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LAYING PERFORMANCE, HEALTH STATUS and SOME PHYSIOLOGICAL TRAITS of MANDURAH LAYING CHICKENS AFFECTED BY THE ADDITION OF DIFFERENT LEVELS of ANISE AND CARAWAY SEED POWDER

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ABSTRACT:

This study was conducted to evaluate the effects of varying levels of Anise and Caraway seeds powder on laying performance, some blood constituents, egg quality traits, yolk cholesterol content, immunity response and total count aerobic bacteria of Mandurah laying chicken. For this purpose, a total number of 140 Mandurah laying chickens at 26 weeks of age were used in this experiment having a completely randomized design, with 7 equal treatment groups, which replicated 4 times with 5 hens per replicate. Each bird was housed in individual wire cage measuring (50 × 50 × 45Cm^2), where each bird in each cage allowing 510 Cm^2 space/hen). A basal control diet was formulated and supplemented with 0.1, 0.2 and 0.3 % Anise and 0.1, 0.2 and 0.3 % Caraway seeds in powder form. Birds were fed on isocaloric (2751 Kcal ME /Kg) and isonitrogenous (16.14% CP) diets. Results showed that laying performance significantly (P<0.05) improved when inclusion Anise and Caraway seeds powder compared with the control group. However, it was clearly observed that plasma lipids were significantly reduced (P <0.05) by supplementing diets with different levels of anise and caraway seeds. In the same trend, the addition of Anise and Caraway seeds powder led to a clear and significant decrease in egg yolk and blood cholesterol compared with the control group (P<0.05). However, Anise and Caraway seed powder incorporation caused positive effects on the parameters related to immune response and overall total antioxidant capacity (TAC) compared with the control group. While, total count aerobic bacteria significantly (P < 0.05) decreased for birds fed diets inclusion Anise and Caraway seeds powder as compared with the control group.

Conclusively, Anise and Caraway seeds powder can be included in the diet of laying hens as a safe and effective feed additive to improve laying performance and reduce total blood lipids and thus reduce cholesterol content in egg yolk.

Key words: Laying performance, health status, and physiological traits.

INTRODUCTION:

The industrialization of poultry husbandry and the improvement of feed nutritional efficiency has speeded up the introduction of the feed additives that have become widely used in animal feed for many decades. Therefore, phytogenic feed additives have been extensively studied to improve poultry performance, where phytogenic products are derived from a group of popular medicine herbal plants (Bozkurt *et al.*, 2014). However, phytochemicals in herbal plants have stimulating effects on poultry digestive system and exhibit antioxidant, antimicrobial, antifungal, and anti-inflammatory, as well as anti-parasitic and antiprotozoal properties (Hajati *et al.*, 2014; Achilonu *et al.*, 2018).

Nowadays, comprehensive investigations of phytogenic plants have indicated their growth promoting, antimicrobial, antioxidant and anti-inflammatory functions (Gheisar and Kim, 2017). The herbal plants have recently gained increasing interest, due to its bioactive content of phytochemicals which comprise phenolic, polyphenols, alkaloids, lectins, terpenoids, polypeptides and essential oils (Gheisar and Kim, 2018). Among these species, Caraway (*Carum carvi*) is one of the most appreciate spices for its seed richness, plethora of bio-logically active compounds and medicinal agent (Laribi *et al.* 2010). However, Seger *et al.* (2019) indicated that the inclusion of Caraway seed powder at levels 4, 6 and 8 g / kg of Japanese quail diet did not affect the growth and development of the gonads as well as the proportion of lipids in the blood. However, Anise (*Pimpinella anisum L.*) is an annual aromatic herb belonging to the Apiaceae family and has been widely used to maintain or improve animal and human health (Al-Shammari *et al.*, 2017). Thus, Christaki *et al.* (2011) found that neither the supplementation of Anise nor that of α-tocopheryl had any effect on the performance of the birds or the quality of their eggs, except for a significant change of the color of the egg yolk. They added that cholesterol concentration in the serum tended to decrease with the addition of Anise to the diet.

Therefore, the present study aimed to evaluate the effect of inclusion different levels of Anise and Caraway seed powered on laying performance,

some blood constituents, egg quality traits, yolk cholesterol content, immunity response and total count aerobic bacteria of Mandurah laying chickens.

MATERIALS AND METHOD

Site, duration, and the aim of the present study.

The present study was carried out at the Anshas Poultry Breeding Research Station belonging to the Animal Production Research Institute (APRI), Agriculture research center, Cairo, A.R.E., between the periods of September 2015 to February 2016. Therefore, the present study aimed to evaluate the effect of inclusion different levels of Anise and Caraway seed powered on laying performance, some blood constituents, egg quality traits, yolk cholesterol content, immunity response and total count aerobic bacteria of Mandurah laying chickens.

Husbandry, experimental design and feeding practice.

Before the start of the experiment the experimental unit which will be used in this study was cleaned and fumigated with a mixture of potassium permanganate (17.5g) and formalin solution (35 mL) to eliminate any pathogen can affect bird's health afterwards. After fumigation the unit, batteries and tools were prepared for the experiment. A total number of 140 Mandurah laying chicken with uniform body weight were randomly assigned to a completely randomized design, with 7 equal treatment groups replicated 4 times with 5 hens per replicate. Each bird was housed in individual wire cage measuring (50 × 50 × 45Cm^2), where each bird in each cage allowing 510 Cm^2 space/hen). Laying hens in the control group were given a corn-soybean-basal diet in pellet form without any supplementation. While, the other treatment groups were given the same basal diet supplemented with 0.1, 0.2 and 0.3 % Anise seed powder (ASP) and 0.1, 0.2 and 0.3 % Caraway seed powder (CSP). All diets were isocaloric (2751 Kcal ME /Kg) and isonitrogenous (16.14% CP). Diets were formulated to provide the nutrient requirements according to NRC (1994).

