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Growth Performance, Blood Components, Immune Response, and Carcass Traits in Broiler Chickens Fed with *Eucalyptus globulus*

Research Article

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ABSTRACT

The research was conducted to determine the effect of various concentrations of *Eucalyptus globulus* leaves powder on growth performance, blood components, immune response, and carcass traits in one-day-old male (Hubbard) chickens. A total of 180-day-old broiler chicks were randomly divided into four groups i.e., R1, R2, R3, and R4. Group R1, as control, whereas groups R2, R3, and R4 were fed at the dose of 0.25, 0.50 and 0.75% of eucalyptus leaves powder/kg feed of broilers, respectively. The Results showed a significant ($P<0.05$) increased in body weight and maximum water consumption by treated groups. The highest feed intake was consumed by control group. Feed conversion ratio was improved in all treated groups. Carcass weight and dressing percentage were improved significantly ($P<0.05$) in treated groups. Weights of edible and non-edible organs showed non-significant ($P>0.05$) differences among treated groups. White blood cells significantly ($P<0.05$) increased in treated groups. The values of red blood cells and hemoglobin were non-significant ($P>0.05$) in treated groups. Glucose levels significantly ($P<0.05$) increased in treated groups, whereas cholesterol levels were recorded to decreased as non-significant ($P>0.05$) among treated groups. Levels of serum glutamic-pyruvic transaminase (SGPT) recorded a significant ($P<0.05$) increased among all treated groups as compared to control group. Treated groups with *Eucalyptus globulus* powder were observed to improve the immunity against Newcastle disease and infections bronchitis in birds as compared to control group. It is concluded that *Eucalyptus globulus* 0.75%/kg in feed showed as a useful replacement for antibiotics and would improve growth performance, blood components, and immune response of broiler chickens.

KEY WORDS blood components, broiler, carcass traits, *Eucalyptus globulus*, growth performance.

INTRODUCTION

Since few decades, the use of prophylactic medications and antibiotic growth promoters in animal feed has resulted in consequences such as the development of antibiotic-resistant and antibiotic residues in meat and other livestock products, poses a significant threat to public health and the environment (Al-Snafi, 2015). Usage of natural growth promoters and medicinal herbs in poultry is increasing day by day which also improves the production and health for both humans and animals, these herbal plants are rapidly increasing to replace the antibiotics and improve health and feed efficiency (Mancini *et al.* 2018; Mancini *et al.* 2019). Medicinal plants contain the bioactive compounds (i.e., esters, alcohols, acids, hydrocarbons, phenols, and steroids) which have positive effects on animal health and produc-

tion, other characteristics of phytogenic feed additives include an increase in the production of digestive secretions such as endogenous digestive enzymes, saliva, bile, and mucin. Use of herbal product is increasing in poultry diets due to better results in body weight gain, higher production rate, improvement in feed efficiency also improve processing and invigorate the resistant capacity in broilers (Ghazalah and Ali, 2008). Eucalyptus is one of those plants which can be used as growth promoter and its leaf extracts have antibacterial antiviral, antifungal, anti-inflammatory, valuable in treatment of oral disease, also contains antihistaminic and antioxidant activities (Salari *et al.* 2006). Eucalyptus is considered an evergreen tree cultivated in gardens, parks and on roadsides in the world and aromatic plant, used for food as synthetic flavoring agent, also used for preservation (Potts *et al.* 2000). Leaves of eucalyptus also contain polyunsaturated fatty acids, it's high in vitamin E and vitamin C, both of which help with oxidative stress resistance also comprising omega-3 and omega-6 due to their generalized beneficial health effects (Guimaraes *et al.* 2009). In addition, the leaves contain minerals like Zn, Cu, Mn, Na, K, P, Fe, Ca and Mg, which may help a good balance of nutrients (Nagpal *et al.* 2010; Leite *et al.* 2011). The improvement of feed conversion is due to the active metabolites and the valuable nutrients that found in eucalyptus leaves causing greater efficiency utilized of the assumption of powder and essential oil of some herbs like *Eucalyptus* might improve the palatability of feed due to their aromatic (1, 8-cineole) characteristics in eucalyptus could promote feed consumption when added to diets of poultry (Windisch *et al.* 2008). Nutritionally and therapeutically the *Eucalyptus* is very important due to comprehensive chemical composition like esters, ethers, carboxylic acids, ketones, aldehydes, alcohols, and hydrocarbons along with monoterpenes and sesquiterpenes (Hayat *et al.* 2015). Eucalyptus leaves supplemented significantly enhanced immunity due to linked the tannin concentration in *Eucalyptus* leaves, which has been shown to improve immunological response by having an immunomodulatory effect (Parisi *et al.* 2018; Fathi *et al.* 2019). Diet of broilers which was supplemented by eucalyptus leaf powder had better antibody response, have a special effect on IgM production in primary antibody response (Farhadi *et al.* 2017). In a diabetic animal show that extract of eucalyptus may decrease the glucose level in blood and it also proven by the rise of glutathione peroxide, catalase and superoxide action, and reduction of lipid peroxide in kidneys and liver (Khalaji *et al.* 2011). Supplementation of eucalyptus makes macrophages active and functional, also it activates broilers indicates that antibody titer of Bronchitis, infectious bursal disease and Newcastle was literally improved (Mesquita junior *et al.* 2010). *In vivo* study on broiler birds, gamma octalactone, which derived from leaf extracts of eucalyptus viminalis, shows that increased the blood plasma activity of digestive enzymes and had significant effect on antioxidant and metabolism (Duskaev *et al.* 2020).

