



PRODUCTIVE, PHYSIOLOGICAL AND IMMUNOLOGICAL EFFECT ROSEMARY LEAVES MEAL (ROSEMARINUS OFFICINALIS) SUPPLEMENTING TO BROILER DIET.

Asmaa Sh. Elnaggar¹; Mervat A. Abdel-Latif²; M.I.El-Kelawy³ and H.S. Abd EL-Hamid⁴

¹ Dep. of Anim. and Poultry Prod., Fac. of Agric., Damanhour Univ.

² Dep. of Nut. and Vet. Clinical Nut., Fac. of Vet. Med., Damanhour Univ.

³ Dep. of Poultry Prod., Fac. of Agric. (New Valley), Assiut Univ.

⁴ Dep. of Poultry and Fish Dis., Fac. of Vet. Med., Damanhour Univ.

Corresponding author: Asmaa Sh. Elnaggar. Email: asmaaelnaggar85@yahoo.com

Received: 12/08/2016

Accepted: 07/09/2016

ABSTRACT : This study was conducted to evaluate the effect of rosemary leaves meal as a natural antioxidant in broiler diet on growth performance, blood parameters and the immune response of broiler chickens. A total of 150 Cobb chicks were assigned equally into five treatment groups. The chicks were fed the same basal diet and were submitted to the following dietary treatments: the first group fed a basal diet (control), while the other four groups were fed basal diet supplemented with 0.25, 0.5, 0.75 and 1.0 % of rosemary leaves meal. Chicks fed diet with 0.25% rosemary leaves meal had significantly ($P < 0.05$) greater production performance than the control group. Feed intake and total cost were significantly decreased in chickens fed diet with 0.25% and 0.5% rosemary leaves meal than those fed diet with 0.75 and 1.0 % of rosemary leaves meal and control group. Rosemary leaves meal had significantly improved the digestibility of crude protein and Ash. Feeding diet with rosemary leaves meal significantly decreased serum urea, creatinine, alanine amino transferase, triglycerides, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) while increased glucose, total protein, triiodothyronine, thyroxine, glutathione, hemoglobin, packed cell volume, red blood cell, white blood cell, lymphocyte, monocytes, mean corpuscular hemoglobin concentration (MCHC), globulin, globulin- γ , bacteriophage activity, lymphocyte transformation test (LTT), immunoglobulins (IgY, IgM and IgA), interferon-gamma ($IFN\gamma$), interleukin-2 (IL2), interleukin-10 (IL10), phagocytic activity and index compared to control group. Therefore, rosemary leaves meal at 0.25% could be considered as a natural antioxidant in broiler diet, potential growth promoter and immune stimulant for poultry.

Keywords: Rosemary- Performance- Hematology- Immunology- Broiler.

INTRODUCTION

The oxidation of lipids in food results in the development of spoilage, off-flavor, rancidity and deterioration of such products turning them unacceptable for the consumer. There is an increasing interest in the use of natural antioxidants, such as phenols isolated from plants to avoid undesired food borne diseases (Shylaja and Peter, 2004). Herbs can improve digestion, metabolism, antibacterial actions and immune function of animals. Rosemary (*Rosemarinus officinalis*) is an aromatic plant contains phenolic acids; phenolic diterpenoid bitter substances; triterpenoid acids; flavonoids; 1.2 to 2.5% volatile oil and tannins (Leung and Foster, 1996). Tomei *et al.* (1995) found that the most important constituents of the essential oil obtained from rosemary leaves were camphor (32.33%), 1, 8-cineole (14.41%) and alpha-pinene (11.56%). Also, Ghazalah and Ali (2008) reported that the main active components in essential oil of rosemary leaves were camphor (11-16%), aloha-pinene (15-20%) and cineole (30-35%) whereas, essential oil of rosemary leaves ranged between 1.4 - 1.6%. Rosemary and its constituents were known to have powerful antioxidant activity (Al-Kassie *et al.*, 2011), antimicrobial, antiviral, anti-inflammatory and anticarcinogenic activities (Aherne *et al.*, 2007). Also, it can delay the rancidity in poultry products (Karpinske *et al.* 2000). Feeding diet with rosemary significantly improved feed conversion ratio (Al-Kassie, 2008), nutrients digestibility of broiler diets (El-Husseiny *et al.*, 2002), improve productively, immunological performance and carcass characteristics (Osman *et al.*, 2010). This study aimed to investigate the effects of rosemary leaves meal as a natural antioxidant in broiler diet on performance, blood hematological and biochemical

content as well as the immune response of broiler chickens.

MATERIALS AND METHODS

The study was conducted at the Poultry Research unit, Damanshour University from April to May 2015.

Chicks and supplements

One hundred and fifty unsexed one-day-old Cobb broiler chicks obtained from commercial hatchery, were randomly distributed into five groups, each in 5 replicates of 6 birds per replicate and reared on similar managerial conditions. The chicks were fed the same basal diet and were submitted to the following dietary treatments: the first group fed a commercial broiler basal diets without supplementation (control), while the other groups fed such basal diet supplemented with 0.25, 0.5, 0.75 and 1.0 % of rosemary leaves meal. The experimental diets were formulated according to NRC (1994). Chicks were fed basal diet containing 22.9% and 3042, 21.4% crude protein and 3103 kcal/kg during the starter and grower periods, respectively.

Housing and husbandry

Chicks were housed on deep litter (10 birds/1m²) in semi-opened house. Chicks were fed *ad libitum* the experimental diets and given free access to water. A light schedule similar to commercial condition was 23 h light until 7th day followed by 20 h light from 8th day to through the experimental period until 3 day before slaughter test (8-36 days of age). The average outdoor minimum and maximum temperature and relative humidity during the experimental period was 22C° and 24 C° and 55.7 % and 58.7%, respectively. The brooding temperature (indoor) was 32, 30, 27 and 24-21 C° during 1-7, 8-14, 15-20 and 21-39 days of age (declined gradually).

