J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian
kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
48(2):101-112, June 2023
DOI: 10.14710/jitaa.48.2. 101-112

Evaluation of *Ruta graveolen*, coriander and basil seed extracts as natural additives on productive performance, blood profiles and egg quality of White Leghorn layers

S. Tessema[1], M. Girma[1], M. Yirgalem[3*], N. Ameha[1], and T. Zeryehun[2]
[1] School of Animal and Range Sciences, Haramaya University, P O Box 138, Dire-Dawa, Ethiopia
[2] College of Veterinary Medicine, Haramaya University, PO Box 138, Dire Dawa, Ethiopia
[3] Department of Animal Sciences, Wolkite University, P.O.Box 07, Wolkite, Ethiopia
**Corresponding E-mail: ymetages257@gmail.com*

Received March 14, 2023; Accepted May 12, 2023

ABSTRACT

The study was conducted to investigate the effect of mixtures of *Ruta graveolen* (Rg), coriander (C), and basil (B) seed extracts on productive performance, blood profiles, egg quality and egg chemical composition of White Leghorn (WL) layers. One hundred eighty WL layers at twenty- six weeks of age were randomly distributed to four treatments, each replicated three times with fifteen layers per replications in a completely randomized design and kept on a deep litter system for eight weeks. The treatments (T) were water containing mixtures of *Ruta graveolen*, coriander, and basil seed extract with the proportion of 0 ml (T1), 2 ml (T2), 4ml (T3), and 6 ml (T4) per liter of water. The hen day egg production (HDEP) was significantly (P<0.05) higher for layers in T3 (50.75%). Serum cholesterol and LDL (low-density lipoprotein) cholesterol levels decreased significantly while, HDL (high-density lipoprotein) cholesterol and globulin increased significantly (P<0.01) when compared with the control. There were no significant differences (P>0.05) among treatments in egg weight, albumen weight, yolk weight, shell weight and thickness. Generally; the mixtures of Rg, C, and B seed extracts in 4ml/1lt in drinking water could increase HDEP and egg quality and reduce serum cholesterol.
Keywords: Basil seed, Coriander seed, Layer performance, Ruta graveolen seed, White Leghorn

INTRODUCTION

Antibiotic growth promoters have undoubtedly improved animal performance and health status However, the inclusion of antibiotics as a principal growth promoter pose a serious health problem for consumer often resulted in the incidence of antibiotic resistance among pathogens and a source of residues in animal body tissues (Abd El-Hack *et al*., 2022). Hence, the European Union banned the use of antibiotics as a growth promoter in animal feeds. As a result, scientists searched for locally available phytogenic feed additives intended to improve gut health and functions by fighting pathogenic bacterial infection (Sapsuha *et al*., 2021; Abd El-Hack *et al*., 2022). Various types of phytogenic products– such as, garlic, anise, cinnamon, coriander, oregano, chili, basil, pepper, rosemary, rosehip and thyme – may be used as additives to enhance performance and modulate gut health in poultry (Criste *et al*., 2017). Medicinal plant extracts that were used as natural antimicrobials have a positive effect on performance parameters, including egg production, egg quality (Khan *et al*., 2012), the immune system and antioxidant status (Abd El-Hack and Alagawany, 2015). In addition, the extract improved performance by increasing the digestibility and retention of nutrients, increasing the secretion of digestive enzymes and mucous production, and improving gut health status and microbial population to maintain production performance during heat stress (Criste *et al*., 2017).

The seed of *Ruta graveolen* is a rich source of secondary metabolites mainly: coumarins, alkaloids, volatile oils, flavonoids, and phenolic acids. It has been used to reduce inflammation abundantly worldwide due to its diverse medicinal properties or presence of essential oil obtained from this plant species have been shown to possess various pharmacological activities, such as antioxidant, anti-inflammatory, spasmolytic, sedative, antibacterial, antifungal, and antidiabetic effects (Szewczyk *et al*., 2023). Furthermore, it has anti-rheumatic, anti-diarrheic, anti-febrile, antiulcer, and antimicrobial properties reported in the recent pharmacological trials (Jianu *et al*., 2021).

The seeds of coriander (*Coriandrum sativum*) contain 0.5% - 1.0% essential oil rich in beneficial phytonutrients including flavonoids, carvone, geraniol, limonene, borneol, camphor, lemon, and linalool. Flavonoids compound in coriander includes phenolic acid; it has been used to treat infections with worms due to its presence of anti-parasitic and antifungal (Matasyoh *et al*., 2009). Similarly, the seeds of basil *(Ocimum basilicum)* have biologically active compounds including ursolic acid, apigenin, and luteolin that activate the cell-mediated immune response and stimulate appetite responses and counterattack the deterioration of feed due to the presence of antioxidant (Shahrajabian *et al*., 2020). Generally, as different researcher explained the use of medicinal plant extracts in poultry feeding have a positive effect on animal health, productivity, appetite stimulation, enhancement of enzyme activity and secretion related to diet digestion and absorption such as trypsin, amylase and jejunal chime (Windisch *et al*., 2008; Ghazaghi *et al*., 2014). In layer chickens, several studies indicated the possible beneficial effects of plant oils on egg production performance, improving egg quality and also supporting health status as a single compound or as mixed preparations (Al-Shaheen *et al*., 2023). According to Hadi and Jassim (2013) study, adding 1.5 g/kg of basil leaves powder to the diet of growing quail resulted in better body weights, less serum cholesterol and increase levels of serum total proteins and globulins from 1st week up to 6th week of age compared to the control group. Basil seeds at 0.3% and 0.6% level also led to enhance the blood biochemical parameters and health status of broilers (Kadhim, 2016). Similarly, Supplementation of coriander seeds 1%-3% significantly increased yellowness in yolk color without affecting other quality parameters (Habiyah *et al*., 2016). On the other hand, synergistic effects of phytogenic compounds have been reported in studies with essential oils and a combination of herbal powders might tend to be more effective than a single herb administration (Khaligh *et al*., 2011). However, there is limited information on the utilization of mixtures of *Ruta graveolen*, coriander, and basil seed extracts in layers feeding. Therefore, this study was designed to evaluate the effect of mixtures of *Ruta graveolen*, coriander, and basil seed extracts on productive performance, blood profiles, egg quality, egg chemical composition, and economic benefit of WL layers.

