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Egg Lipid Profile, Growth Traits, Blood Biomarkers, and Physical Egg Characteristics of Heavy Ecotype Laying Hens Fed Oregano (*Origanum vulgare*) Meals

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ABSTRACT

Investigation on the effect of dietary oregano on growth traits, egg physical characteristics, blood biomarkers, and egg lipid profile of heavy ecotype laying hens was conducted. Two hundred and forty eighteen weeks old, heavy ecotype hens were randomly assigned to two (T1; control - zero oregano meals, and T2; 3g oregano meals kg^{-1}) dietary treatment of twelve replicates with ten hens per replicate. Birds fed oregano meals improved body weight and weight gain (P<0.05) compared with those fed the control diet. The heavy ecotype-laying hens fed zero oregano consume more (P<0.05) diets with a poor FCR. Egg production and egg weight were higher for birds fed oregano meals. Birds fed dietary oregano supplementation had a better (P<0.05) shell thickness than those fed the control diet. Red blood cell (RBC) and hemoglobin (Hb) values were higher for birds fed dietary oregano, as shown in Table 6. White blood cell (WBC), lymphocytes, monocytes, and eosinophil counts were increased (P<0.05) for birds fed the control diet in contrast to those that received oregano inclusion. Cholesterol, high-density lipoprotein (HDL), as well as low-density lipoprotein (LDL) were affected by the dietary oregano. Cholesterol and LDL levels were higher (P<0.05) for birds fed the control diet. At the same time, those that received oregano inclusion had a higher HDL value as well as a reduced (P<0.05) cholesterol and LDL values. It was concluded that oregano improved egg mass, egg quality, and quantity. It also enhanced the immunity of the hens.

Key words: Blood, Egg, Heavy ecotype, Immunity, Lipid profile, Oregano.

INTRODUCTION

The demand for poultry products, including eggs, is constantly rising just as the world population increases. In modern society, the production processes and contents of human food today are highly discussed and questioned. The growth-promoting antibiotics (GPAs) are used as sub-therapeutic doses in the poultry industry. They are used to improve poultry products (meat and egg) and to prevent infection (Lillehoj et al. 2018) by changing gut microbiota, metabolism, as well as the activities of the intestine (Costa et al. 2017). Feed is one of the essential items needed in poultry production. Feeding contributes over 70% of animal production costs. To achieve the maximum feed value, it becomes necessary to use GPAs for efficient production in the poultry industry (Oyeagu et al. 2019a; Hussein et al. 2020). However, this has contributed to increased antimicrobial resistance in poultry production (Tang et al. 2017; Kumar et al. 2018). The use of GPA has also increased the antibiotics residue in meat and eggs, which is a concern to consumers' health (Swanny et al. 2021). Consequently, there are new strategies from international health organizations to develop natural products to fight antimicrobial resistance to promote humans, animals, and ecosystems (Camou et al. 2017).

The poultry industry is currently searching for alternatives to GPA as feed additives in poultry production to improve animal and human health. Most importantly, the quality and quantity of the product appeared as the main priorities (Gerzilov et al. 2015). Oregano is one of the

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several herbal-origin feed additives. Improved health and productivity of broilers were attributed to dietary oregano (Roofchaee et al. 2011). Oregano meal contains some essential oil. It has antioxidant properties such as carvacrol (5-isopropyl-2-methyl phenol) and thymol (2-isopropyl-5-methyl phenol) (Basmacioglu-Malayoglu et al. 2010; Migliorini et al. 2019). Carvacrol and thymol enter the circulatory system, spread to muscles and other tissues (Bozkurt et al. 2012). Oregano (*Origanum vulgare*) belongs to the family Lamiaceae. It is essential as a phytotherapeutic agent. Consequently, the essential oil in oregano is toxic against phytopathogenic bacteria (Eler et al. 2019) and fungi (Bedoya-Serna et al. 2018). The vital compounds found in oregano can provide anti-inflammatory processes that exhibit antioxidant (Eler et al. 2019) and anti-tumor activity (Zeytinoglu et al. 2003; Arpášová et al. 2014). They also stimulate enzyme secretion (Feng et al. 2021) and modulation of microbial colonization of the gastrointestinal tract and intestinal morphology (Zou et al. 2016; Bauer et al. 2019). Feed and food industries make use of oregano due to its beneficial properties. They also significantly inhibit common food pathogens and their subsequent immune activation in the recipient's body (Carvalho et al. 2019).

