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EFFECT OF HERBS ON PRODUCTIVE PERFORMANCE OF LAYING HENS, SOME BLOOD CONSTITUENTS AND ANTIOXIDANT ACTIVITYIN EGG YOLK

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ABSTRACT: The present study aimed to evaluate the comparative effect of ginger, cinnamon, thyme, cumin black seed and pomegranate peels on productive performance, egg quality and some blood constituents of laying hens. Ninety white Hi-Sex layers at 30 week of age were randomly allocated into six treatment groups for ten weeks experimental period. The layers groupsfed six different diets as, basal diet (T1, control)or basal diets supplemented with 1% of five different dried herbal plants , cumin black seeds (T2), thyme (T3), cinnamon (T4), ginger (T5) and pomegranate peels (T6). Thyme wasthe most effective supplementation in improving egg production followed by cinnamon. Average egg weight reduced significantly by feeding dietary thyme, cinnamon, ginger or pomegranate peels. Herbal plants have no significant ($P \leq 0.05$) effects on feed consumption or feed conversion ratio. The percentages of egg yolk, albumin or egg shell did not differ due to treatments. There were reductions in plasma and yolk levels of total cholesterol and LDL. Total phenolic content in different herbs were significantly similar. Free radical scavenging capacity (DPPH reduction %) was approximately similar for different herbs plants, and in egg yolk. Egg yolk contents of carotenoids increased significantly due to inclusion different herbal plants into laying hens diets. It can be concluded that, herbal plants can be employed as natural antioxidants due to its high contents of phenols. As well, inclusion of herbal plants into hen diets can improve the nutritive values of produced eggs via increasing its content of carotenoids and antioxidants.

Key words: Laying hens,herbs, antioxidant , egg quality , blood constituents

INTRODUCTION

Phytogenic feed additives have been studied extensively to improve the performance of ppoultry. Phytogenic products are derived from a group of popular medicine herbal plants (Bozkurt et al., 2014). The most famous phytogenic–derived plants are ginger, cinnamon, thyme, cumin seeds, and pomegranate peels (Dhama et al., 2015). These herbal plants have recently gained increasing interest, due to its bioactive content of phytochemicals which comprise phenolics, polyphenols, alkaloids, lectins, terpenoids, polypeptides and essential oils (Gheisar and Kim, 2018). Phytochemicals in herbal plants have stimulating effects on poultry digestive system in addition to its activities as antimicrobial, coccidiostatical, anti-inflammatory and immune stimulator (Hashemi and Davoodi .2011; Hajati et al., 2014).

The antioxidants and antimicrobial properties of herbal plants are mainly related to its content of phenolic and polyphenolic compounds (Sikora et al., 2008. ; Munguía et al., 2016).

Herbal plants were studied extensively for enhancing laying hens performance. In this concern, Paskudska et al., (2018) and Abdel-Wareth et al., (2013), stated that, thyme oils have a positive effect on laying performance and egg production of laying hens. Khana, et al., (2013); and Abd El-Hack and Alagawany (2015) reported that, feeding dietary black cumin seeds improved egg production, egg weight, and feed conversion ratio of laying hens. As well, feeding dietary cinnamon improved hen-day egg production, feed conversion ratio and egg shell percentage (Şimşek et al., 2015). Nasiroleslami and Torki (2010) observed an improvement in egg shell weight and thickness of laying hens fed diets supplemented with essential oils of ginger. Also, Moeini et al., (2011) concluded that, feeding 1% ginger has positive effects on egg production and egg mass of laying Hens. Yassein et al., (2015) noted significant increase in egg number, egg weight, egg mass and egg shell weight of laying hens fed diet supplemented with pomegranate peel.

Despite, the large numbers of studies have been conducted to evaluate the separate effect of herbal plants, the comparative effect of herbal plants on laying performance is still lacking. Therefore the present study aimed to evaluate the comparative effect of ginger, cinnamon, thyme, cumin black seed and pomegranate peel on productive performance, egg quality and some blood constituents of laying hens. As well as, the antioxidants activity of these selected plants have been studied.

MATERIALS AND METHODS

The current study conducted at the Experimental Poultry Farm, Animal Production Department, Faculty of Agriculture Sciences and Nutrition, King Faisal University, Saudi Arabia kingdom.