Anise and Caraway seeds powder was first mixed with premix which was later mixed with other ingredients and then pelleted. Samples of rations and both seeds powder were taken in to perform routine and bioactive chemical analysis according to AOAC (1994) as shown in Tables (1 and 2). The laying hens were kept in optimal and standard bioclimatic and welfare conditions with *ad libitum* feeding and watering. The temperature degree was between 25 to 30 ^{o}C with relative humidity of 60–65 %, which were recorded by using a digital thermo-hygrometer at 9:00 a.m. to 7:00 p.m. Chickens were provided 16 hour of light:

Table (1): Ingredients and nutrients composition of the basal diet (air-dry basis).

Ingredients	Control diet	Anise level %			Caraway level %		
		0 .1	0 .2	0 .3	0 .1	0 .2	0 .3
Yellow corn (8.5 %)	65.5	65.42	65.35	65.27	65.43	65.37	65.31
Soybean meal (44 %CP	25.0	24.97	24.9	24.92	24.96	24.93	24.89
Limestone ($CaCo_3$)	7.10	7.10	7.10	7.10	7.10	7.10	7.10
Di-calcium phosphate($CaHPO_4$)	1.50	1.50	1.50	1.50	1.50	1.50	1.50
DL-Methionine 99%	0.10	0.10	0.10	0.1	0.10	0.10	0.10
Sodium chloride (NaCl)	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vit & Min. Premix*	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Anise seed powder	0.00	0.10	0.20	0.30	0.00	0.00	0.00
Caraway seed powder	0.0	0.0	0.00	0.00	0.10	0.20	0.30
Total (Kg)	**100**	**100**	**100**	**100**	**100**	**100**	**100**
a-Calculated analysis*							
Crude protein (%).	16.14	16.22	16.19	16.15	16.12	16.20	16.19
Metabolizable energy (Kcal/Kg.)	2752	2750	2751	2753	2750	2753	2751
Calcium (%).	3.04	3.08	3.06	3.03	3.05	3.04	3.07
Available phosphorus (%).	0.41	0.45	0.43	0.44	0.43	0.45	0.42
Lysine (%).	0.89	0.88	0.90	0.87	0.88	0.89	0.86
Methionine (%)	0.39	0.40	0.38	0.37	0.41	0.39	0.40
Methionine + Cystine (%.)	0.56	0.57	0.60	0.59	0.56	0.55	0.57
b-Chemical analysis							
Crude protein (%).	16.24	16.33	16.22	16.25	16.17	16.21	16.24
Crude fiber (%).	3.22	3.23	3.33	3.19	3.31	3.27	3.25
Ash (%).	2.24	2.26	2.28	2.22	2.28	2.30	2.34

*Vitamin and mineral premix supplied per kg of diet: Vitamin A 12,000 IU, vitamin D_3 3,000 IU, vitamin E 40 mg, vitamin K_3 3 mg, vitamin B_1 2 mg, vitamin B_2 6 mg, vitamin B_6 5 mg, vitamin B_{12} 0.02 mg, niacin 45 mg, biotin 0.075 mg, folic acid 2 mg, pantothenic acid 12 mg, manganese 100 mg, zinc 600 mg, iron 30 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1 mg.
Calculated analysis was performed according to (NRC, 1994**)

Eight hours of dark cycle's program using automatic timers to schedule the lighting regimens with light intenesity15 to 20 lx throughout the entire experimental period. The cages were equipped with a nipple drinker and trough feeders, where all feeding conditions among treatments were the same

Table 2: Proximate analysis and bio-active components of Anise and Caraway seeds powder.

Items	**Medicinal plants**	
	Anise seed powder	**Caraway seed powder**
Chemical composition (%)		
Moisture	10.40	9.78
Dray matter	89.60	90.22
Crude protein	17.40	20.03
Ash	10.14	11.61
Crude fiber	14.7	20.16
Ether extract	27.09	20.96
Bio-active components (%)		
Limonene	1.4199	2.35
a-phellandrene	1.4546	2.406
Fenchene	1.4807	-
y-Terpinene	1.4878	-
Carveol, dihidro	1.5082	2.496
d-Camphene	1.5382	-
Cyclofenchene	1.5667	-
Isoanethole	1.6402	2.775
Caryophyllene oxide	1.8256	-
Linalool	2.1405	3.503
Anisole,	2.1673	-
Retinol	2.2265	3.760
Anisole, o-(l-ethylvinyl	2.2432	-
B-Caryophyllene	2.2873	3.819
Champaca camphor	2.5565	4.169
a-Caryophyllene alcohol	2.5913	4.320
Vertioiol	2.6156	4.433
o-Isoeugenol	2.6938	4.663
Engenol	2.8171	4.746
2,4-Dimethoxy-3-methylbenzaldehyde	2.8675	4.840
Anisyl acetate	2.9241	4.886
Vitexin	2.9521	-
Isoretinene	3.0846	5.244
Asterol	3.1682	5.509
Benzyl alcohol,a-ethyl-p-methoxy	3.3285	-
Retinamide, N-(4-hydroxyphenyl	3.4121	-
Dienestrol	3.6687	-
3,4-Dimethoxycinnamic acid	3.8699	-
Diethylstibestrol	3.8905	-
Fisetin	3.9079	-
Sinapic acid	3.9740	-
Coniferyl aldehyae	4.0147	6.652
(-)-a-Tocopherol	4.0778	-
Resveratrol	4.1495	-
Vinpocetine	4.1786	-
Cannabinol	4.2704	-
Longlpinocarvone	-	3.581
9-cis-Retinal	-	3.917