However, many field trials on essential oils, either single oil or in combination, showed better performance of birds and enhanced body weight gain, feed efficiency, among others (Bozkurt et al. 2009). Different studies reported on the inclusion of aromatic herbs extract and its essential oils. According to several reports, dietary herbal essential oils affect egg production rate, egg weight, FI, and FCR (Bölükbaşı et al. 2007; Bölükbaşı et al. 2008; 2009; Orhan and Eren 2011). The mixture of different essential oils added to the diets improved egg yield and the reproductive performance of laying hen compared to dietary GPAs (Bozkurt et al. 2009; 2012). There is a paucity of evidence on the effects of dietary oregano meal on laying hens, but their influence on the performance of broilers is promising (Isabel and Santos 2009; Brenes and Roura 2010). The phytogenic feed additives may benefit broilers' immune-modulatory activities and anti-inflammatory potentials (Mohiti-Asli and Guanaatparast-Rashti 2017). Therefore, this study defines how oregano extract affects growth, laying performance, physical egg quality, blood biomarkers, and egg lipid profile of heavy ecotype layers.

MATERIALS AND METHODS

Ethical Statement

Ethical principles were taken into consideration during the study to adhere to the national and international standards governing research of this nature with regards to the use of research animals. The permission to use animals was obtained from the Ethical Clearance Committee of University of Nigeria, Nsukka, Nigeria.

Study Site

The location of the present research trial was at the local chicken research unit of Animal Science Departmental Teaching and Research Farm, University of Nigeria, Nsukka, Nigeria. The research trial lasted for 12 weeks.

Experimental Material

The test oregano-product (Orego-Stim®) was sourced from the Animal Health and Production Department, University of Nigeria, Nsukka. The product powder was stored on a dry platform at room temperature before use.

Experimental Diet

Table 1 represents the contribution of different ingredients that make up the experimental diet. The chemical analysis of the experimental diet was conducted according to AOAC (2006) and shown in Table 2.

Management of Experimental Birds

A total of 240 eighteen weeks old local laying hens (heavy ecotype) with approximately 1.40kg weight was used for the study. 120 hens (twelve replication of 10 hens in each replicate per treatment) was allocated randomly to one of the two dietary groups (T1; control- zero Oregano meals, and T2; 3g of Oregano meals kg^{-1} of feed). The hens were housed in pens, while the bedding material used was wood shavings. Diets were supplied *ad libitum* together with clean water throughout the twelve-week feeding trial. General poultry management and routine vaccinations were given. The research trial followed the Ethics of animal use of the University of Nigeria, Nsukka, Nigeria.

Data Collection

The feeding trial lasted for 12 weeks. Data collection was done for the following parameters.

Feed Intake

The daily feed intake per hen was measured throughout the feeding trial (week 18 to week 30 of age) as follows;

$$Daily\ Feed\ Intake\ = \frac{Feed\ Supplied\ - Feed\ Refusal}{Total\ number\ of\ hen}$$

Growth Traits

The initial weight was determined at the beginning of the research trial and, subsequently, every week. The live weight of all the birds in each pen was determined using a 10,100g (10.1kg) industrial weighing balance made in Japan.

Feed Utilization Efficiency

The ratio of the feed consumed by the birds to their corresponding weight gains was documented for each experimental pen as their feed conversion ratio (FCR) values.

Hematological Studies

Blood samples were drawn at week 18 of hens age (when the experimental feeding trial started) and week 30 of hens age (when the experimental feeding trial ended). Blood samples (2.5mL each) were drawn from three hens per replicate into EDTA bottles to determine the hematological indices. Basophils, neutrophils, eosinophils, monocytes, lymphocytes, RBC, WBC, PCV and hemoglobin concentrations were determined.