Data collection

Performance parameters including body weight at 7 and 39 days of age, voluntary feed intake, feed conversion ratio and production index were measured weekly throughout the experimental period (7-39d) of age (Attia *et al.*, 2012). Apparent digestibility of dry matter, crude protein, ether extract, crude fibre, and crude ash was done using five birds per treatment housed individually in metabolic cages /treatment using total collection method as cited by Abou-Raya and Galal (1971). Nitrogen, ether extract, crude fibre and ash content of the excreta as well as those of feed were determined according to AOAC (2004). Economical evaluation for all experimental treatment diets was made (Zeweil, 1996). At 39 d of age, 3 chicks were taken randomly from each treatment, slaughtered and the dressed weight was calculated. The carcass organs and parts were expressed as relative to live body weight.

At 39 d of age serum samples were collected from three birds of each treatment. Glucose concentration (mg/dl) was measured according to Trinder (1969). total protein (g/dl) (Henry *et al.*, 1974), albumin (g/dl) (Domas, 1971), globulin (g/dl) (Coles, 1974) and different types of globulin (α -globulin, β -globulin and γ -globulin) were determined according to Bossuyt *et al.* (2003). In addition, serum samples were assigned for determination of creatinine and urea (Bartles *et al.*, 1972), triglycerides (Fossati and Prencipe, 1982), total cholesterol (Stein, 1986), HDL (Lopez-Virella, 1977), while LDL was determined according to (Friedewald *et al.*, 1972). The activity of serum aspartate amino transferase, and serum alanine amino transferase, were estimated according to Reitman and Frankle (1957).

Besides, five blood samples were collected from each treatment to determine number of red blood cell, white blood cell and different types of leukocytes. Packed cell

volume (%), Hemoglobin concentration and red cell indices (MCH and MCHC) were determined according to the following equations:

Mean Corpuscular Hemoglobin (MCH) (Pg) = $\text{Hb} \times 10 / \text{Red blood cell}$

Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl) = $\text{Hb} \times 100 / \text{Packed cell volume}$

Total antioxidant capacity was determined according to Koracevic *et al.* (2001), Superoxide dismutase activity (Misra and Fridovich, 1972), Glutathione peroxidase activity (Paglia and Valentine, 1967) and Glutathione activity (Ellman, 1959). Phagocytic activity and index was determined according to Kawahara *et al.* (1991). Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells.

Phagocytic index (PI) = $\text{Number of yeast cell phagocytized} / \text{Number of phagocytic cells}$.

Serum immunoglobulins (IgY, IgM and IgA) were determined using commercial ELISA kits (Kamiya Biomedical Company, USA) according to Bianchi *et al.* (1995).

The contents of IL-2, IL-10 and IFN- γ were measured using chicken ELISA Kits (R&D Systems, Minneapolis, MN, U.S.A.). Measurements were conducted according to the manufacturer's instructions. Lymphocyte transformation test was determined following the method described by Balhaa *et al.* (1985). Serum bactericidal activity to *Aeromonas hydrophila* strain was determined according to Rainger and Rowley (1993). Serum lysozyme activity was measured with the turbidimetric method described by Engstad *et al.* (1992) and the results are expressed as one unit of lysozyme activity that defined as a reduction in absorbance at 0.001/min. Lysozyme activity = $(A_0 - A) / A$.

Statistical analysis

Data were analyzed by the GLM procedure (Statistical Analysis System (SAS), 2002) using one-way ANOVA with the following model:

$$Y_{ik} = \mu + T_i + e_{ik}$$

Where Y is the dependent variable; μ the general mean; T the effect of experimental treatments; e the random error.

Before analysis, all percentages were subjected to logarithmic transformation ($\log_{10} x + 1$) to normalize data distribution. The difference among means was determined using Duncan's new multiple range test (Duncan, 1955) at $P < 0.05$.

RESULTS

Chemical analysis of the experimental rosemary leaves

The results of chemical analysis shown in Table 1 indicated that the experimental rosemary leaves contain 8.62% moisture, 5.08% crude protein, 16.0% ether extract, 7.52% ash, 18.94% crude fiber and 43.84% nitrogen free extract. The cell wall of rosemary leaves contained high level of cellulose (16.08%), hemicellulose (6.82%) and lignin (6.03%). Furthermore, there are moderate amounts of some macro (Calcium, 2.45%; Potassium, 1.31%) and micronutrients (Zinc, 31.20 mg/kg; Manganese, 14.60 mg/kg; Copper, 3.40 mg/kg and Zinc 31.2 mg/kg).

Broiler chickens Performance

The production performance, economical efficiency and production index of broiler chickens fed diet supplemented with rosemary meal leaves during days 7-39 of age are shown in Table 2. Chicks fed basal diet supplemented with 0.25% of rosemary leaves had significantly greater body weight and body weight gain followed by those fed basal diet supplemented with 0.5, 0.75 and 1.0 % compared to the control group. Feed intake and total cost were significantly and similarly decreased in broiler chickens fed diet with 0.25% and 0.5% of rosemary leaves compared to those fed diet with, 0.75 and 1.0 % and control group. Chicks fed basal diet supplemented

with 0.25% of rosemary leaves had significantly better feed conversion ratio and total revenue followed by those fed basal diet supplemented with 0.5, 0.75, 1.0 % and the control group. Chicks fed basal diet supplemented with 0.25% of rosemary leaves had significantly better economical efficiency and production index followed by those fed basal diet supplemented with 0.5 % of rosemary leaves, both are higher than the control group.

Apparent digestibility of nutrients

Data concerning the effects of the dose of the rosemary leaves on the apparent digestibility of the nutrients of broiler chicks are shown in Table (3). The dose 0.25% of the rosemary leaves had a significant effect on the digestibility of crude protein and ash. Basal diet supplemented with 0.25% of rosemary leaves significantly increased the digestibility of crude protein compared to those fed diet with, 0.75 % of rosemary and control group. In addition, ash retention was significantly increased in broiler chickens fed diet with 0.25% of rosemary leaves than those fed diet with 0.50 % of rosemary leaves and control group.