Caryophllene oxide	-	4.051
Champaca camphor	-	4.250
Linolenin l-mono	-	4.480
Vitexin	-	4.886
Retinyl propionate	-	5.364

throughout the whole experiment. The bird was provided with programmable adequate ventilation, where the air is removed from the house by means of exhaust fans. The experiment was started at 26 weeks of age and terminated at 47 weeks of age.

Preparation of Anise and Caraway seeds powder, experimental diets and bioa-active chemical analysis.

One batch 50 kg of both fresh Anise and Caraway seeds were obtained from the local market. Seeds dried naturally in a sunny then they were ground using a mixer and stored in bags at ambient temperature (27–30^{o}C) before being supplemented into diets. The proximate chemical compositions and bio-active components are shown in Tables 2 according to method of AOAC (1994). The analysis of bio-active components was carried out using a GC (Agilent technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000 equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 mx 0.25 mm i.d. and 0.25 um film thickness). The carrier gas was helium with the linear velocity of 1 ml/min.

The injector and detector temperature were 200^{o}C and 250 ^{o}C respectively. Volume injected 1ul of the sample. The MS operating parameters were as follows: ionization potential 70ev, interface temperature 250 ^{o}C, and acquisition mass range 50-800.The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Santana *et al.,* 2013).

Data recorded:

Laying performance:

The birds were weighed at the start and at the end of the experiment. Body weight changes were calculated as the difference between the initial and final body weight. However, feed intake was recorded and calculated as the decrease, in grams, of feed over 7 days, divided by the number of bird days. Feed conversion ratio (FCR) was calculated as the amount of feed consumed (g) required to produce a unit (g) of egg mass (feed conversion = g feed/g egg).

Also, eggs were daily collected and the egg number was calculated on a hen-day basis. Egg weight (EW) and egg number were daily recorded to calculate the egg mass (egg number × egg weight). All production variables were determined on a replicate basis.

Blood biochemical parameters:

At the end of experiment 3 birds were randomly selected from each treatment after fasting for 12 h for blood samples. Approximately 5.0 mL of fresh blood were taken from the jugular vein using a 5mL syringe fitted with a 24-gauge sterile hypodermic needle. Each blood sample from each individual was drawn in tubes which containing using Ethylenediamine-tetraacetic acid (EDTA) as an anticoagulant and then centrifuged at 3500 rpm for 15 min to obtain plasma. The following biochemical parameters levels were determined spectrophoto-metrically using commercial diagnostic kits provided from Bio diagnostic Company (Giza, Egypt). Plasma was separated and used for determination total protein (Gornal *et al.,* 1949), albumin (Doumas *et al.,* 1971) total lipids (Zollner and Kirsch, 1962), triglycerides (Richmond, 1973), cholesterol (Richmond, 1973), glucose (Trinder, 1969), and total antioxidant capacity (TAC) (Koracevic *et al.,* 2001). The values of globulins were obtained by subtracting the values of albumin from the corresponding values of total protein. Also, A/G ratio was obtained by dividing the values of albumin / globulin.

Egg quality traits:

Some of egg quality traits were done on the same day once all samples were collected. At the end of experiment (47 weeks of age), 15 eggs was randomly collected from each group (3 eggs per replicate) to assess interior and exterior egg quality parameters. Freshly laid eggs were individually weighed on an electric balance, accurate to 0.01 g, the egg length and width were determined before breaking. Shell thickness was determined, where the mean value of measurements at 3 locations on the egg (air cell, equator and sharp end) by using a dial gauge micrometer according to Yannakopoulos and Tserveeni-Gousi (1986). Albumen index, yolk index, Haugh unit, were measured as internal egg quality parameters. Egg shape index (%) was recorded according to Romanoff and Romanoff (1949). Egg yolk visual color was measured by matching the yolk with one of the 15 bands of the Roche yolk color fan. Haugh units, a measure of the height of the albumen of eggs broken out on a flat surface were measured using a tripod micrometer. Haugh units were calculated as follows according to the formula proposed by Cotta (1997):

$$HU = 100 \text{ Log } (h - 1.7\ w + 7.6),$$

Where, HU = Haugh units, h = Albumen height (mm) and w = Egg weight (g).

Yolk extraction and cholesterol determination:

At the same time of conducting egg quality traits, yolk of eggs was taken from each group alone for determination the cholesterol content (mg of cholesterol /g of egg yolk). For yolk fat extraction one gram of yolk was placed into a centrifuge tube, homogenized with 15 ml of polar solvents chloroform: methanol mixture, 2:1 (v/v), vortexed, filtered and evaporated according to Folch *et al.* (1957), as modified by Washburn and Nix (1974). Cholesterol concentration was determined by ultraviolet spectrophotometer using commercial kits according to the methods described by Kaya *et al.* (2001).