Egg Quality Assessment

Hen day performance, egg weight, shell thickness, and percentage shell were routinely recorded for each experimental pen. At week 18 of hens age (when the experimental feeding trial started) and week 30 of hens age (when the experimental feeding trial ended), five eggs per replicate were assayed for their lipid profiles. Cholesterol, triglyceride, HDL and LDL contents of the eggs were determined using cholesterol diagnostic kits (Sigma Diagnostics, Catalog No. 435- Sigma Chemical Co., St. Louis, MO 63177-9926).

Statistical Analysis

The experimental design that was used for the study was t-distribution with the model

$$t = \frac{\bar{x}_i - \bar{x}_j}{S\bar{x}_i - \bar{x}_j}$$

Where, t = t-value

x_i and x_j = are the means of the two treatment groups being compared

S = pooled standard error

Data were analyzed using a statistical package (SAS 2010) windows version 8.0

RESULTS

Growth Traits

Table 3 showed the growth traits of heavy ecotype-laying hens fed dietary oregano. The growth characteristics considered are the final body weight (FBW), weight gain (WG), feed intake (FI), and feed conversion ratio (FCR).

All the growth traits were affected (P<0.05) with the inclusion of oregano meal, except for initial body weight. Compared with the control, birds fed oregano meal improved (P<0.05) FBW and WG. The heavy ecotype hens fed zero oregano consume more (P<0.05) diets with a poor feed conversion ratio.

Egg Physical Traits

Table 4 shows the physical egg parameters from heavy ecotype hens fed diets with and without oregano inclusion.

Egg production and egg weight were higher (P<0.05) for birds fed oregano meals. Birds fed dietary oregano supplementation had a better (P<0.05) shell thickness than those fed the control diet. Shell percentage did not differ (P>0.05).

Blood Biomarkers

Tables 5 and 6 shows the blood biomarker parameters. The initial blood traits were determined at week 18 of hens age (before applying the dietary treatments), and all the initial blood parameters measured were the same (P>0.05), as shown in Table 5.

The blood analysis was repeated towards the end of the experiment (at week 30 of hens age). The blood traits considered in the present study were significant (P<0.05), except for basophils, neutrophils, and packed cell volume, as shown in Table 6. RBC and Hb values were higher (P<0.05) for birds fed dietary oregano (Table 6).

On the other hand, WBC, lymphocytes, monocytes, and eosinophil concentration increased (P<0.05) for heavy ecotype laying-hens fed the control diet in contrast with their oregano inclusion counterparts (Table 6).

Table 1: Ingredient composition of experimental diets

Ingredients	(%)
Maize	37.90
Soybean meal	24.60
Fishmeal	2.90
Wheat bran	25.50
Bone meal	2.40
Oyster shell	6.00
Vitamin premix*	0.25
Salt	0.25
DL-methionine	0.10
Lysine	0.10
Total	100
Calculated composition	
Crude protein (%)	16.92
Crude fibre (%)	4.90
Energy (Kcal/Kg ME)	2553.00

*Bio-mix Layer supplied/kg: Vit A = 3,400,000.00 IU; Vit D3 = 600,000.00 IU; Vit E=4,000.00mg; Niacin=6,000.00mg; B1=600.00mg; B2=1,800.00mg; B6=1,200.00 mg;B12=6.00mg; K3=400.00mg; Pantothenic acid=1,600.00mg; Biotin=200.00mg; Folic acid=240.00mg; Choline Chloride=70,000.00mg; Cobalt=80.00mg; Copper=1,200.00mg; Iodine=400.00mg; Iron=8,000.00mg; Manganese=16,000.00mg; Selenium=80.00mg; Zinc=12,000.00mg; Antioxidant=500.00mg.

