, vitellogenin, and VLDL may be decreased due to dysfunction of hepatic cells because of impaired membrane structure (Bollenger-Lee et al., 1998). Hence, adding excess amounts of α-tocopheryl acetate may promote the release of vitellogenin from liver by protecting cell membranes of hepatocytes from oxidative damage, allowing regular or improved yolk precursor formation and ovulation (Whitehead et al., 1998).

Compared to other treatments, hens that received green tea extract had lower ($p<0.05$) feed conversion ratio than those in the control group. This is in agreement with results of Unganbayar et al. (2005) who found no significant differences in feed conversion ratio among layers fed diets containing 1.0, 1.5 and 2.0% green tea

Table 2. Effect of dietary α-tocopheryl acetate and powder and extract of green tea and marigold on the means of intake and productivity performance.

Dietary treatments	Feed Intake (g/d)*	Egg Production (%)*	Egg weight (g)^{ns}	Egg mass (g/d/hen)*	Feed conversion (kg/kg)*	Feed conversion (kg/dozen)^{ns}	Body weight Change (g)^{ns}
Control	106.49^{ab}	72.36^{b}	63.87	46.23^{b}	2.32^{a}	1.73	-55.75
α-TA	108.99^{a}	82.00^{a}	64.43	52.38^{a}	2.08^{ab}	1.61	+23.94
Marpow	104.18^{ab}	73.67^{ab}	63.23	46.57^{b}	2.27^{ab}	1.72	-13.72
Marext	106.49^{ab}	78.18^{ab}	64.60	50.54^{ab}	2.14^{ab}	1.66	-44.06
Gteapow	104.35^{ab}	73.76^{ab}	63.86	48.08^{ab}	2.22^{ab}	1.70	-39.52
Gteaext	102.45^{b}	79.90^{ab}	64.50	51.50^{ab}	2.02^{b}	1.54	-44.50
SEM	1.98	3.31	0.93	2.14	0.09	0.07	30.37

*Significant at 5% levels. $^{a\ b}$ Values without a common superscript are significantly different within a sub-column ($p<0.05$), ^{ns} = Non significant at 5% levels. α-TA: α-tocopheryl acetate, Marpow: Marigold powder, Marext: Marigold extract, Gteapow: Green tea powder, Gteaext: Green tea extract.

Table 3. Effect of dietary α-tocopheryl acetate and powder and extract of green tea and marigold on means of egg quality characteristics.

Dietary treatments	Yolk weight (g)*	Yolk index*	Yolk color score^{ns}	Albumen height (g)^{ns}	Haugh units^{ns}	Shell thickness (mm)^{ns}	Egg shell strength breaking (kg/cm^2)^{ns}
Control	17.28^{ab}	37.30^{b}	8.77	5.75	72.86	0.344	3.260
α-TA	17.57^{a}	39.06^{a}	8.74	6.12	77.19	0.347	3.397
Marpow	17.38^{ab}	38.43^{ab}	8.60	5.55	72.26	0.348	3.380
Marext	17.38^{ab}	37.96^{ab}	8.91	5.66	72.70	0.349	3.383
Gteapow	16.76^{b}	37.74^{ab}	8.93	5.85	75.36	0.333	3.218
Gteaext	16.76^{b}	38.46^{ab}	8.82	6.10	77.32	0.336	3.222
SEM	0.26	0.52	0.12	0.29	2.01	0.01	0.080

*Significant at 5% levels. $^{a\ b\ c}$ Values without a common superscript are significantly different within a sub-column $p<0.05$), ^{ns} = Non significant at 5% levels. α-TA: α-tocopheryl acetate, Marpow: Marigold powder, Marext: Marigold extract, Gteapow: Green tea powder, Gteaext: Green tea extract.

powder and the control groups. There were no differences in body weight change among treatments.