MATERIALS AND METHODS

Study Area

The study was conducted at Haramaya University poultry farm, which is located 505 km from Addis Ababa, capital city of Ethiopia. The site is situated at an altitude of 1980 meters above sea level, 9 ^{0} 26 ' N latitude, and 42 ^{0} 3' E longitude. The area has an average annual rainfall of 741.6 millimeters the mean annual minimum and maximum temperatures are 8.25 ^{0} C and 23.4 ^{0} C, respectively (Mishra *et al*., 2004).

Collection and Preparation of Seed Extracts

Ruta graveolen (Rg), coriander (C), and basil (B) seeds were purchased from Harar market and the dried seeds were pulverized at Haramaya University's feed processing plant. The powder was preserved in an airtight plastic container until it was directly used for the preparation of water extract. Then 20 g Rg + 20 g C+ 20g B (a total of 60 g of the three seeds powder were added to 1L of distilled water and was shaken and infused overnight at room temperature, then filtered for all experimental days according to the protocol described by Mollah *et al.* (2012).\

Management of Experimental Chickens

A total of 180 White Leghorn layers were randomly selected for the study from Haramaya University Poultry farm. The birds were weighed and randomly distributed into four treatments, each treatment being replicated three times. Each replicate was kept in 2x2 m pens wire-mesh partition on deep litter housing covered with a wheat straw. Before the announcement of the actual experiment, watering, feeding troughs, and laying nests were thoroughly cleaned and disinfected. The experimental pen was sprayed against ecto-parasites. The birds were acclimated to rations for 7 days and then fed for 60 days for evaluation of egg parameters. During the experiment, feed was offered *ad libitum* and water was available all the time throughout the experimental period after they had finished *Ruta graveolen*, coriander and basil infusion offered in drinking water daily at 6:30 hours.

Experimental Design and Treatments

A completely randomized design with three replications was used for each treatment. Each treatment was replicated three times with fifteen layers (Table 1). Using commercial layer ration of the nutrient requirements of 2800-2900 kcal ME/kg DM and 16-17% CP (NRC, 1996).

Measurements and Observations

Chemical analysis. Representative samples of 20g of *Ruta graveolen*, 20g of coriander, and 20g of basil seed powder were further analyzed before starting the experiment for chemical composition. According to the proximate analysis method AOAC (1996) chemical analysis of experimental feeds were carried out for dry matter (DM), ether extract (EE), crude fiber (CF), ash, and Nitrogen (N) content was determined by Kjeldahl procedure and crude protein (CP) was calculated as Nx6.25. The analysis was conducted in the animal nutrition laboratory of Haramaya University. Metabolizable energy (ME) of the experimental diets was determined by indirect method according to the formula given by Wiseman (1987) as follows. ME (Kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 Ash.

Feed intake. Feed was weighed and offered twice daily at 8:00 and 17:00 hours. The orts were collected the next morning and weighed after removing external contaminants by visual inspection and handpicking. The feed offers and refusal were recorded for each replicate. Feed intake was determined as the difference between the feed offered and refused.

$$\text{Feed Intake} = \frac{(\text{Feed offered} - \text{Feed refused})}{\text{No of birds}}.$$

Body weight. The initial body weight of laying hens was individually measured and the final body weight was recorded at the end of the experimental study using sensitive balance to determine the body weight change and the mean value of the pen was taken. Bodyweight change per pen per bird was determined as the difference between the final and initial body weight. Average daily gain or loss was calculated as BW change divided by the number of experimental days.

Egg production. Eggs were collected twice a day from each pen at 10:00 and 15:00 hours. The sum of the two collections was recorded as daily egg production. The rate of lay for each replicate was expressed as the average percentage of hen-day egg production following the method of (Hunton, 1995).

% Hen egg production =

$$\frac{No\ of\ egg\ collected\ per\ day}{No\ of\ hens\ present\ that\ day} X\ 100$$

Egg weight and egg mass. Daily collected eggs immediately after collection were individually weighed for each replication and average egg weight was computed by dividing the total sum egg weight by the total number of eggs. After the mean weight has been determined, the following formula was employed to calculate the egg mass per pen on daily basis (North and Bell, 1984).

