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Productive Performance and Immunocompetence of Commercial Laying Hens Given Diets Supplemented with Eucalyptus

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Abstract: A study was conducted to determine the effect of Eucalyptus leaf powder supplementation on productive performance and immune response of brown Hy-line laying hens. Sixty brown Hy-line layers were equally divided into four groups. They were fed diet containing 0, 1, 2 and 3g Eucalyptus leaf powder/kg diet form 46 to 54 weeks of age. Egg production (weight and number) was recorded daily. Body weight and feed consumption were recorded every 4 weeks. Cell mediated immunity was determined at 54 weeks of age. At the end of the experiment, internal and external egg quality measurements were determined. The present results revealed that supplementation of Eucalyptus at the rate of 3g/kg diet significantly increased egg number compared to the other groups. Likewise, the hens fed a diet containing 3g Eucalyptus produced significantly higher egg mass compared to remaining groups. However, there was no significant difference among treated groups for egg weight. There was no significant difference among treated groups for all egg quality traits, except for breaking strength. Whereas the eggs produced from hen fed a diet containing 3g Eucalyptus had a significantly higher breaking strength compared to other treated groups. With respect to immune response, it could be noticed that the hens fed diets supplemented with 2 or 3g Eucalyptus were significantly hyper responder to PHA-P injection compared to other groups. Moreover, the Eucalyptus supplementation at the rate of 2 and 3g/kg diet significantly decreased the H/L ratio of laying hens. In summary, the current experiment has shown that the inclusion of Eucalyptus at the level of 3g/kg diet is useful for improving the productive performance and immunity of commercial laying hens.

Key words: Laying hens, immune response, eucalyptus

Introduction

In the commercial egg-type chicken industry, profit depends on the cost and nutritive value of the feed. In Egypt, egg production plays an important economic role in livestock production and agricultural sector. Nowadays, the inclusion of medicinal plants and residues of plants are widely used in chicken rations to improve productive performance.

In fact, plant essential oils have been used for many years as pharmaceuticals in alternative medicine and as a natural therapy. Many plants can be used as a source of essential oils particularly those of the Labiates family like rosemary, sage and oregano. The oil of Eucalyptus is used as antiseptic, antispasmodic and stimulant agents in bronchitis, asthma and minor respiratory complaints. A 1% ointment has been used in rhinitis and a 25% liminents as a rubefacient. The vapors from boiled leaves are often inhaled in asthma, diabetes, measles and rheumatism (El-Amary, 1993). Hmamouchi *et al*. (1992) indicated that oil of Eucalyptus globules has antibacterial activities against 9 microorganisms, including *salmonella type, Klebsiella spp, Streptococcus A, Proteus sp. Staphylococcus aureus*). Also, Mahran (1967) indicated that the oil of Eucalyptus globules has antiseptic properties. Leaves of Eucalyptus globules are used as an astringent in form of cigarettes for asthma. Medic *et al*. (1992) found some antibacterial activities of Eucalyptus species including Eucalyptus globules against *Escherichia coil*.

Therefore, this experiment was designed to evaluate the effect of inclusion of Eucalyptus leaf powder in diet on the productive performance and immune response of laying hens.

Materials and Methods

This experiment was conducted at poultry breeding farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. Sixty commercial brown Hy-line laying hens aged 46-week old were randomly assigned to four groups (15 each). The hens were individually housed in individual cages and kept in an open-sided house. All chickens were reared under the same environmental, managerial and hygienic conditions. Feed and water were supplied *ad libitum*. The hens received a typical layer diet containing 2800 ME kcal/kg and 18% CP to meet or slightly exceed the nutrient requirements recommended by NRC (1994). The hens were fed basal diet (control) or basal diet containing 1, 2 and 3 g Eucalyptus leaf powder/kg. The composition and calculated chemical analysis of the experimental diet are presented in Table 1.

Table 1: Composition and calculated chemical analysis of the experimental diet

Ingredient	%
Yellow corn	61.80
Soybean meal 44%	19.30
Corn gluten meal	2.90
Decorticated cottonseed meal	2.00
Corn gluten feed	4.00
Bone meal	1.80
Limestone	7.42
Salt	0.32
Vitamin and mineral premix*	0.40
DL-Methionine	0.04
L-Lysine	0.02
Total	100
Calculated chemical analysis	
ME (kcal/kg)	2800
Crude protein (%)	18.00
Crude fat (%)	2.90
Crude fiber (%)	2.80
Calcium (%)	3.75
Available phosphorus (%)	0.40

*Each 2.5 kg of vitamin and minerals premix contain: vit. A, 12 mIU; vit. D3, 4 mIU; vit E, 15g; vit. K, 2g; vit. B1, 1g; vit B2, 8g; vit. B6, 6g; vit B12, 10mg; niacin, 30g; bioten, 150mg; folic acid, 1g; pantothenic acid, 10g; choline chloride, 40mg; zinc, 60g; manganese, 70g; iron, 15g; copper, 5g; iodine, 1g; selenium, 0.15g.