Table 2: Chemical compositions of the experimental diet

Components	%
Dry matter	97.07
Crude protein	16.98
Crude fibre	4.95
Ether extract	5.19
Ash	13.40
Nitrogen-free extract	59.48
Gross energy (Kcal/g)	2892.75

Table 3: Growth performance of the heavy ecotype laying hens fed Oregano meals

Parameters	T1	T2	Sig. (2 tailed)
IBW (g/bird)	1487.68±8.04	1485.08±8.00	0.30^{NS}
FBW (g/bird)	1694.08±6.17	1735.13±6.04	0.01*
WG (g/bird)	206.40±3.07	250.05±3.00	0.01*
FI (g/bird)	577.92±4.85	416.61±4.86	0.05*
FCR	2. 81±0.02	1.67 ±0.04	0.01*

*=Significant. ^{NS}=Non-significant. FCR=Feed conversion ratio. T1=control group. T2=oregano meal supplemental group. IBW=Initial body weight. FBW=Final body weight. WG=Weight gain. FI=Feed intake.

Table 4: Egg physical characteristics of the heavy ecotype laying hens fed Oregano meals

Parameters	T1	T2	Sig. (2 tailed)
Hen – Day (%)	66.70±0.64	77.03±0.70	0.02*
Egg weight (g)	60.85±0.39	72.43±0.34	0.04*
Shell thickness (mm)	0.33±0.00	0.39±0.01	0.51*
Percent shell (%)	8.23±0.04	8.22±0.03	0.24^{NS}

*=Significant. NS=Non-significant. T1=control group. T2=oregano meal supplemental group.

Egg Lipid Profile

The Initial egg lipid profiles were determined before the feeding trial (at week 18 of hens age), and all the lipid profile parameters were the same (P>0.05), as shown in Table 7.

The analysis of egg lipid profile was subsequently evaluated towards the end of the feeding trial (at week 30 of hens age). Cholesterol, HDL and LDL were affected (P<0.05) by the dietary oregano, as displayed in Table 8. Triglycerides were the same (P>0.05) among the treatments.

Table 5: Hematological indices of the heavy ecotype laying hens measured before the experimental feeding trial (at week 18 of hens age)

Parameters	T1	T2	Sig. (2 tailed)
Packed cell volume (%)	33.20±0.22	35.20±0.04	1.00^{NS}
Red blood cell (10^6µL^{-1})	9.93±1.00	10.06±0.97	0.22 ^{NS}
White blood cell (10^3µL^{-1})	9710±1.06	9680±1.95	0.32^{NS}
Hb (g dL^{-1})	10.38±1.07	11.18±1.26	0.86^{NS}
Lymphocytes (%)	7.20±1.89	8.80±1.06	0.30^{NS}
Monocytes (%)	1.00±0.29	1.20±0.16	0.58^{NS}
Eosinophils (%)	0.60±0.81	0.80±1.24	0.19^{NS}
Basophils (%)	0.00±0.00	0.00±0.00	-
Neutrophils (%)	21.60±0.84	23.20±1.44	0.16^{NS}

*=Significant. ^{NS}=Non-significant. Hb=Hemoglobin concentration. T1=control group. T2=oregano meal supplemental group.

Table 6: Hematological indices of heavy ecotype laying hens fed Oregano meals measured at the end of the study (at week 30 of hens age)

Parameters	T1 (control)	T2 (3 g Orego-Stim® kg^{-1})	Sig. (2 tailed)
Packed cell volume (%)	35.40±0.36	36.00±1.05	0.14
Red blood cell (10^6µL^{-1})	10.46±0.04	15.46±1.42	0.05*
White blood cell (10^3µL^{-1})	10860±7.99	10190±6.05	0.01*
Hb (g dL^{-1})	10.94±0.30	16.10±1.90	0.04*
Lymphocytes (%)	13.40±2.35	8.00±2.86	0.14*
Monocytes (%)	0.89±0.50	0.50±0.83	0.44*
Eosinophils (%)	0.90±2.08	0.49±2.39	0.22*
Basophils (%)	0.20±0.00	0.20±0.01	1.00^{NS}
Neutrophils (%)	20.40±1.33	19.40±2.63	0.65^{NS}

*=Significant; ^{NS}=Non significant; Hb=Hemoglobin concentration

Table 7: Egg lipid profile of the heavy ecotype laying hens measured before the experimental feeding trial (at week 18 of hens age)