Egg mass (g/hen/day) =

$$\frac{Number\ of\ egg\ per\ day\ X\ average\ weight\ of\ egg\ per\ replicate}{Number\ of\ hens\ per\ replicate}$$

Feed conversion ratio. The feed conversion ratio was calculated as the ratio of grams of feed to grams of egg mass according to the following formula.

$$FCR = \frac{\text{Feed consumed (gram / hen / day)}}{\text{Egg mass (gram / hen / day)}}$$

Egg quality parameters. Egg quality was assessed in terms of egg weight, albumen quality (albumen height and albumen weight), yolk quality (yolk height, yolk weight, yolk diameter, yolk color, and yolk index), external quality of shell weight and thickness, and Haugh Unit Score (HUS). For internal egg quality measurement, (15 eggs per treatment and 5 eggs per replication) were taken randomly and the average was computed for each quality parameter once every two week.

Albumen height and (Haugh Unit). The sample eggs were individually weighed, coded, and broken on a flat tray, the height of the thick albumen of each egg was measured with a tripod micrometer, and the average Haugh Unit value for each replicate was calculated by using the formula given by (Haugh, 1937).

Haugh Unit (HU) =100 x log (H-1.7W$^{0.37}$+7.6)

Where, H= albumen height (mm); W= weight of egg (g)

Eggshell weight and thickness. The shells of the broken eggs were separated from their shell membrane, air-dried, and weighed. The measurement of shell thickness was carried out with a micrometer gauge having a sensitivity of 0.001 mm, at three points of the eggshell (air cell, equator, and sharp end).

Yolk quality evaluation. The yolk of each of the sample eggs was separated from the white and yolk height and the diameter was measured using a tripod micrometer and ruler respectively. Then, the yolk was thoroughly mixed and a sample droplet from each egg was placed on a piece of white paper. The Roche color fan consisting of a series of fifteen colored plastic strips were used as a reference to determine yolk color, with 1 rated as very pale yellow and 15 as deep reddish-orange. The mean for each replicate was calculated by taking the average reading from the three sample eggs. Yolk index was also computed using the following formula:

Yolk index = (yolk height) / (yolk diameter) x 100

Hematological and serum biochemical parameters. At the end of the experiment, blood samples (5 ml each) were collected from the wing vein of 12 hens from each treatment. Hematological and serum biochemical analysis was conducted at Haramaya University veterinary physiology laboratory and Higher clinic laboratory, respectively. For, the blood analysis, 2.5 ml of blood was collected using EDTA (Ethylene Diamine tetra acetic acid) tube while the remaining 2.5 ml was collected in a plain tube and left to coagulate. Blood samples were analyzed for total red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), total protein (TP), and serum cholesterol

Table 1. Experimental layout

Treatments		Number of replications	Layer/replication
T1	0 ml Infusion of Rg, C, and B/L water	3	15
T2	2 ml Infusion of Rg, C, and B/L water	3	15
T3	4 ml Infusion of Rg, C, and B/L water	3	15
T4	6 ml Infusion of Rg, C, and B/L water	3	15
	Total	12	60

Rg=*Ruta graveolen*, C= coriander B= basil and T=treatment and ml=milli liter, L=litter

Table 2. Effect of mixtures of ruta graveleon, coriander, and basil seed extracts on bodyweight gain, egg production performances

Parameters	Treatments				SEM	SL
	T_1	T_2	T_3	T_4		
Feed Intake (g/hen/day)	99.77^{b}	100.53^{ab}	103.23^{a}	98.53^{b}	0.9647	*
Initial BW (g)	1133.29	1169.98	1186.16	1178.1	15.741	NS
Final BW (g)	1244.4^{b}	1292.2^{b}	1360.6^{a}	1304.7^{ab}	20.378	*
Body wt. change	111.11^{b}	122.22^{b}	174.44^{a}	126.89^{b}	9.9355	**
BW gain(g/hen/day)	1.85^{b}	2.04^{b}	2.91^{a}	2.11^{b}	0.1656	**
Total egg/bird(No)	27.13^{b}	29.07^{ab}	30.44^{a}	27.82^{b}	0.6390	*
HDEP (%)	45.23^{c}	48.42^{ab}	50.75^{a}	46.37^{bc}	0.9302	*
Egg weight(g)	50.77	52.54	54.10	51.78	0.9963	NS
EM (g/hen/day)	22.97^{b}	25.44^{ab}	27.48^{a}	24.01^{b}	0.8391	*
FCR(egg bases)	4.34^{a}	3.96^{ab}	3.77^{b}	4.10^{ab}	0.1178	*

$^{a, b, c,}$ row means with different superscripts are significantly different,**=p<0.01, *= p<0.05, SL = Significant level, SEM = Standard error of mean, g = gram, BW = Body weight, HDEP = Hen day egg production, FCR = Feed conversion ratio, EM = egg mass

concentration. RBC and WBC were determined by using an improved Neubauer hemocytometer chamber (Dacie and Lewis, 1991). Hemoglobin concentration was determined by using acid hematin or Sahli's methods. The packed cell volume (PCV) by microhematocrit (capillary) tubes method and centrifuged at 3000 rpm for 5 minutes. Finally, Serum was harvested from blood collected in a plain tube which was transferred to an Endorphin tube and stored at -20c^{o} and analyzed for serum chemistry parameters (serum total protein and albumin, total cholesterol count, HDL-C, and LDL-C) with an automated chemistry analyzer (Douglas *et al*., 2010). The globulin value was determined by the difference between serum total protein and albumin (Doumas *et al*., 1981).