Measurements and observations

Productive parameters: Body weight was individually recorded at 46, 50 and 54 weeks of age. Also, egg production (number and weight) was recorded daily from the 46 to 54 weeks of age. Internal and eggshell quality measurements were determined at 54 weeks of age.

The dimensions of eggs (width and length) were measured using digital caliper to calculate shape index. After the all eggs were first weighed to the nearest 0.1g and broken open, each egg yolk was separated from the albumen using a plastic egg separator, rolled on a damp paper towel to remove any adhering albumen and weighed. Albumen yield was determined by subtraction of the yolk and shell with shell membranes intact from the whole egg weight. The percentage of egg components (yolk, albumen and shell) was calculated as the ratio of egg component to egg weight multiplied by 100. Yolk diameter was determined with caliper (mm). Haugh units were calculated according to Williams (1997). Eggshell was weighed after drying to the nearest 0.01g. The thickness (mm) of the shell was measured at three different points in the middle part of the egg using a dial gauge micrometer. The shell breaking strength (kg/cm^2) was determined according to Fathi and El-Sahar (1996) using their eggshell strength apparatus.

Blood constituents: At 54 weeks of age, blood samples were taken from the brachial vein into heparinzed tubes from all birds. A portion of the fresh blood was used for hematocrit determination using capillary tubes and a microhematocrit centrifuge. The hematocrit figures were measured after spinning microhematocrit for 12 min. Plasma was obtained from blood samples by centrifugation for 10 min. at 4000 rpm and was stored at -2^{0}C until the time of analysis. The frozen plasma was allowed to thaw at room temperature prior to analysis. Plasma calcium, phosphorus, total protein, albumin, cholesterol, GOT and GPT were determined by enzymatic colorimetric methods using available commercial kits. The plasma globulin was calculated as the difference between plasma total protein and albumin.

***In vivo* cell-mediated immunity:** A phytohemagglutinin-P (PHA-P) injection assay (Cheng and Lamont 1988) was used to evaluate *in vivo* T-cell-mediated immune response of Hy-Line laying hens. Birds were injected intradermally in the wattle with 0.5 mg of PHA-P (Sigma Chemical Co., St. Louis, Missouri) in 0.1 ml of phosphate buffered saline (PBS) after marking the injection site. The thickness of wattle was measured (to nearest 0.01mm) at 0, 24, 48 and 72hrs after PHA-P injection. Wattle swelling was calculated as the difference between the thickness of the wattle prior to and after injection of PHA-P.

Heterophils / lymphocytes ratio: At 54 week of age, blood samples were obtained from each treatment for heterophil (H) and lymphocyte (L) enumeration based on the procedures of Gross and Siegel (1983). Briefly, one drop of blood being smeared on each of glass slides. The smears were stained using Wright's stain. Two hundred leukocytes, including granular (heterophils) and nongranular (lymphocytes) ones, were counted on different microscopic fields representing 200 cells and the heterophil to lymphocyte ratio was calculated.

Statistical analysis: Data were subjected to a one-way analysis of variance with treatment group effect using the General Linear Model (GLM) procedure of SAS User's Guide, 2001. When significant differences among means were found, means were separated using Duncan's multiple range tests.

Results and Discussion

Productive parameters: Impact of supplemental Eucalyptus on body weight, egg production parameters, feed consumption and feed conversion ratio of laying hens is presented in Table 2. It could be noticed that the body weight of laying hens did not significantly affected by supplemental Eucalyptus. However, the supplemental Eucalyptus at 3g significantly increased egg number compared to other groups. Inversely, there was no significant difference among treated groups for egg weight. Moreover, the hens fed a diet added 3g

Table 2: Productive parameters of laying hens as affected by Propolis supplementation