Immune response against Newcastle disease (ND) and total count of aerobic bacterial (CFU/g):

In order to measure the antibody titer response against Newcastle disease virus (NDV), blood samples were collected from jugular vein at different periods of egg production for Haemagglutination Inhibition (HI) test (OIE, 2012) to determine the antibody titer response. Titers were expressed as the $\log^2$ of the reciprocal of the highest dilution giving visible Haemagglutination. The immunoglobulin's G (IgG), M (IgM) and A (IgA) were determined according to Granfors (1979). To measure the total count of aerobic bacterial digesta from 21 birds (3 birds per treatment) were collected and used for microbial assays using spread plate technique method described by Quinn *et al.* (1994). Microbial level were expressed as colony forming unit (CFU g^{-1}) per gram of sample.

Data analysis:

Data analysis according to Snedecor and Cochran (1982) was performed using General Linear Models (GLM) procedure of SPSS software program package) Version 16 (SPSS, 2010). All data were analyzed based on a completely randomized design using one way ANOVA. All percentages were first transformed to arcsine to approximate normal distribution before ANOVA. Data were presented as means ± SEM. When the treatment effect was significant at $P \leq 0.05$, Duncan test was applied to identify significant differences among groups. All obtained data were analyzed by using the following model Equation;

$$Y_{ij} = u + S_i + e_{ij}$$

Where, Y_{ij} = is the analyzed measurement

u = Overall mean, S_i= Effect of experimental diets (i= 1 …to 7), e_{ij}= Experimental error.

Duncan's multiple range test was used to detect any significant differences among the experimental means (Duncan, 1955).

RESULTS

Proximate chemical analysis and bio-active components:

The results of proximate and phytochemical compositions of Anise and Caraway seeds powder are shown in Table 2. The result of proximate chemical analysis showed that the moisture content of Anise and Caraway were found to be 10.40 and 9.78 % respectively, where Anise seed recorded slightly higher value than those recorded for Caraway. However, Caraway had an appreciable higher values of crude protein (CP) content (20.03 %), ash (11.61 %), and crude fiber (20.16%) than those values recorded for Anise (17.40, 10.14 and 14.7%) respectively. While, Caraway had low content of ether extract (20.96%) than those recorded for Anise (27.09%). On the other hand, results presented in the same Table indicated that both Anise and Caraway have variable bio-active components. The Anise has higher content of bio-active components i.e., cannabinol, vinpocetine, resveratrol, a-tocopherol and coniferyl aldehyae than other bio-active constituents present in the Anise. However, Caraway contains higher value of bio-active constituents including coniferyl aldehyae, retinyl propionate, asterol, vitexin than those other active constituents observed in the Caraway.

Effects of anise and caraway supplementation on:

1-*Laying performance*:

Results of laying performance, as impacted by Anise and Caraway powder are clarified in Table 3. From the present results it is observed that at the end of the experiment (47 weeks of age), laying hens fed diets supplemented with 0.3 and 0.2% Caraway tended to have a higher body weight gain than those values recorded for other levels of both seeds or control group. However, egg production significantly ($P < 0.05$) improved for birds fed diet supplemented with 0.1% Caraway seed powder compared with other levels or control group. Also, egg weight, egg mass and feed intake showed significantly higher ($P < 0.05$) values for birds fed diets supplemented with different levels of both Anise and Caraway compared with the control group. On the other hand, the values of feed conversion ratio exhibited the best records for groups fed different levels of Anise and Caraway compared with the control group. From the results it is very interesting to note that dietary inclusion of Anise and Caraway caused a significant improvement in all laying performance along the experimental period.

2-*Blood biochemical parameters:*

The effect of Anise and Caraway seeds powder on blood biochemical parameters measured at the end of the experiment have been presented in Table 4. As can be seen, the addition of Anise and Caraway seeds powder significantly increased protein profile including total protein, albumin and globulin of the blood compared with the control group ($P<0.05$). While, the values of A/G ratio showed the converse trend, where the highest records observed for birds present in control group or those birds fed 0.1% Anise seed powder compared with other levels of both seeds. However, the Anise and Caraway supplementation in the diets led to a significant ($p<0.05$) decrease in lipid profile including total lipids, triglyceride and cholesterol concentration as compared with the control group. Clearly, data recorded of glucose concentration indicated that incorporation of Anise in laying hen diets at 0.3% showed the highest value; while groups fed 0.1 and 0.2 % Caraway have the lowest concentration compared with other groups. Concerning the values of total antioxidant capacity (TAC), the analysis of variance indicated that laying hens fed diet inclusion 0.3% Caraway showed high value , followed groups fed 0.1, 0.2 Caraway and 0.3, 0.2 and 0.1% Anise or control groups. Clearly observed from these data that birds fed diet supplemented with different levels of Caraway and Anise seeds powder positively decreased lipid profile.

3-*Egg quality traits and yolk cholesterol contents:*

The effect of dietary treatments on egg quality traits and yolk cholesterol contents are shown in Table 5. It is observed that incorporation different levels of Anise and Caraway seeds powder in laying hen diets had no significant effect on egg shape index and shell thickness. While, there was a significant ($P < 0.05$) differences observed among the experimental groups concerning albumin index, yolk index, Haugh unit and yolk color. The analysis of variance showed that yolk color pigmentation significantly ($P < 0.05$) improved due to incorporation of both Anise and Caraway seeds powder as compared with the control group. On the other hand, we find that the great effect of both seeds is the great ability to reduce the level of cholesterol concentrations in the egg yolk compared with the control group ($P < 0.05$), where the lowest levels detected observed for hens fed 0.3% Caraway seed powder.