A total number of 90 White Hi-Sex layers which were randomly sited from 30 to 40 weeks of age in battery cages located in close sided laying house. The hens were allocated into six treatment groups with 5 replicates of 3 layers per cage each. During the ten weeks experimental period the six group of layers fed 6 different diets as, basal diet only (T1, control), or basal diets supplemented with 1% of five different dried herbal plants , cumin black seeds (T2), thyme (T3), cinnamon (T4), ginger (T5) and pomegranate peels (T6). The basal diet was formulated (Table1) to meet all nutrient requirements of laying hens according to NRC (1994). The five

herbal plants were purchased from local market of El Hassa city, Saudi Arabia Kingdom and grinding before included into laying hen diets. Mash feed and water were provided *ad lib.* Lighting hours were controlled as 17 hours per day.

Performance Parameters

Egg weight in grams was recorded daily for each cage and calculated for each hen throughout the experimental period. Average egg weight, egg production percentage were calculated for each hen and treatment group. Feed consumption in grams per cage was recorded weekly and average daily feed consumption per layer was calculated. Feed conversion ratio was calculated as gram feed per gram egg produced (g. feed/g. egg). Body weight gain was calculated for each hen and treatment group by subtracting individual body weight of hen at 30 weeks from that at 40 weeks of age.

Egg components percentages were assessed at 40 weeks of age by using 15 eggs per treatment representing three consecutive eggs from each cage. Eggs were individually weighted, broken, yolk and albumin were separated weighed and calculated as percentage to whole egg weight. Egg shell with membrane were cleaned, weighed and related as percentage to the whole egg. Yolk color index was determined by matching yolk color with the bands of the Roche yolk color fan.

Blood and egg yolk analysis

At the end of the experiment blood samples were obtained from wing vein of 5 hens per treatment representing the five replicates. Plasma was separated and used for different determinations. Total lipids, triglycerides, cholesterol, high density lipoprotein (HDL) were measured using special kits (United Diagnostic industry, Dammam , Saudi Arabia kingdom). Low density lipoprotein (LDL) values were calculated by subtracting HDL from total cholesterol values.For yolk fat extraction three grams of egg yolk was homogenized in 15 ml, 2: 1 of chloroform: methanol mixture and yolk fat was isolated according to methods of Haug and Hostmark (1987). Yolk concentration of total lipids, triglycerides and cholesterol was determined by ultraviolet spectrophotometer using commercial kits (United Diagnostic industry, Dammam , Saudi Arabia kingdom).

Total phenols were determined in herbal plants extract according to the methods described by Boyer and Hai Liu (2004). The free radical scavenging capacity of herbal plants and egg yolk extractagainst DPPH (1,1-diphenyl-2 picrylhydrazyl) was estimated according to Zhang and Hamauzu (2004). Carotenoids was determined in egg yolk extract according to the methods described by Mosquera et al., (1991) the carotenoid fraction at470 nm.was measured in a UV spectrophotometer.

Statistical analysis:

Statistical analysis was conducted by one way ANOVA using statistical analysis system program SAS (2003). Duncan's multiple range tests was used to separate means (Duncan. 1955).

RESULTS AND DISCUSSION

Performance aspects of laying hens are presented in table 2. Thyme was the most effective supplementation in improving egg production followed by cinnamon; however,both are insignificant higher than control group. The improvement in egg production was reported previously due to adding thyme (Abdel-Wareth et al., 2013), and cinnamon (Şimşek et al., 2015). Inclusion of black cumin seeds,

ginger and pomegranate failed to improve egg production percentage. The lack of effect on egg production was reported by feeding dietary cumin seed (Abou-Elkhair et al., 2018) or ginger (Zhao et al., 2011). However, several studies indicated to the improvement effect on egg production percentage of laying hens due to inclusion of ginger (Moeini et al., 2011); black cumin seed (Khana et al., 2013) or pomegranate peel (Abbas et al., 2017).

As compared with control, average egg weight reduced significantly($P \leq 0.05$) by feeding dietary thyme, cinnamon, ginger or pomegranate. The current result is in harmony with those of Arpášová et al., (2013) who noted a reduction in average egg weight as result of adding thyme oil into diets. However, several studies indicate to increase average egg weight by adding ginger, black cumin seed or pomegranate peel (Moeini et al.,2011; Khana et al., 2013; Abbas et al., 2017). Generally, disagreement between the current results and previous studies may be related to the differences in inclusion ratio and type either powder or extracted materials (Moeini et al., 2011) in addition to the experimental conditions.

Inclusion of herbal plants has no significant ($P \leq 0.05$) effects on, feed consumption or feed conversion ratio. Several previous studies did not record any significant effects on feeding parameters due to supplementing laying hen diets with cumin black seed (Hossain et al., 2016)thyme (Abd El-Hack and Alagawany, 2015), cinnamon(ọọọŞİmşek et al., 2015), ginger (Zhao et al., 2011), or pomegranate (Saki et al., 2014). The lack of herbal effect on feeding performance was illustrated previously. Because of, Laying hens are adult birds, have developed digestive system with more stable intestinal bacteria which able to resist and do not change by oral administration of herbal plants (Bozkurtet al., 2014).