	Eucalyptus (g/kg)					
Age (wk)	0	1	2	3	Pooled SEM	Prob.
Body weight, g						
46	1574.8	1560.8	1577.6	1572.1	42.15	NS
50	1596.7	1590.0	1592.5	1597.6	51.14	NS
54	1610.0	1615.12	1620.4	1633.7	52.48	NS
Egg number, no.						
46-50	23.54	23.64	23.69	23.80	0.22	NS
50-54	22.43^{b}	22.72^{b}	22.70^{b}	24.15^{a}	0.28	0.01
46-54	45.97^{b}	46.36^{b}	46.39^{b}	47.95^{a}	0.27	0.01
Egg weight, g						
46-50	63.53	63.57	63.60	63.86	0.17	NS
50-54	63.72	63.77	63.81	63.21	0.20	NS
46-54	63.62	63.67	63.70	63.53	0.21	NS
Egg mass, g						
46-50	1495.50	1502.79	1506.68	1519.87	15.22	NS
50-54	1429.24^{b}	1448.85^{b}	1448.49^{b}	1526.52^{a}	21.14	0.01
46-54	2924.74^{b}	2951.64^{b}	2955.17^{b}	3046.39^{a}	27.16	0.01
Feed consumption, g						
46-50	3559.29^{a}	3501.50^{a}	3480.43^{ab}	3358.91^{b}	57.14	0.01
50-54	3487.35^{a}	3564.17^{a}	3476.38^{a}	3327.81^{b}	62.75	0.01
46-54	7046.64^{a}	7065.67^{a}	6956.81^{a}	6686.72^{b}	61.92	0.01
Feed conversion ratio						
46-50	2.38^{a}	2.33^{a}	2.31^{a}	2.21^{b}	0.02	0.01
50-54	2.44^{a}	2.46^{a}	2.40^{a}	2.18^{b}	0.03	0.01
46-54	2.41^{a}	2.39^{a}	2.35^{a}	2.19^{b}	0.03	0.01

^{a,b} Means within the same row with different letters are significantly differ.

Table 3: Interior and eggshell quality parameters as affected by Propolis supplementation

	Eucalyptus (g/kg)					
Item	0	1	2	3	Pooled SEM	Prob.
Egg weight, g	63.83	63.80	63.84	63.46	0.14	NS
Albumen, %	60.45	60.24	60.41	60.12	0.64	NS
Yolk, %	29.52	29.57	29.81	29.87	0.15	NS
Haugh unit	79.78	80.17	80.14	80.12	0.57	NS
Shape index	75.30	75.30	76.14	75.25	1.15	NS
Shell, %	9.87	10.19	9.78	10.01	0.24	NS
Shell thickness, mm	0.343	0.343	0.344	0.345	0.01	NS
Breaking strength, kg/cm^{2}	3.36^{b}	3.35^{b}	3.34^{b}	3.56^{a}	0.10	0.02

^{a,b} Means within the same row with different letters are significantly differ.

Eucalyptus produced significantly higher egg mass compared to remaining groups. With respect to feed consumption, the present results revealed that the laying hens fed a diet containing 3g Eucalyptus significantly consumed less feed compared to other treated groups. Supplemental Eucalyptus at 3g significantly improved the feed conversion ratio. Improve of feed conversion ratio in the Eucalyptus group (3g) may be due to improved birds health. Data presented in Table 3 showed that there was no significant difference among treated groups for all egg quality traits, except of breaking strength. Whereas the eggs produced from hen fed a diet added 3g Eucalyptus were significantly higher breaking strength compared to other treated groups.

Hematological parameters: Effects of supplemental Eucalyptus on some hematological parameters of laying hens are presented in Table 4. The present results showed that there was no significant difference among treated groups for all hematological parameters, except of plasma globulin, plasma calcium and GOT. Supplemental Eucalyptus at 3g significantly increased plasma globulin and plasma calcium compared to control-group. The laying hens fed a diet added 2g Eucalyptus were intermediated. With respect to liver function, it could be noticed that supplemental Eucalyptus at 3g significantly reduced GOT activity compared to control-group. Similar trend did not observe for GPT.

Table 4: Hematological parameters of laying hens fed different levels of propolis

Item	Eucalyptus (g/kg) 0	1	2	3	Pooled SEM	Prob.
Hematocrit level,%	33.15	33.56	33.17	33.40	2.10	NS
Total protein, g/dl	6.42	6.30	6.51	6.40	0.52	NS
Albumin, g/dl	3.67	3.56	3.51	3.21	0.14	NS
Globulin, g/dl	2.75[b]	2.74[b]	3.00[ab]	3.19[a]	0.16	0.01
Calcium, mg/dl	20.41[b]	20.51[b]	21.10[ab]	22.61[a]	0.54	0.05
Phosphorus, mg/dl	9.15	9.40	9.51	9.37	0.31	NS
Cholesterol, mg/dl	130.14	130.17	131.24	128.84	3.41	NS
GOT, U/L	40.30[a]	40.25[a]	40.00[a]	37.62[b]	0.98	0.01
GPT, U/L	15.20	15.21	15.42	14.67	0.12	NS