Due to concerns about harmful effect of antibiotic growth promoters the interest of consumers is increasing in natural herbs. So, to reduce the feed cost and to improve the growth it is suggested that vegetable, herbs and plants should be used as growth promoters in poultry diets (Hashemi and Davoodi, 2012).

The medicinal and nutritional part of the eucalyptus is aromatic leaves, which has many benefits, especially in the medical field, (Sallam *et al.* 2010). Considering the importance, it has been planned to explore the outcome of eucalyptus leaves on production and health and immunity of broilers in our local conditions.

MATERIALS AND METHODS

A total of 180-day-old male (Hubbard) were purchased from the commercial hatchery and taken to poultry research station Sindh Agriculture University, Tandojam. Weighed the new arrival chicks and then divided them into four groups: R1, R2, R3, and R4. Three replicates were included in each group (15 birds per replicate). All chick groups were offered the deep litter housing system, one square foot area. The artificial brooder preparation was completed 24 hours before, the arrival of day-old chicks, with each group receiving one brooder. The brooding temperature was kept between 90 and 95 ˚F for the first week, then dropped weekly by 5 ˚F until the house temperature reached 70 ˚F. During brooding, 100-watt electric lamps were installed in each electric brooder. To regulate the brooding temperature, one thermometer was put near the brooder at a height of 6-12 inches. The lighting was provided by 60-watt LEDs mounted on an 8-foot-high ceiling. Chickens were vaccinated against Newcastle disease + Infectious Bronchitis at the day 3, on day 12 infectious bursal disease, against hydro pericardium syndrome at 17 days, against infectious bursal disease at 22 days, and against Newcastle disease at 24 days of age.

Eucalyptus globulus leaves were first dried in a room shadow for three days, then kept in an oven at 60 ˚C to decrease the moisture level up to 5%. The leaves were then ground manually with the help of a pestle and mortar. The ground powder was sieved to get a fine powder. In the end, it was mixed and supplemented in the basal diet. The chemical composition is present in Table 1 as described by Bouzabata *et al.* (2014).

All groups were fed on iso-caloric and iso-nitrogenous diet basal. The feeding program consisted of a starter diet of 1-21 days, and a finisher diet of 22-42 days (Table 2).

Group (R1) were fed a basal diet without supplementation of eucalyptus leaves powder.

The concentration of eucalyptus leaves powder in group (R2, was at the level of 0.25%/kg added in feed, group (R3) was 0.5%/kg in feed, and group (R4) was 0.75%/kg in feed, respectively. From 1 to 21 days, the birds were fed a starter diet, followed by a finisher diet from 22 to 42 days *ad-libitium*, and water were provided twice a day.

Table 1 Chemical composition of *Eucalyptus globulus*

Compounds	%
Pinene	0.2
Cymene	1.5
Limonene	1.3
Cineole	8.2
Pipertone	0.5
Eudesmol	0.2
Terpineol	0.4
Phellandral	3.8

Table 2 Composition of experimental diet

Ingredients	Starter feed (g/kg)	Finisher feed (g/kg)
Rice	316	400
Maize	100	100
Rice polish	150	160
Fish meal	85	80
Soya bean	70	55
Guar meal	50	40
Canola meal	115	80
Rape seed meal	33	30
Sunflower	70	44
Limestone	11	11
Nutritive values of experimental diet		
Crude protein (%)	21.2	19
Metabolized energy (kcal/kg)	2800	2950

Performance analysis of broiler

Weekly live body weight was recorded by selecting 10 birds from each group and weight was measured by electronic weighing balance. Feed consumption and water intake were recorded on daily basis. Feed conversion ratio (FCR) was recorded by dividing weight gain with feed intake while at the end of trial period, two birds were selected from each replicate and slaughtered for dressing percentage, the relative weight of edible and no edible organs. were recorded by electronic weighing balance.