4-*Immune response and total count aerobic bacteria*:

To evaluate the effects of dietary supplementation of Anise and Caraway seeds powder on the immune response and total count aerobic bacteria data presented in Table 6, the analysis of variance indicated that there was a

Table (6): Effect of supplementation Anise and Caraway seeds powder on immunity response (log ^{2} value) and total count aerobic bacteria (CFU/g) for whole experimental period.

Treatments	Items				
	Immunity response (HI Titer log^{-2} value)	Immunoglobulin for whole period (mg / dL)			Total count of aerobic bacteria (CFU/g)
		IgG	IgM	IgA	41-47
C (0.0%)	77.52^{c}	1.74^{d}	10.52^{e}	77.63^{e}	7.20^{a}
C+ 0.1 % ASP	8.19^{b}	1.92^{c}	10.81^{d}	80.36^{d}	6.27^{b}
C+ 0.2 % ASP	8.37^{b}	1.92^{c}	11.29^{c}	83.41^{c}	5.44^{c}
C+ 0.3% ASP	8.66^{a}	2.14^{b}	11.75^{b}	84.63^{b}	5.15^{c}
C+ 0.1% CSP	8.92^{a}	2.29^{a}	12.83^{a}	85.44^{ab}	5.18^{c}
C+ 0.2 % CSP	8.88^{a}	2.34^{a}	12.87^{a}	86.48^{a}	4.48^{d}
C+ 0.3% CSP	8.87^{a}	2.34^{a}	12.92^{a}	86.21^{a}	4.11^{d}
SEM*	**0.11**	**0.05**	**0.21**	**0.69**	**0.22**
P-value**	**268.886**	**47.539**	**311.715**	**80.961**	**20.229**
Sig. test	*	*	*	*	*

^{ab} Means with different superscripts within a column are significantly different at $P < 0.05$.
*SEM = Standard error of means, ** P –value= Significant effect, Sig. test= Significant test, * $P < 0.05$

gradual increase in serum antibody titer response, which was statistically significant ($P < 0.05$) measured at the end of experiment. Also, immunoglobulin classes (IgG, IgM and IgA) showed the same trend, where Anise and Caraway supplementation significantly ($P < 0.05$) increased the values of IgG, IgM and IgA recorded at the end of experiment. On the other hand, data revealed that total count f aerobic bacteria showed the converse opposite trend, where laying hens fed diet inclusion different levels of Anise and Caraway seed powered recorded lower value than those values observed for the control group ($P < 0.05$).

DISSCUSION

***Proximate chemical analysis and bio-active components*:**

Interestingly, the chemical composition is the first step to evaluate the nutritive and medicinal value for any feedstuff. The objective of this study was to investigate the use of Anise and Caraway seeds as a feed additive in laying chicken nutrition. The result of proximate analysis showed that the CP,

ash, CF, and DM contents of Caraway recorded slightly higher values than those recorded for Anise. While, Anise has higher content of EE and moisture contents than those values observed for Caraway. The variation in chemical analysis may be due to soil nutrients and environmental factors which have effects on the nutrients availabilities for plants (Adewole *et al.,* 2017). In this context, Kaki *et al.* (2018) noted that the proximate chemical analysis of crushed Caraway seed of DM, OM, CP, CF, EE, ash, NDF and ADF were 93.64, 83.84, 19.25, 28.70, 3.81, 11.69, 67.64 and 40.13 %, respectively. However, Zidan *et al.* (2019) found that the proximate chemical composition of star Anise (mg/g) was determined in terms of protein, fat, fiber, carbohydrates, ash, dry matter, where the contents were 6.41, 3.93, 27.74, 58.56, 3.36 and 86.65% respectively.

However, Phytochemicals or bio-active components are of benefit to health and play an active role in the management of some diseases. Therefore, phytochemicals referred to phytogenic, which are natural bioactive compounds that are derived from plants and incorporated into animal feed to enhance productivity (Gadde *et al.,* 2017). According to Franz *et al.* (2005) indicated that Anise seeds contain 2 - 6% essential oil and also phenolic acids, flavonol and flavone glycosides. The predominant constituent of the essential oil is trans-anethole (80 - 95% of total oil), a powerful flavouring which belongs to the phytoestrogens.

The present study indicates that Anise contains a number of bioactive compounds, more than detected in Caraway, where bioactive components including coniferyl aldehyae, a-tocopherol, resveratrol, vinpocetine and cannabinol are the most active substances in terms of their percentage. While, Caraway contains bio-active components i.e., isoretinene, asterol, and retinyl propionate with greater proportions than the rest of the other active components. In this context, Ciftci *et al*. (2005) indicated that Anise seeds have a number of active compounds, particularly volatile oil (1- 4%), which consists of largely trans-anethol (70- 90%) with estragole (methylchavicol), Anisealdehyde, bcaryophlline, Anise ketone (methyloxyphenylacetone) and the polymers of anethole. Also, Sedláková *et al.* (2003) showed that Caraway seeds contain 1–9% essential oils consisting of more than 30 compounds, where the carvone and limonene were account the main portions. In addition, Abou El-Soud *et al.* (2014) found that the essential oil compounds of Caraway were included (%) α-Pinene 0.3, Camphene0.2, β-Pinene 0.1, β-Myrcene 0.1, Limonene 5.1, γ-Terpinene 12.6, β-Ocimene 0.1, p-Cymene 0.1,Terpinolene 0.1, limonene oxide 0.1, Camphor 0.2,Linalool 0.7, Linalyl acetate 0.3, Terpinene-4-ol 0.1, β-Caryophyllene, Dihydrocarvone 0.2, α-Terpineol 0.1,Germacrene-D 0.1,