[a,b] Means within the same row with different letters are significantly differ

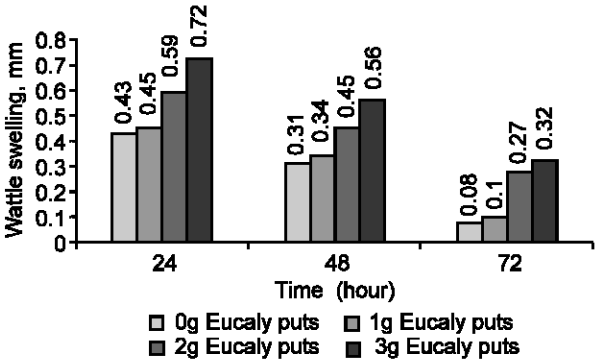

Fig. 1: Wattle swelling of laying hens as affected by Eucalyptus supplementation.

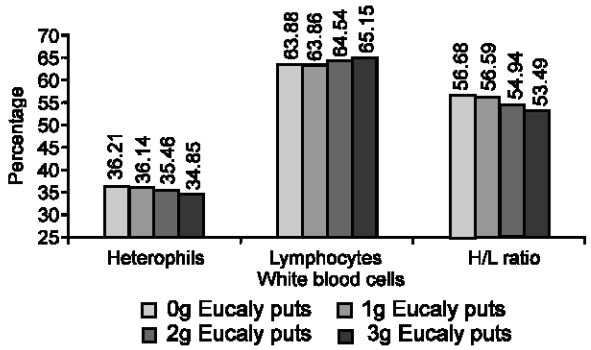

Fig. 2: Heterophils (H), lymphocytes (L) and H/L ratio of laying hens fed different level of Eucalyptus.

Immunocompetence parameters

***In vivo* cell-mediated immunity:** The PHA intradermally reaction, a T-lymphocyte-dependent response, has been well researched and has been shown to be a reliable indicator of *in viva* cellular immunity in poultry (Goto *et al*., 1978; McCorkle *et al*., 1980). The skin response reflects a complex series of physiological events such as mitogen-receptor and lymphocyte-macrophage interactions, release of chemical mediators, cellular proliferation and changes in vascularity (Chandra and Newberne, 1977). Histologically, PHA is strongly mitogenic to T-lymphocytesa and intradermali injections elicit macrophage infiltration and dense perivascular accumulations of lymphocytes 24h post-injection in chickens (Goto *et al*., 1978; McCorkle *et al*., 1980). The increased infiltration by basophils and eosinophils 24h post-injection has been described as a cutaneous basophil hypersensitivity response (Stadeckerm *et al*., 1977). *In vivo* cell-mediated immune response as measured by PHA stimulation (wattle) is presented in Fig. 1. It could be noticed that the hens fed diet added 2 or 3g Eucalyptus diet had significantly hyper responder to PHA-P injection compared to other groups. Many reports in the literature showed that essential oils stimulate digestive enzymes and have an in vitro antimicrobial activity against many bacteria. In fact, there are many studies regarding the antibacterial effects of *Origanum vulgare, Piper nigrum, Syzygium aromaticum and Thymus vulgaris* and essential oil components thymol, carvacrol, curcumin, piperin and eugenol against various strains of Clostridia including C. perfringnes and other bacteria such as *E. Coli, S. aureus, Salmonella typhimurium, Listeria monocytogenes and Y. enterocolotica* (Dorman and Deans, 2000; Fabio *et al*., 2003).

White blood cells differentiation: White blood cells differential count for laying hens fed different levels of Eucalyptus are summarized in Fig. 2. The present results revealed that the medium and high level of Eucalyptus significantly decreased the heterophils count and increased the lymphocytes count when compared with the control-group. In birds, the heterophil are phagocytic cells whose main is protection against invading microorganisms, whereas primary functions of lympho-involve cell-mediated and humoral immunity. Heterophils increase and lymphocytes decrease when are stressed, so that the ratio between them is a index of response to a stressor (Gross and Siegel, 1985). In accordance to H/L ratio, our results showed that the Eucalyptus supplementation at 2 and 3g/kg diet significantly decreased the H/L ratio of laying hens. The H/L ratio is a recognized measure of stress in birds (Davison *et al*., 1983; Gross and Siegel, 1983; Maxwell,

1993) that has become a valuable tool in stress research especially when combined with the convenience and repeatability of automated blood cell counts.

In summary, the current experiment has shown that the inclusion of Eucalyptus at the level of 3g/kg diet is useful for improving the productive performance and immunity of commercial laying hens.

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