Egg characteristics

Egg quality aspects such as yolk color, albumen height, haugh units, egg shell strength breaking and shell thickness were unaffected by treatments (Table 3). Although albumen height was not affected by dietary treatments, Bravo (1998) showed that albumen tended to be thicker in groups fed green tea powder and attributed this effect to possible transfer of green tea powder polyphenols into β-ovomucin. Beta-ovomucin increases albumen durability by forming complexes with proteins and polysaccharides. Yolk weight and yolk index (the ratio of the yolk height to the yolk width) were significantly ($p<0.05$) changed by the dietary treatments. Eggs produced by layers fed α-tochopheryl acetate had a greater yolk weight than those from the green tea powder and extract diets (+0.82 and +0.80 g, respectively); however eggs produced by layers fed control diet had lower yolk index compared to α-TA. There were no statistical differences among the other treatments for yolk weight and index ($p>0.05$). The effect of α-tochopheryl acetate may be attributed to release of vitellogenin from liver to improve those measurements (Whitehead et al., 1998) and/or vitamin E regeneration at the expense of other anti-oxidants such as green tea catechins (Bowry et al., 1999).

Serum biochemical variables

Experimental diets had significant ($p<0.05$) effects on serum HDL, LDL, triglyceride, and cholesterol concentrations (Table 4). Adding green tea extracts to the diet, significantly ($p<0.05$) reduced serum cholesterol (130.8 vs. 145.9) and triglycerides (1291.1 vs. 1393) in comparison to control. The exact mechanism by which herbal extracts decreases yolk cholesterol is not known as yet. Cholesterol is primarily biosynthesized in the liver of laying hens and incorporated into vitellogenin and very low density lipoprotein particles, which are secreted into the bloodstream and subsequently taken up by growing oocytes through receptor-mediated endocytosis (Elkin,

Table 4. Effect of dietary α-tocopheryl acetate and powder and extract of green tea and marigold on the means of serum cholesterol, LDL, HDL, triglyceride.

		g/dl				Ratio*	
Dietary treatments	**Glucose^{ns}**	**Cholesterol***	**HDL***	**LDL***	**Triglyceride**	**HDL/Cholesterol**	**HDL/LDL**
Control	229.30	145.90^{a}	52.60^{b}	54.20^{a}	1393.00^{a}	0.36^{b}	0.97^{b}
α-TA	258.20	138.10^{ab}	57.30^{a}	50.50^{b}	1358.20^{ab}	0.42^{a}	1.14^{a}
Marpow	242.80	134.40^{b}	56.50^{ab}	52.00^{ab}	1326.40^{ab}	0.43^{a}	1.09^{a}
Marext	244.40	135.10^{b}	57.70^{a}	51.60^{b}	1330.50^{ab}	0.43^{a}	1.12^{a}
Gteapow	253.40	139.40^{ab}	55.60^{ab}	50.20^{b}	1343.60^{ab}	0.40^{a}	1.11^{a}
Gteaext	230.70	130.80^{b}	56.80^{a}	49.90^{b}	1291.10^{b}	0.44^{a}	1.14^{a}
SEM	10.89	3.59	1.38	1.09	22.39	0.01	0.02

*Significant at 5% levels. $^{a\,b\,c}$ Values without a common superscript are significantly different within a sub-column ($p<0.05$), ^{ns} = Non significant at 5% levels. α-TA: α-tocopheryl acetate, Marpow: Marigold powder, Marext: Marigold extract, Gteapow: Green tea powder, Gteaext: Green tea extract.