Egg composition percentage (Table 3) showed a non-significant increment in egg yolk due to feeding dietary thyme, the differences were not obvious for other treatments compared to control. Vali and Mottaghi (2016) observed an increase in yolk weight of Japanese quail due to adding thyme into their diets. Several studies indicated that, egg yolk weight was not affected by feeding dietary black cumin seed (Abou-Elkhair et al., 2018), cinnamon (ọọọŞİmşek et al., 2015),ginger (Incharoen. and Yamauchi, 2009) or pomegranate (Saki et al., 2014). As compared to control, egg albumin and egg shell percentages were not differed significantly. Similar results were reported by Abou-Elkhair et al., (2018), Abdel-Wareth et al., (2013)Santos et al.,(2019), Wen et al., (2019) and Saki et al., (2014), who did not note any effect on albumin ratio due to feeding dietary black cumin seed, thyme, cinnamon, ginger or pomegranate respectively. Yolk color index increased significantly due to adding black cumin seed into laying hen diets. Hassan and Alaqil (2014) observed insignificant increase in yolk color score due to inclusion black cumin seed into laying hens diets. The increment in yolk color may be attributed to the pigmentation substances in black cumin seed (Abou-Elkhair, et al., 2018).

Lipids and their derivatives values in blood and egg yolk are shown in Table 4. Triglycerides values in blood and yolk were not differed significantly due to adding herbal plants into laying hens diets. Plasma total lipids reduced due to feeding different herbs, the reduction was significant for cumin black seed and

thyme addition. There were reductions in plasma and yolk levels of total cholesterol. The reduction in total cholesterol levels are related to the decrease in LDL, while HDL values were not affected clearly by treatments except ginger. Most of previous studies indicate to hypolipidimic and hypocholesterolimic effects of herbal plants cumin black seed (Azeema et al., 2014), thyme (Abd El-Hack and Alagawany, 2015), cinnamon (Abo Ghanima et al., 2020), ginger (Khan et al., 2012) and pomegranate (Vashan andGhaznavi 2018).However similar effects was not clear and lacked significance in the current study, that may be related to 1% addition herbs is not enough to exert its beneficial effects on laying hens. As well as, supplementation of herbal plants in form of dried powder in the present study is less effective than herbal essential oil, or extract. The reduction in blood and yolk total lipids, triglycerides and cholesterol levels may be due to the inhibitory effect of herbal plants on fatty acids and cholesterol synthesis in liver hepatocytes (Aydin et al., 2008). Furthermore, herbs can reduce dietary cholesterol absorption (Azeema et al., 2014).

The total phenolic substances in different five herbal plants were significantly similar (Table 5). Black cumin seed contained the highest values of phenols than other herbal plants. Phenols have antioxidant properties exerted their action by reacting with a free radical and delocalization of the gained electrons, as a results avert the continuation of the free radical chain reaction (Lee et al., 2017). Due to its high contents of phenols, herbal plants such as thyme and ginger can be employed as natural antioxidants (Lee et al., 2017). Free radical scavenging capacity was determined as DPPH reduction% to evaluate antioxidants activity spectrophotometrically (Garcia et al., 2012). The values of DPPH % (Table 5) are approximately similar in different herbs except black cumin seed whichwas 50% less than other herbs. However, the values of DPPH reduction% in egg yolk (Table 5) did not responsed totreatments and were approximately similar in all treatments including control group. In this connection the references are still lacking. Egg yolk contents of carotenoids (Table 5) increased significantly due to inclusion different herbal plants into laying hens diets. Carotenoids substances are source for yolk color, and have several functions as, antioxidants agent, pro-vitamin A and immune stimulator (Benakmoum et al., 2013). Accordingly, inclusion of herbal plants into hen diets can improve the nutritive values of produced eggs via increasing its content of carotenoids substances (Hammershøj et al., 2010).

CONCLUSION

It can be concluded that, Thyme was the most effective supplementation in improving egg production. Herbal plants can be employed as natural antioxidants feed additives due to its high contents of phenols and antioxidant capacity. As well, inclusion of herbal plants into hen diets can improve the nutritive values of produced eggs via increasing its content of carotenoids and antioxidants.

Table (1): Composition and calculated analysis of the Basal diet.