Blood analysis

The biochemical constituents of broilers having diet with rosemary leaves are shown in Table 4. Rosemary leaves supplementation at all levels decreased serum urea and creatinine compared to control group. Moreover, Urea and creatinine were lower in chickens fed diet with 0.25% of rosemary leaves than that in the others. Serum aspartate amino transferase was lower ($P < 0.01$) in broilers fed diet with 0.25% and 0.50 % of rosemary than that in the others. However, all levels of rosemary supplementation decreased serum alanine amino transferase, triglycerides, cholesterol, HDL and LDL

compared to control group. Furthermore, triglycerides, cholesterol, HDL and LDL were lower in chickens on diet with 0.25% of rosemary than that in the others. Rosemary leaves supplementation at all levels especially 0.25% increased triiodothyronine and thyroxine compared to control group. Glucose and total protein were increased at all levels of rosemary supplementation compared to control group and total protein was higher in chickens fed diet with 0.25% and 0.50 % of rosemary than that in the others. However, albumin was lower in chickens on diet with 0.25% of rosemary than that in the others. Glutathione activity was higher ($P<0.05$) in chickens on diet with 0.25% of rosemary leaves than that in the others. Total antioxidant capacity and superoxide dismutase was higher in chickens on diet with 0.25% and 0.50 % of rosemary leaves than that in the others. Furthermore, glutathione peroxidase was higher in chickens on diet with 0.25%, 0.50 % and 0.75% of rosemary leaves than these on diet with 1.00% of rosemary leaves and control group. Feeding diet with rosemary leaves significantly increased hemoglobin, packed cell volume, red blood cell, white blood cell, lymphocyte, monocytes and MCHC compared to control group and was higher ($P<0.05$) in chickens on diet with 0.25% of rosemary leaves than that in the others. Mean corpuscular hemoglobin (MCH) was higher ($P<0.05$) in chickens on diet with 0.25% of rosemary leaves than those on diet with 1.00% of rosemary and control group. In addition, basophils was higher ($P<0.05$) in chickens on diet with 0.50% and 1.00% of rosemary leaves than that in the others (Table 5).

Immunization parameters

Feeding diet with rosemary leaves significantly increased globulin and globulin- γ compared to control group whereas diet with 0.25% of rosemary leaves gave higher value of globulin, α –

globulin and globulin- γ and lower value of globulin – β than the other groups.

Feeding diet with rosemary leaves significantly increased bacteriocyte activity, lymphocyte transformation test, phagocytic activity and index compared to control diet whereas diet with 0.25% of rosemary gave higher value of lysozyme activity, bacteriocyte activity, phagocytic activity and index than the other groups. Furthermore, feeding diet with rosemary leaves significantly increased IgG, INF γ , IL2 and IL10 compared to control diet whereas diet with 0.25% of rosemary leaves gave higher value of IgA, IgM, IgG, INF γ and IL10 than the other groups (Table 6).

Carcass characteristics

Percentage of carcass dressing was higher ($P<0.05$) in broilers fed diet with 0.25% of rosemary leaves than in those fed diet with only 0.50% of rosemary leaves. Total edible parts was higher ($P<0.05$) in diet supplemented with 0.25% of rosemary leaves than that in control and 0.50 and those having 0.75% of rosemary leaves. Feeding diet with rosemary leaves significantly decreased abdominal fat compared to the control, while no significant effect was observed due to rosemary leaves between different groups regarding percentage of thymus (Table 7).

DISCUSSION

Considerable attention has been paid to herbal plants as favorable alternatives to antibiotic growth promoters in livestock production to improve the growth, feed conversion efficiency and reduce the cost of feed (Zakeri and Kashefi, 2011). The main advantage of these compounds over antibiotics is that they do usually against any risk regarding bacterial resistance or undesired residues in animal products (Peric *et al.*, 2009). Rosemary leaves are among the plants which in some cases, demonstrated positive effect on health and performance of broiler chickens (Al-Kassie *et al.*, 2011 and Onyimonyi *et al.*, 2012). But, reports about the value of

the inclusion of these plants as growth promoters in poultry nutrition are limited and many of researches still under study about the ideal percentage that is used of it. In the present study, different dietary levels of rosemary leaves were evaluated for their effect on performance, haematological, biochemical and immunological parameters.

At the end of the growing period, the analysis of variance of the obtained results indicated that chicks fed basal diet supplemented with 0.25% of rosemary leaves had significantly greater body weight, body weight gain and total revenue and better values of feed conversion ratio, economical efficiency and followed by those fed basal diet supplemented with 0.5, 0.75 and 1.0 % of rosemary leaves compared to the control group. Feed intake and total cost were significantly and similarly decreased in chickens fed diet with 0.25% and 0.50 of rosemary leaves than those fed diet with 0.75 and 1.0 % of rosemary leaves as well as control group. The level of rosemary leaves had significantly improved the digestibility of crude protein and Ash which reflected on the improvement of performance. These results agree with finding of Basmacioglu *et al.* (2004). Similarly, Al-Kassie (2008) showed that supplementation of anise seeds at 1% and rosemary leaves at 1% in broiler diets significantly improved the daily body weight gain and feed conversion ratio. Such herbal plants could be considered as a potential growth promoter for poultry due to digestive stimulating effect, antimicrobial effect and positive effect on performance. The decrease in body weight and body weight gain with increasing rosemary leaves level may be due to impeding the utilization of nutrients in chicks by the high crude fiber content being cellulose in particular from the cell walls of rosemary leaves. On the other hand, Abd

El-Latif *et al* (2013) showed that supplementation of broiler diets with rosemary essential oil had no growth promoting effect.

Supplementing rosemary leaves at all levels decreased serum triglycerides, cholesterol, HDL and LDL. The reduced content of total cholesterol and LDL may reflect the hypocholesterolemic properties attributed to the defatted part of the leaves which are rich in fibrous (25.24 %) content and may block intestinal cholesterol absorption (Lansky *et al.*, 1993). Conversely, some authors observed that dietary rosemary did not significantly affect cholesterol level in broilers (Osman *et al.*, 2010). The effects of herbal plants on blood lipid profile have been shown to be controversial. Hyperlipidemic effects were seen with some plants (Majid *et al.*, 2010) and hypolipidemia was reported with others. All levels of rosemary leaves especially 0.25% increased glucose, total protein, triiodothyronine, thyroxine, glutathione, total antioxidant capacity and superoxide dismutase while, decreasing urea, creatinine, albumin, alanine amino transferase and aspartate amino transferase compared to the other groups. The results showed that rosemary leaves had no deleterious effect on either kidney or liver functions. These results explained the improvement of performance as rosemary and its constituents were known to have antibacterial, antifungal and powerful antioxidant activities due to the presence of phenolic compounds (Al-Kassie *et al.*, 2011). In addition, feeding diet with rosemary leaves significantly increased hemoglobin, packed cell volume, red blood cell, white blood cell, lymphocyte, monocytes, MCHC compared to control group whereas, diet with 0.25% of rosemary gave higher value for all of these parameters. These changes could be attributed either to a direct stimulating

effect of these herbs on the hematopoietic tissue or to the production of specific or non-specific antibodies against different antigens (Khodary *et al.*, 1996). On the other hand, others stated that incorporation of rosemary oils into the diet of broilers did not affect the normal haematological integrity of the birds (Onyimonyi *et al.*, 2012). These effects could be explained by the stimulatory effects of these oils on immune functions and improved immunocompetence of the birds.