Egg chemical composition. A total of 48, twelve eggs per treatment (four per replication) were taken for proximate analysis. The egg yolk and albumen were separated carefully and then each component was mixed thoroughly and poured into the pan and covered with aluminum foliate. The egg yolk and albumen collected were heated in an oven at 55^{o}C for about 72 hours for partial drying. The sample was ground, homogenized, packed, and stored for further proximate analysis. The partially dried samples were weighed and dried in an oven at 105^{o}C for about 12 hours for the determination of the dry matter. The ash, protein, and lipid content of the egg white and yolk were analyzed following the AOAC methods (AOAC, 2000). Egg nitrogen content was determined by the Kjeldahl method and crude protein was calculated using the formula; egg nitrogen × 6.25.

Statistical Analysis

The experimental design used in this study was a completely randomized design. The data collected during the period of the study were subjected to analysis of variance using (SAS 2009 version 9.4) computer software. When the analysis of variance indicates the existence of a significant difference between treatment means, then the least significant difference method was used to locate the treatment means that are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Feed Intake, Egg Production and Egg Mass

Feed intake (FI) was significantly differ ($P<0.05$) among the different levels of mixtures of *Ruta graveolen*, coriander, and basil seed extracts in drinking water. Higher feed intake was recorded in T3 followed by T2, T1, and, T4 (Table 2). The improvement in feed intake with the addition of polyherbal could be due to essential oils and their main component, which stimulated the appetizing and digestive process in animals. Mudalal *et al.* (2021) reported that medicinal plant extracts have appetite and digestion-stimulating properties and antimicrobial and antioxidant effects. The possible reason for lower feed intake in T4 as compared to T2 and T3 might be due to the high concentration of essential oils which is toxic when used at higher doses (Lee *et al.,* 2004). Feed intake and water intake have positive relationship (Leeson and Summers, 2005). Plant extract that was given in drinking water can affect the feed intake of the animal. As Sigolo *et al*., (2021) indicated, different plant extracts were delivered to broiler in drinking water and they had a significant impact on the feed intake of the animal. Body weight gain and egg production in T4 were not significantly different compared to control that could be the feed intake similarity between these treatments (Yaman *et al*., 2020).

Egg production of White Leghorn layers was significantly higher ($P<0.05$) in T3 than in T2 and T1. T4 was numerically higher than in the T1 (control) but, it did not indicate any statistical difference ($P>0.05$) (Table 2 and Figure 1). The result in this study is in line with the study of Ooi *et al*. (2018) who reported dietary local medicinal herbs as feed additives on production performance and fecal parameters in laying hens level of 1% was effective to increase egg production. Similarly better egg production was obtained by using supplementation of medicinal polyherbal extracts supplements at a level of 1%-2% in the production and egg quality of laying Japanese quail hens (Zeweil *et al*., 2006). The exact mechanism through which egg laying performance enhanced is not known. However, according to Zhao *et al*. (2011) the higher egg production performance of the laying hens may be due to antioxidant, antimicrobial and other activities such as increased blood circulation and secretion of digestive enzymes and reduction in the oxidation of feed due to the bioactive components in the plant. In addition the bioactive components play a vital role in the digestion and absorption of nutrients that might have improved the performance parameters of laying hens

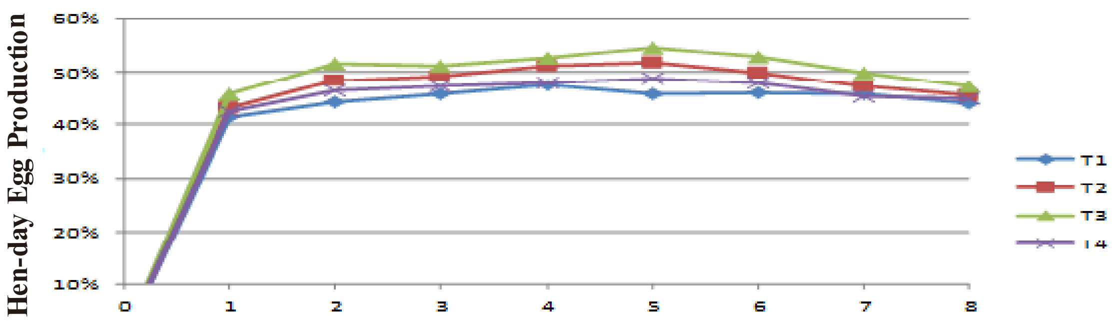

Figure 1. Weekly average hen-day egg production of White Leghorn chick administerd on mixtures of Rg, B and C seed extract via drinking water

Table 3. Effect of mixtures of ruta graveleon, coriander, and basil seed extracts on egg quality measurements of White Leghorn layers.