Ingredients	**%Basic Diet**
Yellow corn	63.44
Soybean meal (48%)	19.20
Corn gluten meal	3.92
Vegetable oil	1
Limestone	9.40
Calcium phosphate	1.98
Common salt	0.45
Vit. & min. premix*	0.30
DL. Methionine	0.20
L-Lysine	0.10
‘Total	100
Calculated analysis	
Crude protein, %	17.04
M.E. Kcal/kg	2828
Calcium, %	4.07
Available phosphorus, %	0.48
Methionine + cysteine, %	0.78
Lysine, %	0.86

*Composition of vitamin and mineral premix. Each 2.5kg of vitamin and mineral mixture contains: 12000000IU vitamin A; 2000000 IU D_3; 10g E; 1g K; 1 g B_1; 5gB_2; 1500mg B_6; 10mg B_{12};10g Pantothenic acid; 20g Nicotinic acid;1g Folic acid; 50mg Biotin; 500g Choline Chloride; 4 g Copper; 300 mg Iodine; 30g Iron; 60g Manganese; 50g Zinc and 100mg Selenium.

Table(2): Effect of herbs on laying hen performance from 30 to 40 week of age

Items	Treatments					
	T1 Control	**T2 Black Cumin**	**T3 Thyme**	**T4 Cinnamon**	**T5 Ginger**	**T6 Pomegranate**
Egg production, %	86.86^{ab} ±2.07	80.09^{b} ±2.79	90.19^{a} ±1.65	87.43^{a} ±1.58	83.81^{ab} ±2.22	83.62^{ab} ±2.93
Av. egg weight, g.	60.49^{a} ±0.412	60.77^{a} ±0.517	58.31^{b} ±0.382	58.50^{b} ±0.365	58.69^{b} ±0.278	59.11^{b} ±0.345
Av. egg mass (g/ hen/day)	52.55 ±1.31	48.38 ±1.59	52.59 ±1.01	51.21 ±1.02	49.15 ±1.29	49.21 ±1.69
Av. feed intake (g./hen/day)	108.09 ±1.72	110.11 ±1.97	107.15 ±2.28	107.16 ±1.46	110.20 ±1.76	106.37 ±1.38
Feed conversion (g. feed/g. egg).	2.12 ±0.064	2.09 ±0.054	2.02 ±0.054	2.14 ±0.052	2.23 ±0.077	2.12 ±0.045
Body weight gain, g.	224.7 ±13.87	236 ±18.28	267.3 ±10.80	247.3 ±15.69	246.0 ±15.33	252.0 ±7.25

Means ± (Standard error) values within a raw with different superscripts are significantly different (P≤0.05)

Table (3): Effect of herbal plants on egg component percentage of laying hens

Items	Treatment					
	T1 Control	**T2 Black Cumin**	**T3 Thyme**	**T4 Cinnamon**	**T5 Ginger**	**T6 Pomegranate**
Yolk, %.	24.19 ±0.576	24.23 ±0.669	25.43 ±0.480	24.38 ±0.425	24.55 ±0.494	24.65 ±0.451
Albumen, %.	63.61^{ab} ±0.512	64.18^{a} ±0.774	62.16^{b} ±0.563	64.11^{a} ±0.478	63.69^{ab} ±0.724	63.26^{ab} ±0.617
Shell, %.	12.20 ±0.293	11.59 ±0.232	12.41 ±0.274	11.51 ±0.232	11.76 ±0.419	12.09 ±0.257
Yolk colour index	4.47^{b} ±0.192	5.27^{a} ±0.248	4.87^{ab} ±0.401	4.67^{ab} ±0.195	4.87^{ab} ±0.192	4.60^{ab} ±0.131

Means ± (Standard error) values within a raw with different superscripts are significantly different (P≤0.05)

Table (4): Effect of herbs on some plasma and egg yolk constituents

Items	Treatment					
	T1 Control	**T2 Black Cumin**	**T3 Thyme**	**T4 Cinnamon**	**T5 Ginger**	**T6 Pomegranate**
Plasma constituents						
Triglyceride, mg/dl.	130.2 ±10.44	120.2 ±8.42	108.0 ±11.92	117.7 ±9.48	138.2 ±12.03	121.8 ±9.72
Total lipid, mg/dl.	288.7^{a} ±27.87	253.7^{b} ±20.52	258.1^{b} ±18.49	269.5^{ab} ±32.36	271.3^{ab} ±13.53	273.4^{ab} ±11.51
Total cholesterol, mg/dl.	135.5 ±5.05	124.9 ±2.96	127.2 ±3.72	132.5 ±3.72	124.0 ±6.14	134.4 ±9.48
Cholesterol HDL, mg/dl.	67.3^{b} ±3.39	68.6^{b} ±4.11	67.9^{b} ±3.45	73.8^{ab} ±1.66	79.2^{a} ±0.937	68.9^{b} ±3.02
Cholesterol LDL, mg/dl.	68.2^{a} ±6.79	56.3^{ab} ±5.21	59.3^{ab} ±7.37	58.7^{ab} ±4.01	44.8^{b} ±7.83	65.5^{a} ±8.51
Egg yolk constituents						
Triglyceride, mg/g. Yolk.	160.9 ±2.32	162.4 ±3.13	153.8 ±1.36	159.4 ±2.41	162.1 ±2.59	153.8 ±9.51
Total lipid, mg/g. Yolk.	314.2 ±19.33	323.4 ±21.01	312.6 ±13.94	342.7 ±15.16	329.8 ±25.36	328.5 ±14.55
Total Cholesterol, mg/g yolk.	13.8 ±0.547	12.1 ±1.05	11.5 ±0.709	12.3 ±0.503	12.5 ±0.896	12.9 ±0.503