Parameters	T1	T2	Sig. (2 tailed)
Total cholesterol (mg dL^{-1})	24.40±2.05	23.69±0.7	0.09 ^{NS}
HDL (mg dL^{-1})	13.60±1.74	13.00±1.92	0.19^{NS}
LDL (mg dL^{-1})	32.20±2.06	31.40±2.34	0.91^{NS}
Triglycerides (mg dL^{-1})	118.40±4.42	117.20±6.96	0.41^{NS}

*=Significant; ^{NS}=Non-significant; HDL=High density lipoproteins; LDL=Low density lipoproteins. T1=control group. T2=oregano meal supplemental group.

Table 8: Egg lipid profile of the heavy ecotype laying hens fed Oregano meals measured at the end of the study (at week 30 of hens age)

Parameters	T1	T2	Sig. (2 tailed)
Total cholesterol (mg dL^{-1})	35.40±3.72	28.80±4.01	0.03*
HDL (mg dL^{-1})	7.60±1.08	11.60±2.73	0.62*
LDL (mg dL^{-1})	24.60±3.60	22.20±4.35	0.95*
Triglycerides (mg dL^{-1})	78.00±6.70	76.00±6.83	0.86^{NS}

*=Significant; ^{NS}=Non-significant; HDL=High density lipoproteins; LDL=Low density lipoproteins. T1=control group. T2=oregano meal supplemental group.

The heavy ecotype laying-hens fed the control diet had a higher ($P<0.05$) concentration of Cholesterol and LDL in contrast to higher HDL concentration as well as a reduced ($P<0.05$) cholesterol and LDL concentration for that fed dietary oregano (Table 8).

DISCUSSION

Growth Performance

Higher feed consumption was reported for heavy ecotype hens fed the control diet, while those supplemented with dietary oregano had a better body weight and FCR. Generally, herbs and spices, including their oil extracts, may improve diet palatability because of their flavorful traits. The flavonoid traits may enhance feed intake in poultry diets (Zeng et al. 2015; Mucha and Witkowska 2021). However, there has not been consistent and repeatable evidence of improved palatability due to oregano in broilers and pigs (Windisch et al. 2008). There was no difference in FI for hens fed four dietary herbs (thyme, oregano, rosemary, turmeric) with an inclusion level of 5 to 10g/kg diet (Radwan et al. 2008). They used 28-week old hens for the study that lasted for 12 weeks. Similar results on black cumin seeds (10, 20, or 30g/kg) (Aydin et al. 2008) and garlic (20, 40, 60, 80, or 100g/kg diet) (Chowdhury et al. 2002) were reported for laying hens. Our study showed that hens fed oregano herb consumed less feed than those fed untreated (control) diet. Oyeagu et al. (2016) reported that animals continue to eat and stop eating when they have satisfied their energy requirements. The investigation of some researchers (Bölükbasi et al. 2010) showed a significant decrease in feed intake for hens fed dietary bergamot essential oil at 0.50 and 0.75ml/kg supplemental levels as compared with 0.25mL/kg inclusion level. Some authors reported similar feed intake of hens when a blend of dietary six herbs containing essential oil of oregano fed at 36 and 24mg/kg (Çabuk et al. 2006; Özek et al. 2011; Bozkurt et al. 2012). Based on the inconsistent results on FI for laying hens, the hypothesis that herbs and their respective essential oil increase FI may not be generally justified. Hens fed dietary oregano had the lowest FI in this study. They also recorded the highest body weight and a better FCR than the control diet. It may be due to the ability of oregano additive to assist in the efficient digestion and utilization of feed. Oregano and its active component stimulate endogenous digestive enzymes. The enzymes include amylase, protease, and lipase, improving digestibility (Safid et al. 2006). Oregano enhances digestion and absorption due to increased intestinal length and depth and width of the villi, allowing the absorption of nutrients (Alçiçek et al. 2003; Abdel-Wareth et al. 2013). Egg number and egg mass are the significant traits for selecting egg-laying birds and not bodyweight. Although, Oyeagu et al. (2015) reported that hens with big body mass tend to produce bigger eggs compared with hens with small body mass. Dietary oregano increases the availability of nutrients in the intestine. The absorption of nutrients increases BW according to birds' genetic potential without compromising the output performance (Bozkurt et al. 2014). The FCR explains the efficiency of transforming consumed feed mass into egg mass over a particular time (Tallentire et al. 2016). According to Feng et al. (2021), oregano promotes FCR and improves nutrient absorption in the intestine. The improved FCR recorded in the present study explains the impact of oregano in enhancing the digestion stimulating properties because of their bioactive effects on physiological and metabolic activities (Hernandez et al. 2004; García et al. 2007; Bozkurt et al. 2012). Through enzymatic stimulation, oregano essential oil improves nutrient digestion and absorption. Oregano can also exert beneficial effects when used in laying hens. The dietary oregano impacts the animals depending on their compounds' level and composition (Zhang et al. 2005).