Dietary supplementation with different levels of rosemary leaves had significantly increased serum content of globulin, globulin- γ , bacteriophage activity, lymphocyte transformation test, IgG, INF γ , IL2, IL10, phagocytic activity and index compared to control group where, diet with 0.25% of rosemary leaves gave higher value for all of these parameters. This indicated that herbal plants had a stimulant effect on the innate and adaptive immunity. In addition, these results demonstrate an immunoregulatory effect of rosemary on the cell mediated immunity through secretions of higher levels of cytokines (IFN- γ , IL-2 and IL-10) which improved resistance to intracellular pathogens. In this respect, Soltan *et al.* (2008) found that dietary anise seeds supplementation at different levels increased phagocytic activity, index and lymphocytes in broilers. These results are in agreement with those obtained by Ghazalah and Ali (2008) and

Abd El-Latif *et al.* (2013). In addition, the increase in the globulin fractions indicate the effective role of rosemary in increasing immunity due to its role in developing and protecting cells and inhibiting non-enzymatic oxidation (Houghton *et al.*, 1995). Supplementation of rosemary leaves at 0.25% increased dressing and total edible parts while decreasing abdominal fat, bursa and spleen percentages. These results are similar to those reported by Osman *et al.* (2010) who reported that rosemary levels at the higher level (1g./ kg diet) significantly increased ($P \leq 0.01$) dressing percentage as compared to those of the control. Similarly, Ghazalah and Ali (2008) found that rosemary supplementation at 0.5% increased carcass % numerically almostly at 1.5% and supplementing 2% of rosemary reduced abdominal fat more than at 0.5% levels.

CONCLUSION

Dietary supplementation with different levels of rosemary leaves (0.25, 0.5, 0.75 and 1%) in broilers diet had beneficial effect on performance, hematological, biochemical and immunological parameters especially 0.25% being was the best inclusion rate. However, increasing the levels of cell mediated immune markers (IFN- γ , IL-2 and IL-10) as a resulted using rosemary leaves needs further research to examine its role in the protection against intracellular pathogens in broiler chickens.

Table(1): Chemical analysis of rosemary leaves

| Components | Amount |
|---------------------------------------|---------------|
| Active components of essential oil, % | |
| Camphor | 13.61 |
| Alpha-pinene | 17.29 |
| Cineole | 34.11 |
| Mineral Elements | |
| Potassium (%) | 1.31 |
| Calcium (%) | 2.45 |
| Copper (ppm) | 3.4 |
| Zinc (ppm) | 31.2 |
| Manganese (ppm) | 14.6 |
| Proximate analysis, % | |
| Moisture | 8.62 |
| Crude protein | 5.08 |
| Ether extract | 16 |
| Crude fibre | 18.94 |
| Ash | 7.52 |
| Nitrogen free extract | 43.84 |
| Cellulose | 16.08 |
| Hemicellulose. | 6.82 |
| Lignin | 6.03 |
| Essential oil | 1.33 |

Rosemary- Performance- Hematology- Immunology- Broiler.

Table (2): Effect of different levels of rosemary leaves (RL) on production performance, economical efficiency and production index of broiler chickens

| Traits | Control | RL 0.25% | RL 0.50% | RL 0.75% | RL 1.00% | <i>P value</i> | SEM |
|-----------------------|--------------------|-------------------|--------------------|--------------------|--------------------|----------------|-------|
| BW 7 d (g) | 173 | 178 | 174 | 180 | 177 | 0.236 | 2.76 |
| BW 39 d (g) | 1884 ^c | 2259 ^a | 2100 ^b | 2073 ^b | 2035 ^b | 0.007 | 41.37 |
| BWG 7-39 (g) | 1711 ^c | 2081 ^a | 1926 ^b | 1893 ^b | 1858 ^b | 0.002 | 39.79 |
| FI 7-39 (g) | 3700 ^a | 3471 ^c | 3515 ^c | 3644 ^{ab} | 3606 ^b | 0.003 | 32.3 |
| FCR 7-39 (feed/gain) | 2.18 ^a | 1.68 ^c | 1.86 ^b | 1.94 ^b | 1.96 ^b | 0.001 | 0.047 |
| Total cost (L.E) | 18.8 ^{ab} | 18.1 ^b | 18.5 ^b | 19.2 ^a | 19.3 ^a | 0.009 | 0.239 |
| Total revenue (L.E) | 24.5 ^c | 29.4 ^a | 27.3 ^{ab} | 26.9 ^{ab} | 26.5 ^{bc} | 0.006 | 0.784 |
| Economical efficiency | 30.4 ^c | 62.5 ^a | 47.8 ^b | 40.1 ^{bc} | 37.4 ^{bc} | 0.001 | 4.756 |
| Production Index | 222 ^c | 348 ^a | 295 ^{ab} | 275 ^{bc} | 269 ^{bc} | 0.002 | 2.181 |

SEM=Standard error of mean's; BW=body weight; BWG=body weight gain; FI=Feed intake; FCR= feed conversion ratio; L.E= Egyptian pound

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

Table (3): Apparent nutrients digestibility and ash retention (%) of broiler chickens fed diet supplemented with different levels of rosemary leaves (RL).

| Item | Control | RL 0.25% | RL 0.50% | RL 0.75% | RL 1.00% | <i>P value</i> | SEM |
|-----------------|-------------------|-------------------|--------------------|--------------------|--------------------|----------------|------|
| Dry matter | 66.9 | 71.3 | 66.8 | 66.2 | 66.2 | 0.273 | 1.81 |
| Crude protein | 59.1 ^b | 68.4 ^a | 63.7 ^{ab} | 61.0 ^b | 63.2 ^{ab} | 0.050 | 2.07 |
| Ether extract | 67.8 | 75.5 | 72.2 | 70.9 | 73 | 0.414 | 2.79 |
| Crude fiber | 12.1 | 15.6 | 12.4 | 13.7 | 13.9 | 0.526 | 1.56 |
| Ash retention,% | 30.2 ^b | 35.3 ^a | 30.3 ^b | 33.0 ^{ab} | 33.6 ^{ab} | 0.016 | 1.12 |

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

SEM, Standard error of mean's.