Carvone 70.1, β- Selinene 0.2, α-Farnesene 0.4, Citronellol 0.1, δ-Cadinene 0.3, γ-Cadinene 0.5, Cuminaldhyde 0.1, Nerol 0.2, Trans-carveol 0.1,Nonadecane 0.1, Spathulenol 0.3, Eugenol 0.2, Thymol 0.5and Carvacrol 0.2 . Also, Neveen *et al.* (2014) showed that the major components of Caraway essential oil were Carvone (70.1%) followed by γ –Terpinene (12.6%) and Limonene (5.5%) in addition to some minor compounds including Linalool (0.7%),Thymol (0.5%), γ –Cadinene (0.5%), α- Farnesene (0.4%), δ- Cadinene (0.4%).

***Effects of anise and caraway supplementation on*:**

1-*Laying performance:*

The present results of diet chemical analysis (Table 2) were formulated to cover the laying hens' diets (16.14% CP and 2751 kcal ME/kg DM feed) as reported by (NRC 1994). Data presented in this study showed that final body weight and change in weight (gain) significantly improved at the end of experiment by feeding diets supplemented with Anise and Caraway seeds powder. This improvement might be due to improved digestion and absorption of diet nutrients by increasing enzymes and saliva secretion and subsequently improved digestion processes (Suganya *et al.*, 2016).

Therefore, the active ingredient present in seeds has positive effects on nutrient digestibility and increasing activities of pancreatic lipase and amylase (Ramakrishna *et al.,* 2003). This observation is confirmed by Kassie (2008) found that the star Anise may be widely used for growth promoting in poultry as a result of stimulating digestion and antimicrobial effects. Also, Ciftci *et al.* (2005) found that Anise essential oil dietary supplementation at 400 mg/kg diet in broilers, resulted in significant better body weight gain. The result of this study demonstrated that egg production, egg weight and egg mass tended to be significantly increased, due to inclusion Anise and Caraway seeds powder. This improvement may attributed to the synergetic effect of bioactive chemical ingredients present in these seeds, which modulating gut microbiota, enhancing nutrient digestibility and absorption, and improving ovarian characteristics resulted in better health status and subsequent improved laying performance (Boka *et al.,* 2014). The result of Yu *et al.* (2018) demonstrated that egg mass, egg weight and feed intake increased by the addition of star Anise oil in the laying hens diets from 200 to 600 mg/kg. This finding is compatible with Yang *et al*. (2017) found that laying hens supplemented with star Anise, salvia miltiorrhiza and ginger root improved laying rate and egg mass of Hy-Line brown laying hens at 40 weeks of age. Also results of this study indicated that birds fed diets inclusion Anise and Caraway significantly improved feed intake and feed conversion ratio. The increased feed intake due to supplementation

Anise and Caraway is likely attribute to a stimulating effect of appetite as a result of aromatic flavour (Wang *et al*., 2011). In this connection, Al-Kassie (2008) showed that broilers fed on a diet containing 1% Anise had higher feed intake than those recorded for the control group. Also, Bayram *et al*. (2007) showed that feed intake and feed conversion ratio was improved for broiler chickens when fed diets supplemented with star Anise seed at 40 g/kg. In general, Windisch *et al.,* (2008) reported that feed additives derived from plants, which called phytogenics or phytobiotics can be included in animals' diets to improve their productivity and the properties of the resulting feed and animal products.

2- ***Biochemical blood parameters***:

Interestingly, biochemical blood parameters are usually related to health status, where these parameters are vital indicators of the nutritional and physiological status of birds. It is observed from obtained data that blood biochemistry profiles were within the expected range, and no signs of toxicity or diseases due to experimental diets. It is notice that blood protein profiles significantly affected by the dietary supplementation reflecting no injury of liver by the addition of both seeds. This effect may attributed to beneficial effects of bioactive components such as flavonoids, phenolic compounds and polyphenols present in both seeds that play a vital role in the preserving of liver cells, (Amit Roy *et al.,* 2014).

In addition, the increase of protein profile due to inclusion Anise and Caraway reflect better hepatoprotective activity of seeds may be owing to the free radical scavenging (antioxidant) properties of its components. It was noted from this study that the use of Anise or Caraway seed powder as additives in laying chicken diets is safe when used by 0.3% without any adverse side effects on liver function. In this context Soltan *et al*. (2008) found that Anise supplementation at 0.5 g/kg of broiler diet increased serum albumin, A/G ratio, while decreased globulin concentration. Also, Al-Shammari *et al.* (2017) found that when supplemented Anise seed powder at 0, 500, 750, and 1000mg/L to the drinking water in broiler significantly improved albumin and globulin compared with the control group. In another study, Jafari (2011) showed that using Caraway powder in diet of Japanese quail at 1.0%, 1.5% and 2.0 had a significant effect on blood biochemical parameters. On the other hand, blood lipids profile depend on the conditions of breeding, nutrition and sexual activity (Itoh *et al.*, 1998). Therefore, in the current study, the addition of Anise and Caraway powder have vital roles in reducing lipid profiles including total lipids, cholesterol and triglyceride compared with the control group. This may be attributed mainly to the inhibitory effects of phytogenic bioactive components on hepatic 3-hydroxy-3-