However, different studies with laying hens recorded no significant in FCR with the inclusion of essential oil of oregano in a 60 days research trial (Florou-Paneri et al. 2005), black cumin for ten weeks (Bölükbaşı et al. 2009) and bergamot for eight weeks (Bölükbasi et al. 2010). The present results are similar to Roofchaee et al. (2011) report that dietary oregano improved BW and FCR. The authors linked these beneficial effects to the antimicrobial activity of phenolic compounds (Bozin et al. 2006). According to Lee et al. (2003), carvacrol is a compound of oregano, and it improved the efficient utilization of feed (FCR) in broilers. According to Windisch et al. (2008), phytogenic compounds improve digestive enzymes and nutrient absorption.

Egg Physical Traits

Hen day production, egg weight, and eggshell thickness were better under dietary oregano. The egg's physical traits are essential both for the farmer/producer and the consumers. Increased number of egg production and egg mass directly increase the economic returns to the farmer/producer. The success of commercial egg-laying farming projects depends on the total number and size of eggs produced (Maoba 2016). Having an improved shell thickness guarantees minimal losses from crack eggs on the farm, during transport, or at the shelves in the shops. Consumers would desire to gain value for the money they spent on one product or the other. Hence, most consumers prefer to buy more giant eggs with rugged shell thickness than medium or smaller egg sizes. Some researchers (Bozkurt et al. 2012) suggested that increased thickness of eggshells may be attributed to higher retention and availability of nutrients in the intestinal tract of hens during the formation of eggshells. The increased eggshell thickness recorded in the present study may result from oregano's bioactive component (thymol). The oregano bio-component has an impact on the metabolic activities of bacteria in the GIT of layers. Consequently, it increases the rate of mineral absorption, particularly Ca^{2+} and Mg^{2+} (Panda et al. 2003; Ramirez et al. 2021).

The increased egg number recorded in our study may be attributed to dietary oregano inclusion, which positively influences the eubiosis of the gastrointestinal tract microbiota, which directly improves uterine health (Zou et al. 2016). It favors the proliferation of non-pathogenic microbes, which enhances nutrient digestibility, immunity, and ability to resist pathogens in birds (Pan and Yu 2014), optimizing energy and nutrient use for the production of eggs (Kers et al. 2018; Diaz Carrasco Juan et al. 2019). Thymol and carvacrol (from oregano) reduce the effect of pathogenic microbes on the bacteria cell wall by denaturing and coagulating its proteins, disrupting its system, and increasing its hydrogen and potassium ion permeability. It disrupts important cellular processes such as protein translocation, phosphorylation, and other enzyme-dependent reactions. These processes lead to a loss of chemosmotic control of the cells, leading to the death of bacteria (Diaz-Sanchez et al. 2015).

Consequently, these compounds (thymol and carvacrol) influence intestinal health, resulting in increased egg production, egg weight, and FCR. Based on the beneficial effects of oregano on poultry, it is safe to support the claim that oregano can serve as an alternative to the antibiotic in the industry. Oregano can be an effective antibiotic alternative because of its bio-activities such as antiseptic, antimicrobial, antioxidant, and anti-parasitic activities (Leyva-López et al. 2017). Some authors have reported positive results concerning the efficacy of oregano essential oils on poultry performance (Isabel and Santos 2009; Brenes and Roura 2010). According to Radwan et al. (2008), 1.0% oregano in the diet increased feed conversion of hens. Therefore, the inclusion of oregano may improve the health of the intestine, enhancing feed digestibility, improving the efficiency of feed utilization, and increasing the egg number and egg weight of laying hens.