Table(4):Biochemical constituents of blood serum of broiler chickens fed diet supplemented with different levels of rosemary leaves (RL).

| Traits | Control | RL 0.25% | RL 0.50% | RL 0.75% | RL 1.00% | <i>P value</i> | SEM |
|------------------------|--------------------|--------------------|--------------------|---------------------|---------------------|----------------|-------|
| Urea (mg/dl) | 21.7 ^a | 18.0 ^d | 20.0 ^{bc} | 19.3 ^c | 20.7 ^b | 0.002 | 0.283 |
| Creatinine (mg/dl) | 1.13 ^a | 0.767 ^c | 0.933 ^b | 0.867 ^{bc} | 0.900 ^{bc} | 0.008 | 0.050 |
| AST(U/L) | 62.3 ^a | 56.3 ^b | 58.3 ^b | 58.7 ^{ab} | 59.3 ^{ab} | 0.029 | 1.189 |
| ALT (U/L) | 71.7 ^a | 66.0 ^{cd} | 68.0 ^{bc} | 64.3 ^d | 68.7 ^b | 0.002 | 0.762 |
| Glucose (mg/dl) | 73.3 ^b | 79.0 ^a | 77.3 ^a | 77.0 ^a | 77.7 ^a | 0.004 | 0.906 |
| Triglycerides (mg/dl) | 186 ^a | 171 ^c | 176 ^b | 175 ^b | 174 ^b | 0.001 | 0.653 |
| Cholesterol (mg/dl) | 216 ^a | 202 ^d | 207 ^c | 212 ^b | 207 ^c | 0.012 | 1.236 |
| HDL(mg/dl) | 52.0 ^a | 38.0 ^c | 43.7 ^b | 44.3 ^b | 45.3 ^b | 0.007 | 0.837 |
| LDL(mg/dl) | 53.3 ^a | 32.0 ^d | 42.3 ^c | 47.3 ^b | 48.3 ^b | 0.007 | 1.042 |
| T3 (ng / ml) | 2.15 ^d | 2.30 ^a | 2.21 ^{bc} | 2.20 ^c | 2.23 ^b | 0.011 | 0.010 |
| T4 (ng / ml) | 1.18 ^d | 1.38 ^a | 1.21 ^c | 1.25 ^b | 1.23 ^{bc} | 0.001 | 0.010 |
| Total protein (g/dl) | 4.97 ^c | 6.57 ^a | 5.83 ^b | 6.23 ^a | 5.70 ^b | 0.007 | 0.115 |
| Albumin (g/dl) | 2.73 ^{ab} | 2.33 ^c | 2.60 ^b | 2.77 ^{ab} | 2.93 ^a | 0.004 | 0.065 |
| TAC (mg/dl) | 411 ^c | 425 ^a | 417 ^b | 413 ^c | 413 ^c | 0.001 | 0.987 |
| GPX (mg/dl) | 0.313 ^c | 0.450 ^a | 0.433 ^a | 0.440 ^a | 0.343 ^b | 0.002 | 0.007 |
| GSH (mg/dl) | 955 ^b | 991 ^a | 972 ^{ab} | 970 ^{ab} | 975 ^{ab} | 0.018 | 3.505 |
| SOD (mg/dl) | 222 ^e | 255 ^a | 243 ^c | 247 ^b | 239 ^d | 0.006 | 1.068 |

a,b Values within a row with different superscripts differ significantly at $P < 0.05$.

SEM, Standard error of mean's; AST=aspartate amino transferase; ALT=alanine amino transferase; HDL=high-density lipoprotein; LDL=low-density lipoprotein; T3= triiodothyronine; T4=thyroxine; TAC=total antioxidant capacity; GPX =glutathione peroxidase; GSH= glutathione; SOD=superoxide dismutase

Rosemary- Performance- Hematology- Immunology- Broiler.

Table(5):Blood hematological of broiler chickens fed diet supplemented with different levels of rosemary leaves (RL).

| Traits | Control | RL 0.25% | RL 0.50 % | RL 0.75 % | RL 1.00 % | <i>P value</i> | SEM |
|--|--------------------|--------------------|--------------------|--------------------|--------------------|----------------|-------|
| RBC's (10 ⁶ /cmm ³) | 1.57 ^c | 2.07 ^a | 1.90 ^b | 1.87 ^b | 1.97 ^b | 0.015 | 0.033 |
| Hemoglobin (g/100ml) | 11.7 ^c | 17.0 ^a | 14.3 ^b | 15.0 ^b | 14.7 ^b | 0.031 | 0.346 |
| PCV % | 36.6 ^c | 45.3 ^a | 41.0 ^b | 42.7 ^b | 41.7 ^b | 0.001 | 0.622 |
| MCH (Ug) | 74.4 ^b | 83.0 ^a | 75.7 ^{ab} | 80.5 ^{ab} | 74.5 ^b | 0.005 | 2.571 |
| MCHC (%) | 31.8 ^b | 37.7 ^a | 35.1 ^a | 35.2 ^a | 35.2 ^a | 0.012 | 1.018 |
| WBC's (10 ³ /cmm ³) | 20.7 ^c | 27.7 ^a | 25.3 ^b | 24.7 ^b | 25.1 ^b | 0.004 | 0.356 |
| Lymphocytes (%) | 35.3 ^c | 45.0 ^a | 41.0 ^b | 41.0 ^b | 41.7 ^b | 0.002 | 0.365 |
| Monocytes (%) | 11.7 ^d | 16.3 ^a | 14.7 ^b | 14.0 ^c | 14.3 ^{bc} | 0.043 | 0.163 |
| Basophils, (%) | 0.333 ^b | 0.333 ^b | 1.00 ^a | 0.333 ^b | 1.00 ^a | 0.014 | 0.141 |
| Eosinophils, (%) | 12.33 ^c | 14.0 ^a | 13.3 ^{bc} | 13.0 ^{bc} | 13.67 ^b | 0.027 | 0.346 |

a,b Values within a row with different superscripts differ significantly at $P < 0.05$.

