Short Paper

Effects of using eucalyptus (*Eucalyptus globulus* L.) leaf powder and its essential oil on growth performance and immune response of broiler chickens

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Summary

The aim of this study was to evaluate the effects of eucalyptus leaf powder (ELP) and eucalyptus essential oil (EEO) on growth performance and immune response of broiler chickens. A total of 160 broiler chicks were assigned randomly into five dietary treatments from 7-42 days of age. Dietary treatments included: a control diet, control diets plus 1,000 or 3,000 mg/kg of ELP, and control diets plus 250 or 500 mg/kg of EEO. Dietary inclusion of ELP decreased body weight gain (BWG) during 7-28 days of age. Broilers fed diet containing 3,000 mg/kg of ELP had lower feed intake (FI) during 7-28 days compared to the other treatments. Broilers fed ELP or EEO had greater primary antibody response to sheep red blood cells (SRBC) compared to the control, but differences in secondary antibody response were not significant. In conclusion, dietary EEO has the potential to enhance immune response of broiler chickens.

Key words: Broiler chicken, Eucalyptus, Immunity, Performance

Introduction

Eucalyptus is a medicinal plant that belongs to Myrtaceae family, originated in Australia but found worldwide, especially in tropical and subtropical regions (Salari *et al*., 2006). In humans, eucalyptus leaf is used to reduce nasal congestion in common cold during cold winter months (Sadlon and Lamson, 2010). Eucalyptol (1,8-cineole) is a terpene oxide and major constituent (more than 70%) of eucalyptus essential oil (EEO), which is used as a flavoring agent in food products (De Vincenzi *et al*., 1996). The essential oil of eucalyptus has applications including nasal congestion relief, flu, sore throats, and bronchitis (Estanislau *et al*., 2001).

Though many aspects of bioactive properties of eucalyptus have been explored (Amakura *et al*., 2002; Soyingbe *et al*., 2013), little is known about its potential nutritive properties in broiler chickens. The aim of this study was to evaluate the effect of eucalyptus (*Eucalyptus globules* L.) leaf powder (ELP) or EEO on growth performance and immune response of broiler chickens.

Materials and Methods

Plant preparation and essential oil extraction

Eucalyptus leaves were purchased from a local supplier. They were air-dried in shade, finely ground using a laboratory mill to pass through 2 mm sieve, and designated as ELP. Eucalyptus essential oil was collected by subjecting ELP to a 3-h hydrodistillation using a Clevenger-type apparatus.

Broilers, management and dietary treatments

A total of 160 one-day-old unsexed broiler chicks (Ross 308) were purchased from a local hatchery and grown on litter until 7 days of age. Then, all broilers were randomly distributed into 20 wire-floored cages, each cage contained eight chicks. The house temperature was kept at 30°C at the first week and then decreased 3°C/week. A 23L:1D lighting schedule was applied.

Dietary treatments included: a control diet, control diets plus 1,000 or 3,000 mg/kg ELP, and control diets plus 250 or 500 mg/kg of EEO. The basal diets (Table 1) were formulated according to breeder recommendations (Ross, 2007). Feed and water were provided *ad libitum*.

Measurements

Body weight gain (BWG) and feed intake (FI) were calculated during 7-28, 29-42, and 7-42 days of age. From these, data feed conversion ratio (FCR) was calculated after correcting for mortality.

All broilers were intramuscularly immunized with killed vaccine of Newcastle Disease and Avian Influenza (H9N2 subtype) viruses at 9 days of age. Also, broilers were orally vaccinated against Newcastle Disease Virus (NDV) at 19 days of age. At 18 and 25 days of age, two broilers per replicate were inoculated via the brachial vein with 0.2 ml of 0.5% sheep red blood cell (SRBC) suspension. At 25 and 32 days of age, blood samples were taken and serum was collected by centrifugation (3,000 ×g, 10 min) and stored at -20°C. Antibody titers against SRBC and NDV were measured as described previously (Wegmann and Smithies, 1966; King and Hopkins, 1983). Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination.

Table 1: Ingredients and nutrient level of basal diets

Item (% unless noted)	Starter (7-28 days)	Finisher (29-42 days)
Ingredients		
Corn	53.23	61.80
Soybean meal (44% CP)	39.83	32.59
Soybean oil	2.35	1.51
Di-calcium phosphate	1.75	1.54
Limestone	1.35	1.14
Salt	0.37	0.37
DL-Methionine	0.36	0.31
L-Lysine HCL	0.26	0.24
Vitamin-mineral premix[a]	0.50	0.50
Calculated nutrients		
Metabolizable energy (kcal/kg)	2,900	2,950
Crude protein	22.50	20.00
Methionine + cysteine	0.94	0.84
Lysine	1.27	1.10
Calcium	1.05	0.90
Available phosphorus	0.45	0.40

[a] Supplied following per kilogram of diet: vitamin A: 9,000 IU; vitamin D_3: 1,000 IU; vitamin E: 18 IU; vitamin K_3: 2 mg; thiamine: 2 mg; riboflavin: 6.5 mg; vitamin B_6: 2 mg; vitamin B_{12}: 0.01 mg; niacin: 30 mg; choline chloride: 500 mg; vitamin C: 50 mg; calcium D-pantothenate: 8 mg; folic acid: 0.5 mg; Mn: 100 mg; Fe: 50 mg; Zn: 70 mg; Cu: 10 mg; I: 1 mg; Se: 0.2 mg

Statistical analysis

Data were analyzed using the GLM procedure of SAS software (SAS, 1996) as a completely randomized design. Differences among treatments were assessed using Duncan's multiple range tests (P<0.05).