Blood Biomarkers

Dietary oregano was expected to improve the hens' egg production without impairing their physiological conditions and health status. Blood traits are good indicators of the physiological, pathological, and nutritional quality of the animal. Changes in hematological traits can be used to explain the impact of diets and additives on any living thing (Toghyani et al. 2010). Blood traits are used to monitor the state of health of the animal. Our results showed that lymphocyte, monocyte, eosinophil, and WBC values were higher for hens under a control diet than dietary oregano-fed birds. The increased production of lymphocyte, monocyte, eosinophil and WBC for control-fed birds may indicate that the hens are fighting a disease or stress. According to Oyeagu et al. (2019b), WBC and its constituents (lymphocyte, monocyte, and eosinophil, among others) are antibodies. Their secretion/production increases when the immune system fights harmful intruders in the body, such as disease or stress. The present study's findings support the results of Davis et al. (2008) and Sugiharto et al. (2014). According to Akinwutimi et al. (2004) and Oyeagu et al. (2019c), increased production of the blood parameters may result from the diets' high fiber content. It may also be attributed to anti-nutrient inhibitors, especially phytate, which chelates divalent metal utilization in non-ruminant metabolism. These drawbacks may have triggered a reduction of egg production and inefficient FCR recorded for hens fed the control diet in this study.

Monocytes engulf or ingest microbes, they are aggressively phagocytic in action and motile. Monocytes exit the bloodstream to overwhelm microorganisms and other antigens found in the tissue. The primary function of lymphocytes is to produce antibodies if toxic foreign materials make their way into the tissue. In contrast, anti-toxins are produced by eosinophil to counter the toxins and their effects produced by pathogens. Eosinophil ingests particles that form when toxic foreign materials and antibodies react (Adeyemo and Longe 2007; Oyeagu et al. 2019c). The WBCs protect the body against infections, and they are the immune cells. According to Toghyani et al. (2010), the production of leucocytes increases more when infection occurs because they are the first line of body defense. It is safe to argue that hens that received dietary oregano in our study were not fighting against diseases or stress because antibody constituents (lymphocyte, monocyte, and eosinophil) were lower than the control diet. The result explains why the heavy ecotype fed dietary oregano increased egg production, egg weight, and efficiency of feed utilization. Oregano can be used as a phytotherapeutic agent because the essential oil of oregano is toxic against phytopathogenic bacteria and fungi. The active ingredients in oregano are known for their anti-inflammatory properties. It also exhibits antioxidant and anti-tumor activity (Zeytinoglu et al. 2003; Balusamy et al. 2018). The properties of oregano and oregano extracts are the reason for their increased usage in food and feed. They also complement the antibodies in the blood in protecting the system against pathogens (Arpášová et al. 2014). The authors also reported that oregano plays a significant role in inhibiting diseases or pathogens in the animal system. They also give more protection to the recipient's body.

Hemoglobin and red blood cells (RBC) increased in heavy ecotype hens fed dietary oregano compared to those provided control diet. The RBC contains components of hemoglobin (heme, iron, globulin, and protein). Hemoglobin forms oxy-hemoglobin as it joins with oxygen in the blood, and it carries the oxygen from the lungs to needy tissues. The RBC and hemoglobin lower concentration recorded in the present study may be the reason for the poor feed efficiency, low egg production with a reduced egg weight of hens fed the control diet. According to Adejinmi et al. (2000) and Mbhele et al. (2019), RBCs (erythrocytes) in animals undergo some degradation process in the presence of anti-nutrients. The lower concentration of RBC recorded for hens fed the control diet may be attributed to different forms of stress, such as anti-nutrients in diets and high fiber diets. The pressure from this stress often causes an oxygen reduction – carrying capacity (anemia) in the blood, which leads to a decrease in performance (lower egg production, egg weight, and inefficient FCR) of the hen (Mohammed and Oloyede 2009; Oyeagu et al. 2019c). Consequently, there is a suggestion that dietary oregano used in the present research trial improved egg weight, egg number, and feed conversion efficiency. There may be physiological or health difficulties that translate to poor feed conversion efficiency, egg number, and egg weight of hens that received the control.