Hemagglutination inhibition (HI) antibody titer

The vaccine was inoculated to all (180) birds, intranasal/intraocular administration. The blood was collected from (three birds of each group) via wing vein on day 14, 28 and 42. The blood was centrifuged for separation of serum then serum was stored at -20 ˚C to check the Infectious Bronchitis, and Newcastle disease antibody titers. HI, test was performed on chicken serum samples for the presence of antibodies as described in the Organization for Animal Health Manual (OIE, 2013).

Blood collection, hematology, and biochemical assay

The blood sample was collected randomly from 5 birds of each replicate from wing web on day 42 in EDTA containing tubes, blood parameters i.e., complete blood count (red blood cell, white blood cell, hemoglobin (Hb), count was analyzed by a manual method using a hemocytometer Sahli's method. Glucose, cholesterol and alanine transaminase level in blood was calculated as suggested by Mondal *et al*. (2011).

Statistical analysis

The collected data were tabulated and analyzed by one-way analysis of variance (ANOVA) through Statistics 8.1 version (SAS, 2001) and in case of significance differences appeared, the means were compared through Tukey's comparison test at 95% level of Probability.

RESULTS AND DISCUSSION

The effect of different levels of *Eucalyptus globulus* powder on live body weight, water intake, feed consumption, feed conversion ratio, carcass weight, and Dressing %in broiler chicken, is presented in Table 3. There was a significant ($P<0.05$) increased in live body weight among all treated groups as compared to control group, highest body weight was noted in group R4, followed by group R3 and group R2. Maximum water intake was consumed by group R4, followed by group R2, and group R3 as compared to group R1. Non-significant ($P>0.05$) difference was observed in treated groups. Maximum feed consumption was consumed by control group R1, followed by groups R2, R3, and group R4.

A better feed conversion ratio in broiler was obtained from group R4, followed by R3, R2 and R1 groups, significantly ($P<0.05$) different among all treated groups. Highest means values of carcass weight were obtained in group R4 followed by groups R3, R2, and R1 (control group). Significant ($P<0.05$) difference among all treated groups, moreover, highest percent of dressing was obtained from group R4 followed by groups R3, R2 as compared to group R1. Significantly ($P<0.05$) difference was noted in all treated groups.

The effect of different levels of *Eucalyptus globulus* powder on relative organs (edible and non-edibles in broiler chickens, is presented in Table 4. The relative weight of the Heart, liver, gizzard, proventriculus, spleen intestine, crop, thymus, and bursa were observed non-significant ($P>0.05$) variation among all treated groups.

Table 3 Effect of eucalyptus powder, on performance traits of broilers at different concentrations

Parameters	Treatment groups				P-values
	R1 (Control)	R2 (EP 0.25%/kg)	R3 (EP 0.5%/kg)	R4 (EP0.75%/kg)	
Live body weight (g/broiler)	1877±21.51^{c}	1943±21.65^{b}	1983±9.29^{b}	2243±14.57^{a}	0.0000
Water intake (mL/broiler)	7133±38.44^{a}	7141±51.03^{a}	7168±66.73^{a}	7217±87.43^{a}	0.7898
Feed consumption (g)	3950±11.26^{a}	3897±7.26a	3767±35.28^{b}	3747±24.04^{b}	0.0005
Feed conversion ratio (feed/gain)	2.1±0.04^{a}	2.0±0.06^{b}	1.9±0.06^{b}	1.7±0.02^{c}	0.0012
Carcass weight (kg)	1063±17.63^{d}	1165±9.29^{c}	1223±18.50b	1407±12.34^{a}	0.0000
Dressing (%)	56.6±1.31^{b}	59.9±0.70^{a}	61.7±0.97^{a}	62.7±0.63^{a}	0.0090

EP: eucalyptus powder.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 4 Effect of eucalyptus powder, on carcass traits (relative weight of organs, g/100 g of live weight) of broilers