Parameters	Treatments				SEM	SL
	T1	T2	T3	T4		
Albumen weight (g)	30.40	30.00	26.80	29.90	1.30	NS
Albumen height (mm)	6.50	6.40	5.70	6.40	1.29	NS
Haugh unit	90.76	90.09	87.75	89.86	2.15	NS
Yolk weight (g)	15.49	16.97	15.36	16.81	0.45	NS
Yolk height (mm)	15.87	16.13	15.71	15.90	0.165	NS
Yolk diameter (cm)	4.12	4.14	4.02	4.10	0.054	NS
Yolk index	0.38	0.39	0.39	0.39	0.001	NS
Yolk color (*RSP)	3.63^{c}	4.13^{ab}	4.45^{a}	4.00^{bc}	0.137	*
Shell thickness (µm)	0.334	0.307	0.335	0.328	0.003	NS
Shell weight (g)	5.471	4.966	4.971	5.056	0.193	NS

$^{a,\ b,\ c,}$ row means with different superscripts are significantly different, *= P<0.05, T1 = 0ml of Rg,C and B seed extracts (Control), T2 = 2ml of Rg,C and B seed extracts ,T3=4ml of Rg,C and B seed extracts,T4=6ml of Rg,C and B seed extracts, g = gram, cm = centimeter, RSP =Roche Scale Point SL = Significant level, SEM = Standard error of mean, Rg=Ruta graveleon,C=Coriander, B=Basil

Table 4. Effect of mixtures of ruta graveleon, coriander, and basil seed extracts in drinking water on egg chemical composition of White Leghorn layers.

Albumen	T1	T2	T3	T4	SEM	SL
Moisture (%)	87.71	87.67	86.84	85.45	0.6807	NS
Protein (%DM)	62.19^{b}	63.15^{b}	64.46^{b}	69.78^{a}	1.2393	*
Lipid(%DM)	0.81	0.62	0.58	0.93	0.1793	NS
Ash(%DM)	3.73	3.65	4.11	3.01	0.6142	NS
Yolk						
Moisture (%)	93.89	94.82	94.55	93.36	0.7774	NS
Protein (%DM)	15.75	20.80	15.41	17.99	1.9677	NS
Lipid(%DM)	49.19	52.52	50.86	55.01	1.2356	NS
Ash(%DM)	5.92	5.21	4.44	4.82	0.7059	NS

$^{a\ and\ b,}$ row means with different superscripts are significantly different *= P<0.05, SL = Significant level, SEM = Standard error of the mean.

(Windisch *et al*., 2008).

The egg mass of layers in T3 was higher (P<0.05) than that of layers in T2, T4, and control group. Similarly, Guler *et al.* (2006) reported that significant (P<0.05) increase in the highest egg mass were in 1% and 2% coriander seed extracts on egg production performance and nutrient retention in laying Japanese Quails. These results are in contrast with the findings of Ooi *et al*. (2018) who reported dietary local medicinal herbs as feed additives on production performance in laying hens level of 1% was effective to increase egg weight but no significant change in egg mass. A possible reason for higher egg mass might be due to positive influence of the extract on the conversion of digested feed into eggs.

Yolk Color

A high yolk color score was recorded in T3, followed by T2, T4, and T1. But yolj color score of T3 was statistically similar to T2 (Table 3). Likewise, Kazem (2013) stated that dietary supplementation of 1%-3% medicinal herbs on diets including 2% medicinal herbs increased egg yolk color. In addition, Kopsell *et al*. (2005) explained that, basil was shown to rank highest among spices and herbal crops for carotenoids. Similarly, supplementation of coriander seeds into the feed was able to increase the absorption of beta-carotene contained in the feed (Habiyah *et al*., 2016). So, the changes in color values, i.e., the increase in yolk color fun (YCF) score from the experimental treatments in the present study are consistent with the changes in the content of carotenoid fractions in the diets (Kljak *et al*., 2021).

As Hernandez *et al*. (2014) reported the carotenoid in such medicinal herbs supported the high yolk color score since it had the same function as xanthophyll.

Albumen Weight, Eggshell, Yolk Weight, Yolk Height, Yolk Index and Yolk Diameter

The result of this study revealed that eggshell, albumen, yolk weight, yolk height, yolk index, and yolk diameter did not show significant difference (p>0.05) among the different treatments (Table 3). Likewise, Habiyah *et al*. (2001), reported no significant (P>0.05) difference in the shell, albumen, and yolk weight by supplementation of the deferent ratio of coriander seed extracts in Lohmann Brown. The current study disagrees with the study of Guler *et al*. (2006) who reported that significantly (P < 0.05) increase in the highest yolk and albumen weights were in 1% and 2% coriander seed extracts on egg production performance and nutrient retention in Laying Japanese Quails**.** .

Egg Yolk and Albumen Chemical Composition

There were no significant (P>0.05) differences in egg yolk and albumen chemical compositions across all the treatments combination except for albumen protein content (Table 4). Higher (P<0.05) albumen protein content was recorded in T4 than those in T3, T2, and T1. But, T3, T2, and T1 were statistically similar. The increase in albumen protein might be the synergetic effect of the active compounds in polyherbal seed (furanocoumarins, flavonoids, and furoquinolines) which have strong, antioxidant, anti-inflammatory, and anti-helminthic properties maybe reduce protein oxidation (Brenes and Roura, 2010). Besides, Rg, C, and B mixtures might have abundant photolytic enzymes, which promote protein digestion, deposition, and improve the transportation of metabolic protein in birds (Wasiyati *et al*., 2018).