SEM, Standard error of mean's; RBC's=red blood cell; PCV=packed cell volume; MCH=mean corpuscular hemoglobin; WBC's=white blood cell

Table(6): Immune indices of broiler chickens fed diet supplemented with different levels of rosemary leaves (RL).

| Traits | Control | RL 0.25% | RL 0.50% | RL 0.75% | RL 1.0% | <i>P value</i> | SEM |
|---------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|----------------|-------|
| Globulin (g/dl) | 2.23 ^d | 4.23 ^a | 3.23 ^b | 3.47 ^b | 2.77 ^c | 0.017 | 0.132 |
| α -globulin (g/dl) | 0.53 ^b | 0.73 ^a | 0.80 ^a | 0.83 ^a | 0.80 ^a | 0.031 | 0.051 |
| globulin- β (g/dl) | 0.73 ^a | 0.467 ^b | 0.67 ^a | 0.70 ^a | 0.70 ^a | 0.001 | 0.028 |
| Globulin- γ (g/dl) | 0.97 ^c | 3.03 ^a | 1.77 ^b | 1.93 ^b | 1.27 ^c | 0.016 | 0.151 |
| LA (IU%) | 0.107 ^b | 0.177 ^a | 0.120 ^b | 0.123 ^b | 0.113 ^b | 0.005 | 0.006 |
| BA (%) | 34.0 ^c | 39.7 ^a | 37.3 ^b | 36.7 ^b | 36.7 ^b | 0.002 | 0.476 |
| LTT(%) | 20.7 ^c | 27.7 ^a | 25.3 ^b | 27.7 ^a | 27.0 ^a | 0.007 | 0.432 |
| PI (%) | 1.43 ^c | 2.10 ^a | 1.83 ^b | 1.80 ^b | 1.80 ^b | 0.021 | 0.048 |
| PA (%) | 16.0 ^d | 21.0 ^a | 19.3 ^b | 19.3 ^b | 17.7 ^c | 0.006 | 0.316 |
| IgA (mg/100 ml) | 77.0 ^{bc} | 84.3 ^a | 78.7 ^b | 76.0 ^c | 75.7 ^c | 0.002 | 0.735 |
| IgM (mg/100 ml) | 226 ^b | 313 ^a | 225 ^b | 226 ^b | 227 ^b | 0.001 | 2.535 |
| IgG (mg/100 ml) | 918 ^d | 988 ^a | 969 ^b | 963 ^b | 946 ^c | 0.012 | 4.127 |
| INF γ (pg/mL) | 4.00 ^c | 4.77 ^a | 4.37 ^b | 4.33 ^b | 4.33 ^b | 0.008 | 0.061 |
| IL.2 (pg/mL) | 6.57 ^b | 7.60 ^a | 7.50 ^a | 7.40 ^a | 7.50 ^a | 0.001 | 0.083 |
| IL10 (pg/mL) | 15.7 ^c | 20.7 ^a | 17.0 ^b | 18.0 ^b | 17.0 ^b | 0.002 | 0.337 |

a,b Values within a row with different superscripts differ significantly at $P < 0.05$.

SEM, Standard error of mean's; LA= lysozyme activity; BA=bactriocide activity ; LTT=lymphocyte transformation test; PI=phagocytic index; PA =phagocytic activity

Table (7): Carcass characteristics and relative weight of immune organs to live body weight of Cobb broiler chickens fed diet supplemented with different levels of rosemary leaves (RL).

| Traits | Control | RL 0.25% | RL 0.50% | RL 0.75% | RL 1.00% | P value | SEM |
|--------------------------|--------------------|--------------------|---------------------|----------------------|---------------------|---------|-------|
| Dressing, % | 71.1 ^{ab} | 72.8 ^a | 70.4 ^b | 71.5 ^{ab} | 71.9 ^{ab} | 0.054 | 0.522 |
| Total edible parts, % | 75.3 ^b | 77 ^a | 75.4 ^b | 75.4 ^b | 76.4 ^{ab} | 0.041 | 0.442 |
| Abdominal fat, % | 1.402 ^a | 0.192 ^c | 0.74 ^b | 0.971 ^b | 0.309 ^c | 0.002 | 0.133 |
| Spleen, % | 0.127 ^a | 0.078 ^c | 0.099 ^{bc} | 0.101 ^{abc} | 0.121 ^{ab} | 0.005 | 0.009 |
| Bursa, % | 0.101 ^a | 0.025 ^b | 0.029 ^b | 0.034 ^b | 0.034 ^b | 0.003 | 0.007 |
| Thymus,% | 0.321 | 0.299 | 0.377 | 0.259 | 0.358 | 0.088 | 0.031 |

a,b Values within a row with different superscripts differ significantly at $P < 0.05$.
SEM, Standard error of mean's.