Means ± (Standard error) values within a raw with different superscripts are significantly different ($P \leq 0.05$)

Table (5): Antioxidants agents in different herbs and egg yolk

Items	Treatments					
	T1 Control	**T2 Black Cumin**	**T3 Thyme**	**T4 Cinnamon**	**T5 Ginger**	**T6 Pomegranate**
Antioxidants in herbs						
Total phenols, mg/100g.	-	348.44 ±31.75	329.58 ±5.14	306.44 ±2.57	314.15 ±2.97	331.30 ± 3.43
DPPH Reduction, %.	-	27.70^{b} ±1.22	65.36^{a} ±0.054	62.90^{a} ±0.031	64.30^{a} ±0.173	60.32^{a} ±5.69
Antioxidants in egg yolk						
Carotenoids, mg/kg.	10.83^{b} ±0.247	15.07^{a} ±0.929	14.40^{a} ±1.73	14.17^{a} ±1.18	17.00^{a} ±1.29	14.57^{a} ±0.851
DPPHReduction, %.	59.52 ±1.2	61.35 ±0.409	61.87 ±0.968	62.31 ±1.09	61.91 ±1.24	62.42 ±0.845

Means ± (Standard error) values within a raw with different superscripts are significantly different (P≤0.05)

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الملخص العربي

تأثير النباتات العشبية على الأداء الإنتاجي للدجاج البياض وبعض مكونات الدم ومحتوى الصفار من مضادات الأكسدة

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تهدف الدراسة الحالية إلى مقارنة تأثير كل منالزنجبيل والقرفة والزعتر والحبة السوداء وقشور الرمان على الأداء الإنتاجي وجودة البيض وبعض مكونات الدم فى الدجاج البياض.أستخدم فى الدراسة عدد تسعين دجاجة بياضة من سلالة الهاى سكس الأبيض عمر 30 أسبوع وزعت عشوائيا إلى ست مجموعات تجريبية تم تغذيتها لمدة عشر أسابيع على ست علائق مختلفة هى عليقة كنترول خالية من الأضافات (T1)، أو عليقة كنترول مضاف لها 1 ٪ من النباتات العشبية ، الحبة السوداء (T2)، الزعتر (T3)، القرفة (T4)، الزنجبيل (T5) وقشور الرمان (T6). لوحظ أن إضافة الزعتر كانت الأكثر فعالية في تحسين إنتاج البيض يليه القرفة. انخفض متوسط وزن البيض بشكل كبير فى الدجاجات المغذاة على الزعتر أو القرفة أو الزنجبيل أوقشرالرمان. النباتات العشبية ليس لها تأثير معنوى على معدل استهلاك االعلف أو معدل التحويل الغذائى. لم تختلف النسبة المئوية لصفار البيض أو الألبيومين أو قشرة البيض بسبب المعاملات. كانت هناك انخفاضات طفيفة في مستوى الكوليسترول فى بلازما الدم وصفار البيض كذلك الكوليسترول منخفض الكثافة LDL. اتضح أن محتوي النباتات العشبية من المواد الفينولية الكلية كان متشابه كذلك تشابهت كفاءتها كمضادات للأكسدة طبقا لقياس(DPPH Reduction %). كذلك زاد محتوى صفار البيض من الكاروتين بشكل كبير نتيجة التغذية على النباتات العشبية.خلصت الدراسة إلى إمكانية استخدام النباتات العشبية كمضادات أكسدة طبيعية فى علائق الدجاج البياض نظرا لمحتواها العالي من الفينولات. إضافة إلى دورها فى تحسين القيمة الغذائية للبيض المنتج بزيادة محتواه من الكاروتينات ومضادات الأكسدة.