Organs	Treatment groups				P-values
	R1 (Control)	R2 (EP 0.25%/kg)	R3 (EP 0.5/ kg)	R4 (EP 0.75%/kg)	
Heart	9.37±0.17^{b}	10.03±0.14^{ab}	10.10±0.36^{ab}	10.80±0.20^{a}	0.0199
Liver	45.6±0.26^{a}	45.9±0.65^{a}	46.2±0.20^{a}	46.7±0.95^{a}	0.6681
Gizzard	50.13±0.38^{c}	52.17±0.61^{b}	52.60±0.38^{b}	54.63±0.40^{a}	0.0010
Proventicular	9.71±0.14^{d}	11.0±0.47^{c}	12.33±0.21^{b}	13.33±0.19^{a}	0.0001
Spleen	2.10±0.10^{a}	2.13±0.09^{a}	2.17±0.05^{a}	2.17±0.04^{a}	0.9024
Intestine	206±2.08^{a}	208±1.17^{a}	208±2.63^{a}	212±2.88^{a}	0.3803
Crop	66.7±0.52^{a}	67±1.53^{a}	68±0.80^{a}	69.0±0.90^{a}	0.3949
Thymus	2.37±0.16^{a}	2.57±0.16^{a}	2.63±0.12^{a}	2.70±0.05^{a}	0.4133
Bursa	1.33±0.07^{b}	1.40±0.01^{b}	1.43±0.012^{b}	1.73±0.03^{a}	0.0006

EP: eucalyptus powder.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The effect of different levels of *Eucalyptus globulus* powder on haematology HI-ND and IB antibody titer in broilers chicken, is presented in (Table 5). Supplementation of *Eucalyptus globulus* significantly (P<0.05) increase the white blood cell in groups R4 and R3 as compared to R2 and control group R1. However, Red blood cell, Hemoglobin, and non-significant (P>0.05) variation in observed among all treated groups. Moreover, glucose level significantly increases in groupsR4, R3 and R2 as compared to R1 control group. The level of cholesterol shows non-significant (P>0.05) variation among all treated groups. serum glutamic-pyruvic transaminase (SGPT)/alanine transaminase (ALT) level shows significant (P<0.05) variation was observed among all treated groups. Supplementation of *Eucalyptus globulus* significantly (P<0.05) improved the immunity of broilers against Newcastle disease and infections bronchitis in all treated groups as compared to the control group.

Eucalyptus leaves concentration increased in broiler diet and feed consumption decreased. The findings of Farhadi *et al.* (2017), are supporting our results who found that utilizing *Eucalyptus* leaves powder at a higher concentration in abroiler diet had a negative impact on feed consumption. This could be due to the availability of higher tannin concentrations. Maximum water consumption was seen in this trail when different concentrations of eucalyptus were mixed with a commercial diet.

According to Lee *et al.* (2014), eucalyptus can activate the thrust center, which causes the birds to increase their water intake, which is necessary for optimal growth performance by managing temperature and electrolyte balance due to alkaloids, this could be due to the presence of micronutrients in eucalyptus. This study was supported by Borges *et al.* (2003), who reported that Na+, K+ supplied with poultry diet can increase the thrust.Our results regarding weight gain showed agreement with a previous study by Mashayekhi *et al.* (2018) in which the highest body weight gain was seen for broiler chickens fed with 0.5% eucalyptus powder. The broilers fed with plant extracts showed a much larger body weight gain than the other groups. Maulod and Delman (2015) in their study agreed that supplementation of 0.1 and 0.2 % eucalyptus leaves powder in the diet of broiler birds increased body weight as compared to other groups. According to the current findings, the antibacterial action of eucalyptus extract boosted broiler weight gain. This finding is strengthened by Dhama *et al.* (2014), who found that several herbal extracts had an antibacterial impact when fed to chicken, avoiding infectious illness, and improving growth performance. Results o fthis study also supported by those working with broilers and Japanese quail, Barbour *et al.* (2011), Hassan *et al.* (2011), and Shao *et al.* (2020) revealed that growth performance can be improved by adding eucalyptus as a feed additive, which they believe is due to gut microflora and immunity.