REFERENCES

- Abd El-Latif, A. S.; Saleh, N. S.; Allam, T.S. and Ghazy, E. W. 2013.** The Effects of Rosemary (*Rosemarinus officinalis*) and Garlic (*Allium sativum*) Essential Oils on Performance, Hematological, Biochemical and Immunological parameters of Broiler Chickens. Brit. J. Poult. Sci., 2 (2): 16-24.
- Abou-Raya, A. K. and Galal, A. G. H. 1971.** Evaluation of poultry feeds in digestion trials with reference to some factors involved. ARE J. Anim. Prod., 11: 207-221.
- Aherne, S. A.; Kerry, J. P. and O'Brien, N. M. 2007.** Effects of plant extracts on antioxidant status and oxidant-induced stress in Caco-2 cells. Brit. J. Nutr., 97: 321-328.
- Al-Kassie, G. A. M. 2008.** The Effect of Anise and Rosemary on Broiler Performance. Inter. J. Poult. Sci., 7: 243-245.
- Al-Kassie, G. A. M.; Abd-Al-Jaleel, R. A. and Mohseen, A.M. 2011.** The effect of a mixture of anise and rosemary on broiler performance. Agric. Biol. J. N. Am., 2: 1279-1282.
- AOAC 2004.** Official methods of analysis. 18th ed., Association of Official Analytical Chemists, Washington, DC, USA.
- Attia, Y. A.; El-Tahawy, W. S.; Abd Al-Hamid, A. E.; Hassan, S. S.; Nizza, A.; El-Kelawy, M. I. 2012.** Effect of phytate with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital. J. Anim. Sci., 11: 303-308.
- Balhaa, R. L.; Hinz, H. H.; Luders, H. and Siegmann, O. 1985.** Clinical experiences with the drugs for lymphocyte transformation in chickens and turkey flocks. Tierarztliche umschau 43: 507-508.
- Bartles, H.; Bohmer, M. and Heierli, C. 1972.** Serum creatinine determination without protein precipitation. Clin. Chim. Acta 37: 193-197.
- Basmacioglu, H.; Tokusoglu, Ö. and Ergül, M. 2004.** The effect of oregano and rosemary essential oils or alpha-tocopherol acetate on performance and lipid oxidation of meat enriched with n-3 PUFA's in broilers. S. Afr. J. Anim. Sci. 34:197-210

- Bianchi, A. T. J.; Moonen-Leusen, H. W. M.; van der Heijden, P. J. and Bokhout, B. A. 1995.** The use of a double antibody sandwich ELISA and monoclonal antibodies for the assessment of porcine IgM, IgG, and IgA concentrations. *Vet. Immunol. Immunopathol.* 44:309–317.
- Bossuyt, X.; Lissoir, B.; Mariën, G.; Maisin, D.; Vunckx, J.; Blanckaert, N. and Wallemacq, P. 2003.** Automated Serum Protein Electrophoresis by Capillarys. *Clin Chem Lab Med;* 41(5):704–710.
- Coles, E. H. 1974.** Veterinary clinical pathology . IST ED. 211-213 W.B. saunder, company, Philadelphia, London, Toronto.
- Doumas, B. 1971.** Colorimetric determination of serum albumin. *Clin. Chim. Acta* 31: 400-403.
- Duncan, D. B. 1955.** Multiple range and multiple “F” test. *Bio- metrics.* 11,1-42.
- El-Husseiny, O.; Shalash, S. M. and. Azouz, H. M. 2002.** Response of broiler performance to diets containing hot pepper and/ or fenugreek at different metabolizable energy levels. *Egypt. Poult. Sci.*, 22: 387-406.
- Ellman, G. L. 1959.** Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, 82: 70-77.
- Engstad, R. E.; Robertsen, B. and Frivold, E. 1992.** Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. *Fish and Shellfish Immun.* 2: 287 - 297
- Fossati, P. and Prencipe, L. 1982.** Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide *Clin. Chem.* 28: 2077-2080.
- Friedewald, W. T.; Levy, R. T. and Frederickson, D. S. 1972.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.* 18: 499-502.
- Ghazalah, A. A. and Ali, A. M. 2008.** Rosemary leaves as a dietary supplement for growth in broiler chickens. *Inter. J. Poult. Sci.*, 7: 234-239.
- Henry, R.; Cannon, D. and Winkelman, J. 1974.** Clinical chemistry, principles and techniques, 2nd edition, Harper and Row, New York , USA
- Houghton, J.; Zarka, R.; Heras, B. I. and Houtt, J. R. S. 1995.** Fixed oil of *Nigella satriva* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.*, 61: 33-36.
- Karpinske, M.; Borowski, J. and Danowska-Oziewicz, M. 2000.** Antioxidative activity of rosemary extract ion lipid fraction of minced meat balls during storage in a freezer. *Nahrung* 44: 38-41.
- Kawahara, E.; Ueda, T. and Nomura, S. 1991.** *In vitro* phagocytic activity of white spotted shark cells after injection with *Aeromonas salmonicida* extracellular products. *Gyobo kenkyu, Japan* 26: 213-214.
- Khodary, R. M.; El-Azzawy, M. H. and Hamdy, I. R. 1996.** Effect of nigella sativa on egg production, hatchability percentage and some biochemical values in laying hens with reference to fertility in cockerels. *Proceedings of the 7th Scientific Congress, November 17-19, Assiut, Egypt*, pp: 91-106.
- Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S. and Cosic, V. 2001.** Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.*, 54: 356-361.
- Lansky, P. S.; Schilcher, H.; Phillipson, J. D. and Loew, D. 1993.** Plants that lower cholesterol. *First World Congress on Medicinal and Aromatic Plants (WOCMAP) for human welfare,*