Blood Analysis

The total serum cholesterol concentration was significantly lower (P < 0.01) in T4 compared with control, T2, and T3. As supplementation of Rg, C, and B seed extracts increased, serum HDL-cholesterol significantly (P < 0.01) increased from 47.57 mg/dl in T1 to 55.95 mg/dl in T_4. In the present study, the serum LDL-cholesterol concentration decreased significantly (P <0.05) from 101.65 mg/dl in T1 and 96.75 mg/dl in T4 (Table 5). The finding in the present study was in line with Khubeiz and Shirif (2020) who observed that coriander as a feed additive to broiler increased HDL-cholesterol level and decreased their LDL-cholesterol level. The decrease in cholesterol levels might be due to the polyherbal oil is an inhibitor of the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-

Table 5. Effect of mixtures of ruta graveleon, coriander, and basil seed extracts in drinking water on hematological and serum biochemistry of White Leghorn layers.

Parameters	Treatments				SEM	SL
	T1	T2	T3	T4		
Total cholesterol (mg/dl)	149.52^{a}	148.53^{a}	146.22^{a}	142.24^{b}	1.08	**
HDL-C (mg/dl)	47.57^{c}	49.43^{bc}	51.96^{b}	55.95^{a}	1.11	**
LDL-C (mg/dl)	101.65^{a}	101.08^{ab}	97.38^{c}	96.75^{bc}	1.22	*
TP (g/dl)	3.50^{b}	3.50^{b}	3.95^{a}	3.51^{b}	0.04	***
Albumin(g/dl)	1.93	1.89	2.00	1.98	0.08	NS
Globulin(g/dl)	1.56^{b}	1.56^{b}	1.95^{a}	1.53^{b}	0.07	**
RBC(10^6/µl)	3.46	3.54	3.56	3.34	0.65	NS
WBC(10^3/µl)	7.10^{a}	6.33^{ab}	6.12^{b}	5.42^{b}	0.30	*
Hb (g/dl)	10.39^{b}	10.78^{ab}	11.33^{a}	10.65^{b}	0.18	*
PCV (%)	33.82	34.47	35.23	33.95	0.42	NS

$^{a, b, c,}$ row means with different superscripts are significantly different,***= P<0.001, **=p<0.01, *= P<0.05, SL = Significant level, SEM = Standard error of mean, RBC=Red Blood Cells, Hb= Hemoglobin, PCV= Packed Cells Volume, WBC= White Blood Cells, TP= Total Protein, HDL-C= High Density Lipoprotein cholesterol, LDL-C= Low Density Lipoprotein Cholesterol, Rg=Ruta graveleon,C=Coriander, B=Basil.

CoA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis by hepatocytes or the fractional reabsorption from the small intestine (Lee *et al*., 2004).

Statistically significant difference was not seen on PCV and total RBC count of the experimental birds among the different treatments. However, significant differences ($P<0.05$) were noted on total WBC count, Hb concentration, serum total protein and globulin. Likewise, Zeweil *et al*. (2006) noted significantly higher serum total protein and globulin with 1% supplementation of medicinal polyherbal extracts to laying Japanese quail hens as compared to a control diet. A higher value serum total protein and globulin might indicate there is enzyme hydrolysis of dietary proteins explained that the blood pool serves as a major source of amino acids needed for the synthesis of proteins as indicated by Scott (1970). Significant difference in blood constituents of hens may be due to associated with the effects of herbs bioactive compounds on improving the antioxidant status of the bird (Rababah *et al*., 2004)

CONCLUSION

From this study, it was concluded that inclusion of 4ml of *Ruta graveolen*, coriander, and basil seed extracts (mixed medicinal herbs powder) in one liter of drinking water significantly increased hen-day egg production, yolk color, feed intake, egg weight, egg mass and HDL-cholesterol level while, it can significantly decrease serum LDL- cholesterol level. Further research was recommended to assess the identification of active chemical compounds in the seeds of *Ruta graveolen*, coriander, and basil as well as their effect at higher proportions on the performance of layers.

ACKNOWLEDGMENTS

Authors would like to thank Haramaya University Research Office for the financial funding to the research project. A special gratitude is going to Haramaya University Animal nutrition and food science laboratory technicians and poultry farm workers for their kind support with critical research inputs and the help in the farm and laboratory.

REFERENCES

Abd El-Hack, M.E., M.T. El-Saadony, H.M. Salem, A.M. El-Tahan, M.M. Soliman, G.B. Youssef, A.E. Taha, S.M. Soliman, A.E. Ahmed, A.F. El-kott, K.M. Al Syaad and A.A. Swelum. 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult. Sci. 101: 1-20.

Abd El-Hack, M.E. and M. Alagawany. 2015. Performance, egg quality, blood profile, immune function, and antioxidant enzyme activities in laying hens fed diets with thyme powder. J. Anim. Feed Sci. 24: 127-133.

Al-Shaheen, S.A., R.J. Abbas and T.I. Majeed. 2023. Effect of different levels of basil and peppermint an essential oils on productive and physiological performance of two quail lines during egg production period. Ann. For. Res. 66: 2526-2546.