Results

Growth performance

Dietary supplementation of ELP decreased BWG during 7-28 days of age, but dietary addition of EEO had no effect on BWG, FI, and FCR during different growth periods. Compared to the control, broilers receiving 3,000 mg/kg ELP in their diet had lower FI during 7-28 days of age. Broiler performance during 7-42 days of age was not affected by dietary treatments (Table 2). Mortality rate was not influenced by dietary treatments (data not shown).

Humoral immune response

Broilers fed diet supplemented with ELP and EEO had greater primary antibody response against SRBC compared to the control (25 days of age), while the highest response was observed in broilers receiving EEO supplementation. Secondary antibody response against SRBC (32 days of age) and NDV (25 days of age) were not affected by dietary treatments (Table 3).

Table 3: Effects of dietary supplementation of ELP and EEO on antibody responses in broiler chickens

Item	Primary SRBC (25 day)	Secondary SRBC (32 day)	NDV (25 day)
Control	1.21[d]	2.50	2.12
ELP (1000 mg/kg)	1.80[bc]	2.07	1.94
ELP (3000 mg/kg)	1.75[c]	2.13	2.10
EEO (250 mg/kg)	2.31[a]	2.05	2.22
EEO (500 mg/kg)	2.21[ab]	1.86	1.96
SEM	0.088	0.087	0.053
P-value	<0.0001	0.313	0.382

[a, d] Means within each column showing different lowercase letters are significantly different (P<0.05)

Discussion

There are few reports regarding the effect of ELP in poultry diet. In these studies, ELP had a positive effect on growth performance, which was associated with the manipulation of gut microbiota and improved immunity (Barbour *et al*., 2011; Hassan *et al*., 2011). In contrast, in the present study, broiler BWG was decreased by supplementation of 3,000 mg/kg ELP to the diet. The reason for this discrepancy may the presence of higher levels of tannins in our ELP sample. Tannins are present in eucalyptus (Moore *et al*., 2005) and other plant species, and usually considered as antinutrients because they interact with feed constituents such as proteins and minerals and make them unavailable (Nyman and Björck, 1989). Moreover, tannins are known to interfere with enzyme activities and to cause morphological damage to the gut (Mansoori *et al*., 2007). These mechanisms appear to decrease FI and nutrient absorption, therefore, decreasing growth performance.

Studies are rare on the effect of dietary EEO on

Table 2: Effects of dietary supplementation of ELP and EEO on growth performance in broiler chickens

Item	Body weight gain (g)			Feed intake (g)			Feed conversion ratio (g/g)		
	7-28 days	29-42 days	7-42 days	7-28 days	29-42 days	7-42 days	7-28 days	29-42 days	7-42 days
Control	1017.8[a]	976.4	1994.2[a]	1611.5[a]	2266.8	3878.3	1.58	2.32	1.94
ELP (1000 mg/kg)	975.4[b]	973.3	1948.5[ab]	1557.0[ab]	2222.7	3779.7	1.59	2.29	1.94
ELP (3000 mg/kg)	979.4[b]	935.3	1915.1[b]	1509.5[b]	2266.7	3776.2	1.54	2.46	1.97
EEO (250 mg/kg)	1017.9[a]	993.7	2011.6[a]	1631.0[a]	2275.6	3906.6	1.60	2.29	1.94
EEO (500 mg/kg)	1032.6[a]	977.1	2009.7[a]	1624.5[a]	2258.8	3883.3	1.57	2.31	1.93
SEM	7.045	9.07	12.82	15.55	29.65	38.75	0.01	0.03	0.02
P-value	0.010	0.390	0.044	0.040	0.990	0.079	0.499	0.789	0.970

[a, b] Means within each column showing different lowercase letters are significantly different (P<0.05)

poultry productivity. In a recent study, Barbour *et al*. (2015) showed that growth performance of coccidia-challenged broilers was improved when a commercial blend containing EEO was added to the drinking water. However, in the present study, supplementation of EEO to broiler diet did not affect their growth performance. This discrepancy may be related to the absence of coccidia (or other diseases) in our study. Nevertheless, the efficacy of a herbal dietary supplement can be affected by other variables as reviewed by Wallace *et al*. (2010).

The positive effect of eucalyptus on primary antibody response against SRBC is in agreement with those of Barbour *et al*. (2008). Though secondary antibody response against SRBC was not affected by dietary treatments in the present study. Similarly, dietary treatments did not affect antibody response against NDV, which was measured only after the second administration of NDV vaccine. In poultry, there are three classes of immunoglobulins, namely IgM, IgG, and IgA, from which IgM and IgG are the most abundant in blood (Danner *et al*., 2011). In primary antibody response, IgM is the main immunoglobulin whereas IgG is the predominant immunoglobulin in secondary antibody response (Mesquita Júnior *et al*., 2010). Taken together, these results indicate that eucalyptus secondary components may have a special effect on IgM production in primary antibody response. This speculation needs to be documented with further studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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