methylglutaryl coenzyme A reductase activity (a critical enzyme in cholesterol biosynthesis), thereby reducing cholesterol synthesis (Crowell, 1999) or by decreasing its fractional reabsorption from the small intestine (Brunton, 1999). Also, the reduction of blood cholesterol could be contributed in some cases to the reduction in some hormones secreted by the cortex of adrenal glands, which in turn causes the reduction in the secretion of fatty acids from adipose tissues or the reduction of fat oxidation that lead to the reduction of level of fatty acids including cholesterol and triglyceride (Ganong, 2005). This finding is consistent with Christaki *et al.* (2011) showed that total cholesterol and triglycerides considerably lowered in Japanese quails when fed diets supplemented with ground Anise seed at 10 g/kg and 20 g/kg. Also, Khajeali *et al.* (2013) indicated that adding 1, 1.5 and 2% of Caraway to broiler diets led to a decrease of triglyceride levels in birds compared to the control group. Also, Ali *et al.* (2007) reported that the addition of Anise in laying gen diet decreased the levels of serum triglyceride and total lipids.

However, results showed that total glucose recorded the highest value for birds fed diets containing 0.3 Anise seed powder, while the lowest values observed for birds fed diets inclusion 0.1 and 0.2 % Caraway. This finding are disagree with results obtained by Tabanca *et al.* (2003) found that Anise supplementation at 0.25 and 0.5 g/kg of broiler diet reduced serum levels of glucose when compared with the control group. On the other hand, Kucukkurt *et al.* (2009) indicated that plasma glucose of laying quails insignificantly affected when fed diet inclusion Anise seeds at 0, 10, 20, 30, 40, and 50 g/kg. However, the statistical analysis showed that the inclusion of both Anise and Caraway seeds powder in diets are beneficial in increasing total antioxidant capacity (TAC) especially with group fed diet inclusion 0.3% Caraway, this may attributed to the ability of Caraway to exhibited high antioxidant activity which has been attributed largely to the presence of monoterpene alcohols, linalool, carvacrol, carvone, flavonoids and other polyphenolic compounds (Najda *et al.*, 2008), and neutralize free radicals which reduce plasma malondialdehyde. In addition, Anise seed possess a potent antioxidant activity that may be attributed to many polyphenol compounds have been detected in extracts from different Anise species and anethole (Kang *et al*., 2013). This finding is agree with Hoda *et al.* (2015) showed that the activity of MDA was decreased, and the activities of SOD, GSH-Px, and CAT were significantly high in the rat groups administered black Caraway oil 5, 10, and 20 mg kg−1 body weight, respectively. Also, Ding *et al.* (2017) found that supplementation of star Anise and its essential oil increased activities of super

dismutase, glutathione peroxidase, and catalase but decreased content of malondialdehyde in the serum of broilers at 21 d and 42 d of age of broiler.

3- ***Egg quality traits and yolk cholesterol contents.***

The improvement in egg quality is of paramount importance in the field of production and management (Mahmoud *et al.,* 2010). Therefore, there is a dearth of literature available concerning the beneficial effects of Anise or Caraway addition on egg quality traits of laying diets. It is cleared that, egg weight, yolk color, albumin index, and Haugh unit significantly improved by the inclusion of Anise and Caraway in laying diets. Interestingly, the increased of egg yolk colour in this study indicated more pigmentation of birds fed diets inclusion Anise and Caraway.

The enhanced pigmentation by Anise and Caraway supplementation is likely attributed to natural pigments in Anise and Caraway that can be absorbed and transferred into the egg yolk and to consumption of zeaxanthin, lutein, alpha-carotene, beta-carotene, and carotenoids present in seeds (Hammershoj *et al.*, 2010). In addition, it is well recognised that egg yolk colour is important to consumers and is one of the main parameters by which the quality of an egg is judged (Hammershoj and Steenfeldt, 2005). In this connection, Christaki *et al.* (2011) found that the egg yolk colour parameter was significantly increased by the dietary addition of Anise seed at 10 g/kg or 20 g/kg but did not modify the L* (lightness) and b* (yellowness) parameter of egg yolk colour of laying Japanese quail. On the other hand, egg yolk cholesterol content was tended to decrease significantly decreased due to feeding hens on diets containing different levels of Anise and Caraway compared with the control group. The decrease in cholesterol content, this may be related to the active components (thyme and carvacrol) present in seeds, where these components reduce the liver enzyme activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which is a key enzyme in cholesterol synthesis (Abdulkarimi *et al.*, 2011). Also the decrease of cholesterol content of eggs for birds fed diets inclusion Caraway and Anise may be due to the reduction of serum total cholesterol and triglycerides concentration in blood compared to the control group (Jafari, 2011).

4- ***Immunity response and total count aerobic bacteria.***

In the poultry industry, it is important to stimulate the immune system to reduce or prevent infectious diseases. Many factors like failure of vaccination and inhibition of antibiotics can induce immunodeficiency. Therefore, the usage of immune enhancers is a key solution to improving immunity and reducing

susceptibility to infectious diseases in poultry farms. The precise mechanism of phytogenic on immunomodulation is still not fully clear. The immune response was mediated by employing HI test to detect the antibody titer against New Castle Disease are presented in Table 6. It is observed the addition of Anise and Caraway to layer diets may improve the immune system due to the increase in immunoglobulin concentrations (IgG, IgA and IgM). It has been reported that under the practical conditions, it is quite common to vaccinate layer chicken at different ages against ND to provide the flocks by protective immunity as maternal immunity wears. Clearly, the results obtained from the study can explain the nutritional and biological effects of examined treatments on immune response. Compared with the control group the values of IgG, IgM and IgA were elevated in birds fed diets supplemented with Anise and Caraway seeds powder. It is interesting to note that Caraway seed which is richness in polyunsaturated fatty acids, which help to produce prostaglandin E1. Prostaglandin E1 has so many functions such as in relation to the immune system.