AOAC (Association of Official Analytical Chemists). 1996. Official Methods of Analysis of AOAC International (16th Edition). Virginia. USA.

Asghariana, S., M.R. Hojjatib, M. Ahrari, E. Bijad, F. Derisc and Z. Lorigooinia. 2020. Ruta graveolens and rutin, as its major compound: investigating their effect on spatial memory and passive avoidance memory in r ats. Pharm. Biol. 58: 447–453. https://doi.org/10.1080/13880209.2020.1762669.

Brenes, B. and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. Anim. Feed Sci. Technol. 15:81-114.

Criste, R.D., T.D. Panaite, C.Tabuc, M. Saracila, C. Șoica and M. Olteanu. 2017. Effect of oregano and rosehip supplements on broiler (14-35 days) performance, carcass and internal organs development and gut health. AgroLife Sci. J. 6: 75-83.

Dacie, J.V. and S.M. Lewis. 1991. Practical Hematology. 7th Edition, Churchil Livingstone, Edinburgh, P. 54-79.

Douglas, J., K. Weiss and J. Wardrop. 2010. Veterinary Hematology. 6th Edn. Blackwell Publishing Ltd. Esteghamat, P. 82 .

Doumas, B.T., D.D. Bayso, R.J. Carter, T. Peters and R. Schaffer. 1981. Determination of total serum protein. Clin. Chem. 27:1642-1643. https://doi.org/10.1093/clinchem/27.10.1642

Ghazaghi, M., M. Mehri and F. Bagherzadeh-Kasmani. 2014. Effects of dietary Mentha spicata on performance, blood metabolites, meat quality and microbial ecosystem of small intestine in growing Japanese quail. Anim. Feed Sci. Technol. 194: 89-98.

Guler, T., O.N. Ertaş, M. Ciftçi and B. Dalkiliç. 2006. Effect of feeding coriander (*Coriandrum sativum* L.) on egg production performance and nutrient retention in laying Japanese quails.J. Appl. Anim. Res. 30: 181 -184. https://doi.org/10.1080/09712119.2006.9706614

Habiyah, U., R. Mutia, and S. Suharto. 2016. Performance and Egg Quality of Laying Hens Fed Ration Containing Coriander Seeds (*Coriandrum sativum* Linn). Media Peternakan. 39: 61-66. https://doi.org/10.5398/medpet.2016.39.1.61.

Hadi M.M. and D.B. Jassim. 2013. Effect of using dried basil leaves powder on some productive and hematological traits of Japanese quail. Euphrates J. Agric. Sci. 5: 57-64. (In Arabic).

Hashemi, S.R. and H. Davoodi. 2010. Phytogenics as a new class of feed additive in the poultry

Haugh, R. 1937. The Haugh unit for measuring egg quality. http://edis.ifas.ufl.edu/pdffiles.

Hernandez, F., J. Madrid, V. Garcia, J. Orengo and M.D. Megias. 2004. Influence of two plant extracts on broiler performance, digestibility, and digestive organ size. Poult. Sci. 83(2): 169-174. https://doi.org/10.1093/ps/83.2.169

Hunton, P. 1995. Egg production, processing and marketing. International World Poultry Science, Elsevier, Tokyo, P. 457-480.

Jianu, C., I. Goleț, D. Stoin, I. Cocan, G. Bujanca, C. Mișca, M. Mioc, A. Mioc, C. Șoica, A.T. Lukinich-Gruia and L.C. Rusu. 2021. Chemical profile of *Ruta graveolen*s, evaluation of the antioxidant and antibacterial potential of its essential oil, and molecular docking simulations. Appl. Sci. 11:11753.

Kadhim, S.K. 2016. Effect of different levels of basil seeds on some blood biochemical traits. J. BioSci. Biotechnol. 5: 477-480.

Kazem, K. 2013. Effect of dietary medicinal herbs on performance, egg quality and immunity response of laying hens. Adv. Environ. Biol. 13

Khaligh, F., G. Sadeghi, A. Karimi and A. Vaziry. 2011. Evaluation of different medicinal plants blends in diets for broiler chickens. J. Med. Plant Res. 5:1971-1977.

Khan, R.U., Z. Nikousefat, V. Tufarelli, S. Naz, M. Javdani and V. Laudadio. 2012. Garlic (Allium sativum) supplementation in poultry diets: Effect on production and physiology. World Poultry Sci. J. 68: 417-424.

Khubeiz, M.M. and A.M. Shirif. 2020. Effect of coriander (Coriandrum sativum L.) seed powder as feed additives on performance and some blood parameters of broiler chickens. Open Vet. J. 10:198-205. DOI: 10.4314/ovj.v10i2.9

Kljak, K., K. Carović-Stano, I. Kos, Z. Janječić, G. Kiš, M. Duvnjak, T. Safner and D. Bedeković. 2021. Plant carotenoids as pigment sources in laying hen diets: Effect on yolk color, carotenoid content, oxidative stability and sensory properties of eggs. Foods. 10*:* 721.

Kopsell, D.A., D.E. Kopsell and J. Curran-Celentano. 2005. Carotenoid and chlorophyll pigments in sweet basil grown in the field and greenhouse. HortScience, 40: 1119 -1119.