Egg Lipid Profile

There were increased high-density lipoprotein (HDL) values and decreased cholesterol and LDL of eggs from hens-fed dietary oregano. Cholesterol can be described as a fat-like material found in all the cells of the body and the blood. Cholesterol production takes place in the liver, and it is also in animal diets. Cholesterol contributes to the body system's proper functioning but increases the risk of coronary artery disease (heart disease) when found excess in the blood. Furthermore, too much cholesterol in the egg poses a higher health risk to the consumers. The bioactive components of oregano include carvacrol and thymol. Feed supplementation with oregano has a positive effect on meat and egg production. It improved performance, enhanced digestive enzyme activities, increased antioxidant enzyme activities, reduced lipid oxidation, and decreased cholesterol content in the egg (Mendoza-Ordoñez et al. 2020) with no antagonistic effect on the birds (Abdel-Wareth 2016). The biological action of oregano on the reduced build-up of cholesterol may be due to the bioactivities of thymol, which prevents the creation of 3–hydroxy–3–methylglutaryl coenzyme A (HMG - CoA) reductase known as a regulatory enzyme that produces cholesterol (Bampidis et al. 2005).

Oregano (thymol and carvacrol) dietary inclusion decreased blood lipid fractions and increased HDL in Japanese quail and their eggs (Cetingul et al. 2009), and reduced LDL content in broilers (Yang et al. 2003). However, dietary carvacrol and thymol (bio-components of oregano) reduce triglyceride, LDL, and cholesterol and increase the HDL concentration in quails (Khaksar et al. 2012). The positive effects of the bio-components of oregano (thymol and carvacrol) on quail and broilers regarding reduced cholesterol, LDL, and increased level of HDL (Govinthasamy et al. 2016) are also found in poultry eggs as reported in the present study. Therefore, feed manufacturers and poultry producers/farmers should be concerned about using phytogenic feed additives such as oregano in poultry egg production to reduce cholesterol and LDL content with a high HDL content. Consequently, this will ensure that consumers purchase healthy eggs with zero health risks. Higher density lipoprotein contains proteins that transport fat molecules (lipids) through the body within the blood. High-density lipoprotein can dissolve cholesterol deposited from the vascular walls and transports the cholesterol back to the liver via the arteries, where it is catabolized. Thus, HDL protects against the harmful effects of LDL on blood vessels and thus prevents cardiovascular disease. The majority (about 70%) of the total cholesterol in the body consists of LDL. Low-density lipoproteins carry cholesterol in the blood from the liver to the body cells. Higher low-density lipoprotein in the body increases cholesterol storage. The stored cholesterol is found on the walls of the blood vessels with higher health risks. These risks can lead to atherosclerosis and an increased risk of cardiovascular diseases such as heart attacks or stroke.

Conclusion

In conclusion, dietary supplementation of oregano improved the growth traits of hens. It promotes the production of eggs, egg weight and shell thickness, which indicates successful egg-laying farming. The blood biomarker analysis showed that hens fed dietary oregano received adequate immune protection. Also, dietary oregano positively influenced the egg lipid profile traits, which will go a long way to reduce the risk of cardiovascular diseases from poultry egg consumers.

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Author's Contributions

DCE, CUO and CE: Conceptualization. MCO, CUO, and CE: Data collation. CEO, DCE, and FBL; Formal analysis. DCE, CEO, AEA, and CE: Methodology. DCE, CEO, and FBL: Supervision. CEO, DCE, and AEA: Writing the original draft. CEO, DCE, AEA, CE, and FBL: Writing, review & editing. All authors have read and agreed to the published version of the manuscript.

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