- Maastricht, Netherlands, Acta-Horticulture, 332: 131-136.
- Leung, A. Y. and Foster, S. 1996.** Encyclopedia of Common Natural ingredients Used in Food, Drugs and Cosmetics, 2nd Ed., John Wiley and sons, Inc., New York.
- Lopez-Virella, M.F.; Stone, S.; Eills, S. and Collwel, J.A. 1977.** Determination of HDL-cholesterol using enzymatic method. Clin. Chem. 23: 882-884.
- Majid, T.; Mohsen, T.; Abas, A. G. and Sayed, A. T. 2010.** Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr. J. Biotechnol. 9: 6819-6825.
- Misra, H. P. and Fridovich, I. 1972.** The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170-3175.
- National Research Council, NRC 1994.** Nutrient requirement of poultry. National Academy Press, Washington, D.C., USA.
- Onyimonyi, A. E.; Chukwuma, P. C. and Igbokwe, C. 2012.** Growth and hypocholesterolemic properties of dry garlic powder (*Allium sativum*) on broilers. Afr. J. Biotechnol., 11: 2666-2671.
- Osman, M; Yakout, H. M.; Motawe, H. F. and Ezz El-Arab, W. F. 2010.** Productive, physiological, immunological and economical effects of supplementing natural feed additives to broiler diets. Egypt. Poultry Sci., 30: 25-53.
- Paglia, D. E. and Valentine, W. N. 1967.** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med , 70(1):158-169.
- Peri, L.; Ziki, D. and Luki, M. 2009.** Application of alternative growth promoters in broiler production. Biotech. Anim. Husbandry 25: 387-397.
- Rainger, G. E. and Rowley, A. F. 1993.** Antibacterial activity in the serum and mucus of rainbow trout, *Oncorhynchus mykiss* following immunization with *Aeromonas salmonicida*. Fish and shellfish Immun., 3: 475-482.
- Reitman, S. and Frankel, S. 1957.** A Method for determination of enzymatic activities. Am. J. Clin. Path., 287: 56-58.
- SAS Institute (2002).** SAS/STAT User's guide statistics. SAS institute INC., Cary. NC, USA.
- Shylaja, M. R. and Peter, K. V. 2004.** The functional role of herbal spices. In: Peter, K.V. ed. Handbook of herbs and spices, Volume 2: CRC Press, Woodhead Publishing, Boca Raton, Florida.
- Soltan, M. A.; Shewita, R. S. and El-Katcha, M. I. 2008.** Effect of dietary anise seeds supplementation on growth performance, immune response, carcass traits and some blood parameters of broiler chickens. Inter. J. of Poultry Sci., 7: 1078-1088.
- Stein, E. A. 1986.** Quantitative enzymatic colorimetric determination of total cholesterol in serum or plasma. In: Textbook of Clinical Chemistry. N. W. Tietz, editor. WB. Saunders, Philadelphia, USA Pp. 879-886.
- Tomei, P. E.; Cioni, P. L.; Flamini, G. and Stefani, A. 1995.** Evaluation of the chemical composition of the essential oils of some *Lamiaceae* from Serrania de Ronda (Andalucia, Spain). J. of Essential Oil Res. 7: 279-282.
- Trinder, P. 1969.** Enzymatic colorimetric determination of glucose in serum, plasma or urine. Ann. of Clin. Biochem. 6: 24-26.

Zakeri, A. and Kashefi, P. 2011. The comparative effects of five growth promoters on broiler chickens, humoral immunity and performance. J. Anim. Vet. Adv., 10: 1097-1101.

Zeweil, H. S. 1996. Enzyme supplements to diets growing Japanese quails. Egypt. Poul. Sci. J., 16: 535-557.

الملخص العربي

التأثيرات الإنتاجية والفسيوولوجية والمناعية لإضافة مسحوق أوراق إكليل الجبل لعلائق كتاكيت اللحم

أسماء شوقي النجار^١، مرفت عبد الحليم عبد اللطيف^٢، محمود إبراهيم الكيلوي^٣، حاتم صلاح عبد الحميد^٤
^١ قسم الانتاج الحيواني والداخلي - كلية الزراعة - جامعه دمنهور
^٢ قسم التغذية و التغذية الإكلينيكية- كلية الطب البيطري- جامعة دمنهور.
^٣ قسم إنتاج الدواجن - كلية الزراعة - جامعة أسيوط (فرع الوادي الجديد).
^٤ قسم أمراض الدواجن والأسمك - كلية الطب البيطري - جامعة دمنهور.

أجريت هذه الدراسة لتقييم ورق إكليل الجبل كمضاد أكسدة طبيعي في علائق كتاكيت اللحم على النمو ومكونات الدم والاستجابة المناعية لكتاكيت اللحم. تم استخدام عدد ١٥٠ كتكوت (كب) عمر ٧ ايام تم تقسيمها بالتساوي عشوائيا الى خمس مجموعات تجريبية. تم تغذيت الكتاكيت على العليقة الأساسية وكانت المعاملات التجريبية كالتالي: المجموعة الأولى تغذت على العليقة الأساسية بدون اي اضافة (كنترول) و الاربع المجاميع التجريبية الأخرى تغذت على العليقة الأساسية مع إضافة ٠,٢٥ و ٠,٥ و ٠,٧٥ و ١,٠٪ من مسحوق أوراق إكليل الجبل. الكتاكيت المغذاه على عليقة تحتوي على ٠,٢٥٪ من إكليل الجبل كانت افضل معنويا في الصفات الإنتاجية مقارنة بالكنترول. انخفض العلف المأكول والتكاليف الكلية معنويا في الكتاكيت المغذاه على عليقة تحتوي على ٠,٢٥٪ و ٠,٥٪ من إكليل الجبل عن تلك المغذاه على عليقة تحتوي على ٠,٧٥ و ١,٠٪ من إكليل الجبل ومجموعة الكنترول. حسنت أوراق إكليل الجبل معنويا معامل الهضم الظاهري للبروتين الخام والرماد. أدت التغذية على ورق إكليل الجبل إلى انخفاض معنويا في تركيز يوريا السيرم و الكرياتينين وانزيم ALT و الدهون الثلاثية والكوليسترول والبروتين الدهني عالي الكثافة (HDL) والبروتين الدهني منخفض الكثافة (LDL)، في حين ادت الي زيادة تركيز الجلوكوز، البروتين الكلي، هرمون T3، هرمون T4، الجلوتاثيون، الهيموجلوبين، حجم كرات الدم الحمراء، كرات الدم الحمراء، كرات الدم البيضاء، كرات الدم البيضاء الأحادية، متوسط تركيز الهيموجلوبين في كريات الدم الحمراء (MCHC)، الجلوبيولين، الجلوبيولين γ ، ومعامل تحويل الخلايا الليمفاوية (LTT) ونشاط مقاومة البكتريا، الجلوبيولينات المناعية (IgY - IgM - IgA)، انترفيرون جاما (IFN γ)، انترلوكين ٢ (IL2)، انترلوكين ١٠ (IL10)، والنشاط البلعمي ودليل النشاط البلعمي مقارنة بمجموعة الكنترول في حين أظهرت التغذية على ٠,٢٥٪ من إكليل الجبل قيم أعلى بالمقارنة مع المجموعات الأخرى.

الخلاصة: يمكن اعتبار ورق إكليل الجبل مضاد أكسدة طبيعي و محفز نمو و منشط مناعي في علائق كتاكيت اللحم.