This improvement in immunity may be due to Anise and Caraway are rich in flavonoids act as antioxidants and may enhance immune function (Acamovic and Brooker, 2005). In addition phytochemicals also exert their action through immunomodulatory effects such as increased proliferation of immune cells, modulation of cytokines, and increased antibody titers (Lee *et al*., 2017). Also, it is observed that many different active components present in these seeds has significantly enhanced immune response. Theses findings are consistent with Al-hajj *et al.* (2015) reported that inclusion of 6 g of Anise seed in broiler diet diet showed higher antibody titer against Newcastle disease virus and infectious bronchitis. Also, Khajeali *et al*. (2012) showed that antibodies titers against New Castle Vaccine was significantly increased when broilers was fed with a diet contains high level of Caraway. Also, Mahmood *et al*. (2014) reported that Aniseed addition to basal diet at the rate of 0.5 g/kg and 1.0 g/kg of feed had best immunomodulatory activity both for humoral and cellular immune response. In this regard, Al-Beitawi *et al.* (2009) found that the use of Anise seeds in broiler chickens resulted in improved antibody titers against NDV.

However, results concerning total count of aerobic bacteria indicated that there was a significant decrease in values for birds fed diet inclusion different levels of Anise and Caraway compared with the control group. This attributed to the appropriate amount of cumin aldehyde can explain the antibacterial effects for Caraway. In addition active components such as pinene and sabinene present in Caraway has antibacterial effects (Gachkar et *al.*, 2007). In addition, the antimicrobial activity of Anise may be attributed to their phenolic contents since

numerous phytochemical studies indicated the presence of noticeable amounts of phenolic compounds in Anise is considered a natural anti-bacterial (Martins *et al.*, 2016). In this context Proestos *et al.* (2006) found phenolic compounds present in Anise act as antimicrobial agents via several mechanisms including the disruption of microbial membranes. This could be considered another reason of improving the performance and immune status of the birds. This finding can be supported by the work done of Lillehoj *et al.* (2011) demonstrated that many of the health-promoting activities of phytochemicals are mediated through their ability to enhance host defense against microbial infections. In general, many studies indicate that the main important function of the active substances in caraway is act as an antioxidant. Thus, it acts as an inhibitor of free radicals and protect tissues from damage (Crowell, 1999).

In conclusion, from these results, it could be concluded that Anise and Caraway seeds powder in the diet of laying hens as a safe and effective feed additive to improve laying performance and reduce total blood lipids and thus reduce cholesterol content in egg yolk.

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الأداء الانتاجى، الحالة الصحية وبعض الصفات الفسيولوجية لدجاج المندره المتأثر بإضافة مستويات مختلفة من مسحوق بذور اليانسون والكراويه

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أجريت هذه الدراسة لتقييم تأثير اضافه مستويات مختلفة من مسحوق بذور الكراوية واليانسون على الاداء الانتاجى ، بعض مكونات الدم ، جودة البيض ، محتوى الكوليسترول فى صفار البيضه ، الاستجابة المناعية ، العدد الميكروبي للبكتريا الهوائيه لدجاج المندره. لهذا الغرض تم استخدام عدد 140 من دجاج المندره عند عمر 26 أسبوعًا من العمر حيث تم تقسيم الطيور عشوائيا الى7 مجموعات متساوية وتم تمثيل كل مجموعه باربع مكررات يحيث احتوت كل مكرره على خمس دجاجات. تم وضع كل دجاجه في قفص فردي (بمساحة 510 سم 2 / دجاجة). تم تكوين عليقه قاعديه وتم اضافه مسحوق الينسون اليها بمستويات 0.1 ، 0.2 ، 0.3٪ وكذلك مسحوق الكراويه بمستويات 0.1 ، 0.2 ، 0.3٪ على الترتيب. كانت جميع العلائق متماثله فى الطاقه (2751 كيلو كالورى /طاقه ممثله/كجم) ، والبروتين (16.14% بروتين خام).

أظهرت النتائج أن الاداء الانتاجى تحسن معنويا عند اضافه مسحوق اليانسون والكراويه مقارنة بمجموعة الكنترول. كما لوحظ بوضوح أن مستوى الدهون الكليه في الدم انخفض معنويا عند اضافه مسحوق اليانسون والكراويه مقارنة بمجموعة الكنترول. وفي نفس الاتجاه انخفض معنويا محتوى كوليسترول الصفار فى البيض وكوليسترول الدم عند اضافه مسحوق اليانسون والكراويه مقارنة بمجموعة الكنترول.

كما تحسنت الاستجابة المناعية ، والسعه الكليه لمضادات الاكسده عند اضافه مسحوق اليانسون والكراويه مقارنة بمجموعة الكنترول. كما انخفض العد البكتيري الكلي للبكتيريا الهوائية للطيور المغذاه على العلائق المحتويه على مسحوق اليانسون والكراوية مقارنة بمجموعة الكنترول.

التوصية:

يمكن اضافه مسحوق بذور اليانسون والكراوية كمضافات غذائيه امنه وفعاله فى علائق الدجاج البياض لتحسين الأداء الانتاجى وتقليل محتوى الدهون في الدم وبالتالي تقليل محتوى الكوليسترول في صفار البيض فى الدجاج البياض.