Table 5 Effect of eucalyptus powder (EP), on blood components and antibody immune response

Parameters	Treatment groups				P-values
	R1 (Control)	R2 (EP 0.25%/kg)	R3 (EP 0.5/kg)	R4 (EP 0.75%/kg)	
WBS (x10^9/µL)	14.3±0.23^{c}	14.7±0.26^{bc}	15.3±0.23^{ab}	16.0±0.20^{a}	0.0044
RBC (x10^9/µL)	2.3±0.11^{a}	2.3±0.05^{a}	2.1±0.10^{a}	2.2±0.11^{a}	0.4752
Hemoglobin (g/dL)	8.3±0.46^{a}	8.3±0.20^{a}	8.4±0.17^{a}	8.5±0.23^{a}	0.9531
Glucose (mg/dL)	208±2.68^{b}	213±3.44^{b}	231±1.20^{a}	234±2.73^{a}	0.0003
Cholesterol (mg/dL)	123±0.44^{a}	123±1.45^{a}	123±1.00^{a}	122±2.16^{a}	0.9434
SGPT/ALT (IU/L)	7.8±0.20^{a}	7.0±0.17^{b}	6.3±0.11^{c}	5.9±0.23^{c}	0.0005
IB titer	2.3±0.23^{c}	2.9±0.11^{b}	3.0±0.11^{ab}	3.0±0.15^{a}	0.0113
ND titer	4.2±0.05^{d}	4.8±0.05^{c}	5.2±0.05^{b}	5.8±0.33^{a}	0.0745

WBC: white blood cells; RBC: red blood cells; SGPT/ALT: serum glutamic-pyruvic transaminase/alanine transaminase; IB: infectious bursal and ND: Newcastle disease
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The better carcass weight was obtained in group R4. Mashayekhi *et al.* (2018) in their study showed that experimental treatments raised carcass weight with broilers fed 0.5 percent eucalyptus having the highest carcass weight.

Due to the improvement in carcass weight, the dressing percentage also improved as seen in our study the highest percentage of dressing was obtained from group R4.Our findings are supported by Kabir *et al.* (2004), who found an increase in carcass and breast weight of broilers fed probiotics, as well as an increase in dressing percentage in diets containing eucalyptus, this could be due to the essential oils' effect on digestion, absorption, and body weight gain.

Abetter feed conversion ratio (FCR) was obtained in supplementation groups as compared to control in which the FCR was not better than intreatment groups. Thisstudy is also supported by, Griggs and Jacob (2005), who revealed that dietary supplementation with herbs may improve feed efficiency, which is due to the presence of antioxidant chemicals, antimicrobial active compounds, linalool, terpineol, and limonene that are present in eucalyptus. According to Mashayekhi *et al.* (2018), eucalyptus has a significant response to broiler growth performance in terms of FCR. Our findings are also consistent with those of Molla *et al.* (2012), who found that herbs have no side effects and show growth promoter activity. Mashayekhi *et al.* (2018) found that broiler chickens fed with 0.5 percent Eucalyptus powder had the highest BWG and the lowest FCR. The weight of edible organs was non-significantly (P>0.05) different from one anotherwhich isalso supported by the study of, Mashayekhi *et al.* (2018) who suggest that effects of treatments on weights of internal edible organs were not significant. The weight of some non-edible organs was significant which are match the results of (Teo and Tan, 2007) who suggest that the Bursa of Fabricius relative weight increased. Increased lymphoid organ weight could imply improve immunity in treated birds, which could be explained by the antibacterial activity of antibiotics, probiotics, phenylpropanoids and flavonoids found in eucalyptus. In a study on broiler chicks, adding probiotic bacillus subtitles dramatically increased the relative weights of the bursa and thymus. Protoxin probiotics also enhanced the weights of the liver and bursa in broilers (Azadegan *et al.* 2014). Khaligh *et al.* (2011) in research of several medical plant mixtures in broiler chicken diets, it was discovered that medicinal plant mixtures had no effect on relative weight. In this study, the results of antibody titer showed a positive response, and an increased in white blood cells (WBC) count also showed increased by treatments. Treatments at 42 days of age (secondary titer) significantly boosted antibody production against sheep red blood cells (SRBC), with the maximum antibody production reported in birds given 0.5 percent eucalyptus powder and probiotic treatment. Eucalyptus powder may improve immunological response by stimulating antibody synthesis. Some researchers claim that taking probiotics and medicinal extracts in the diet boosts immunological response, which is consistent with our findings (Khaksefidi and Ghoorchi, 2006; Moorthy *et al.* 2009; Mustafa, 2019). This analysis revealed that probiotic-rich diets boost immunological response by stimulating the development of B and T cells. Herbal extracts also contain vitamin C and phagocyte activity, which boost immunological response, according to some research (Barbour and Danker, 2005; Hashemi and Davoodi, 2012).

CONCLUSION

It was concluded from the present study that broilers can be fed on eucalyptus leaves at 0.75% per kg feed for better growth, FCR, blood composition, health, and per-bird profit in our local conditions.

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