2006). Therefore, it was suggested that the decrease in the egg yolk cholesterol is dependent on the decrease in cholesterol synthesized in the liver. Hence, the decrease in total lipid and cholesterol may be attributed to the diminishing effect of herbal extracts on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that is needed for cholesterol synthesis in liver. The pure components of essential oils could inhibit the activity of HMG-CoA reductase (Crowell, 1999) and increase the activity of LDL receptor. Therefore, it could strengthen direct absorption of very low density lipoproteins (VLDL) in the liver and reduce the amount of VLDL transformed to LDL to achieve cholesterol reduction (Pitman et al., 1998). Conversion of cholesterol to bile acids occurs exclusively in the liver and represents the major pathway for the elimination of cholesterol from the body. This may also explain the reduced cholesterol levels. Liver weight and liver cholesterol content may also be explained by the disrupting effect of tea catechins on micelle formation. Bile acids can be re-absorbed from the small intestine only in the form of micelles. Tea catechins prevent the re-absorption of bile acids by disrupting the micelle formation and thus increasing bile acid excretion. In order to replenish the loss in bile acids, the conversion of cholesterol to bile acids in the liver will be enhanced and it further reduces the content of liver cholesterol (Shefer et al., 1969; Myant and Mitropoulos, 1977). The results indicated that except for powder form of marigold, adding other supplements could effectively reduce serum LDL. These reducing effects were 8, 8.6, 5, and 7.3% for Gteapow, Gteaext, Marext, and α-TA, respectively. Feeding α-TA, Marext, and Gteaext increased serum HDL compared to control group (8.9, 9.6, and 7.9%, respectively). The ratios of HDL to cholesterol and HDL to LDL were significantly ($p<0.05$) affected by dietary treatments so that these values were greater than that of control group. Based on current results, egg cholesterol contents varied inversely with the HDL/LDL ratio. As shown in Tables 4 and 5, the egg cholesterol decreased with increasing in HDL/LDL ratios. Therefore, it seems that the best way to achieve maximum egg cholesterol reduction will be optimization of HDL/LDL ratios rather than those contents separately.

Egg yolk cholesterol and triglycerides

Egg yolk cholesterol content did not differ among α-TA, Marext, and either form of green tea, but their contents were lower than those of Marpow and control groups ($p<0.05$). Cholesterol content when expressed as milligrams per egg, however, was not affected by treatments except for extracted forms of marigold and green tea compared to control group. Except for powder form of marigold, dietary treatments reduced triglyceride content of egg yolk compared to control.

In agreement with our results, Biswass and Wakita (2000) reported that green tea supplementation to layer diet reduced the cholesterol content of the egg yolk. Yamane et al. (1999) demonstrated that the fat content of egg yolks significantly decreased due to the caffeine and catechin content of green tea. That possible mechanism was discussed extensively in the previous section. Unlike of our results, Unganbayar et al. (2005) reported no significant differences in cholesterol contents of egg yolk between layers fed diets containing 0.5, 1.0 and 1.5% green tea powder and control diets but, administration of 2.0% green tea suppressed egg yolk cholesterol significantly compared to that of the positive control treatment

Conclusions

Overall, dietary supplementation of α-tocopheryl acetate, green tea and marigold had beneficial effects on performance and internal egg quality measurements (yolk weight and index). Egg yolk cholesterol and triglyceride contents also were reduced by green tea and marigold supplementation. Adding 200 IU α-tocopheryl acetate/kg

Table 5. Effect of dietary α-tocopheryl acetate and powder and extract of green tea and marigold on the means of egg yolk cholesterol and triglyceride.

Dietary treatments	Cholesterol mg/g yolk*	Cholesterol mg/egg*	Triglyceride g/100g yolk*
Control	13.12[a]	237.74[a]	31.99[a]
α-TA	11.92[c]	224.84[ab]	30.09[b]
Marpow	12.77[b]	232.90[ab]	31.07[ab]
Marext	12.14[c]	218.10[b]	30.65[b]
Gteapow	12.32[c]	223.07[ab]	30.90[b]
Gteaext	12.07[c]	208.91[b]	30.34[b]
SEM	0.10	5.53	0.33

*Significant at 5% levels. [a b c] Values without a common superscript are significantly different within a sub-column ($p<0.05$), [ns] = Non significant at 5% levels. α-TA: α-tocopheryl acetate, Marpow: Marigold powder, Marext: Marigold extract, Gteapow: Green tea powder, Gteaext: Green tea extract.

on feed was more effective than other treatments to improve feed intake, egg production, yolk weight and index. Diets supplemented with 0.5% green tea extract decreased feed consumption and feed conversion ratio compared with control group. In general, incorporating antioxidant components derived from herbal plants in laying hens diets as alternative options for α-tocopheryl acetate can be effective based on cost and availability.

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