Lee, K.W., H. Everts and A.C. Beynen. 2004. Essential oils in broiler nutrition. Int. J. Poult. Sci. 3: 738-752. https://doi.org/10.3923/ijps.2004.738.752.

Leeson, S. and J.D. Summers. 2005. Commercial Poultry Nutrition. 3rd ed. Nottingham University Press, Canada. 398p.

Matasyoh, J.C., Z.C. Maiyo, R.M. Ngure and R. Chepkorir. 2009. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum.* Food Chemistry. 113: 526-529.

Mishra. B.B, K.M. Kibret and B. Eshetu. 2004. Soil and land resources inventory at AlemayaUniversiry Research farm with referen ce to land evaluation for sustainable agricult ural management and production. Synthesis of working papers, soil sciences Bulletein, 1:123.

Mollah, M.R., M.M. Rahman, F. Akter and M. Mostofa. 2012. Effects of Nishyinda, black pepper and cinnamon extract as growth promoter in broilers. Bangladesh j. vet. med. 29: 69-77. https://doi.org/10.3329/bvet.v29i2.14345

Mudalal, S., A. Zaazaa and J.A. Omar. 2021. Effects of medicinal plants extract with antibiotic free diets on broilers growth performance and incidence of muscles abnormalities. Braz. J. Poult. Sci. 23: 1-8

North, M. and D. Bell. 1984. Breeder management. Commercial Chicken Production Manual. avian. Publishing Company. Inc.Westport, Connecticut, P. 240-321.

NRC (National Research Council). 1996. Nutrient Requirement of poultry, 19th Edition. National Academic Press, Washington D.C. USA. P. 167.

Ooi, P.S., A.R. Rohaida, A.D. Nur Hardy, D. Devina, A.H. Borhan, S. Kartini, J.S. Jupikely, M. Abdul Rahman and A.R. Alimon. 2018. Effect of local medicinal herbs as feed additives on production performance and faecal parameters in laying hens. Malays. J. Anim. Sci. 21:59-67.

Rababah, T.M., N.S. Hettiarachchy and R. Horax. 2004. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola and ginkgo extracts, vitamin E and tert-butylhydroquinone. J. Agric. Food Chem. 52: 5183-5186.

Rahimi, S., Z. Teymouri Zadeh, M.A. Karimi Torshizi, R. Omidbaigi and H. Rokni. 2011. Effect of the three herbal extracts on growth performance, immune system, blood factors, and intestinal selected bacterial population in broiler chickens. J. Agric. Sci. 13:527–53.

Sapsuha, Y., E. Suprijatna, S. Kismiati and S. Sugiharto. 2021. Combination of probiotic and phythobiotic as an alternative for antibiotic growth promoter for broilers chickens - a review. Livest. Res. Rural. Dev. 33: 49.

Scott, A. 1970. Absorption of carbohydrate and protein metabolism. In Duke"s Physiology of Domestic Animals. (18th Eds). (Swenson, M.J. edited). Cornell University Press Limited, London, UK.

Shahrajabian, M.H., W. Sun and Q. Cheng. 2020. Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): a review. Int. J. Food Prop. 23: 1961–1970.

Sigolo, S., C. Milis, M. Dousti, E. Jahandideh, A. Jalali, N. Mirzaei, B. Rasouli, A. Seidavi, A. Gallo, G. Ferronato and A. Prandini. 2021. Effects of different plant extracts at various dietary levels on growth performance, carcass traits, blood serum parameters, immune response and ileal microflora of Ross broiler chickens. Ital. J. Anim. Sci. 20:359-371.

Szewczyk, A., W. Pazdziora, and H. Ekiert. 2023. The Influence of Exogenous Phenylalanine on the Accumulation of Secondary Metabolites in Agitated Shoot Cultures of Ruta graveolens L. Molecules. 28: 727.

Wasiyati, A., F. Pratama and T. Widowati. 2018. Physical and chemical properties of salted egg with addition of coriander seed extract (*Coriandrum sativum* L.). Int. J. Recent Sci. Res. 9: 29878-29880.

Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr. 2008. Use of Phytogenic Products as Feed Additives for Swine and Poultry. J. Anim. Sci. 86: E140–E148.

Wiseman, J. 1987. Feeding of Non-Ruminant Livestock. Butter worth and C. Ltd. P.370.

Yaman, M.A., Y. Usman, C.A. Fitri and H. Latif. 2020. Increase in egg production, egg quality and immunity of local chicken resulted by cross-breeding. IOP Conf. Ser.: Earth Environ. Sci. 425: 012043. doi:10.1088/1755-1315/425/1/012043.

Zeweil, H.S., S.G. Genedy and M. Bassiouni. 2006. Effect of probiotic and medicinal plant supplements on the production and egg quality of laying Japanese quail hens. In Proceeding of the 12th European poultry conference ZWAN, September, 2006. P. 1-6.

Zhao, X., Z.B. Yang, W.R. Yang, Y. Wang, S.Z. Jiang and G.G. Zhang. 2011. Effects of ginger root (Zingiber officinale) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Poult. Sci. 90:1720-1727. https://doi.org/10.3382/ps.2010-01280.