

Effect of Supplementation of Eucalyptus (*Eucalyptus globulus*) Leaf Powder in Diet Containing Phytase Enzyme on Performance of Commercial Laying Hens

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ABSTRACT

A feeding trial was conducted to discern the influence of dietary incorporation of eucalyptus (*Eucalyptus globulus*) leaf powder on nutrient utilization, egg quality, and haemato-biochemical parameters of White Leghorn laying hens for 12 weeks. A total of 72, twenty-eight days old laying hens were randomly assigned into four treatment groups, with 18 hens per treatment having three replicates of 6 birds each. Four treatment groups were control (T₁): basal diet and Treatment T₂, T₃, and T₄ with the incorporation of 0.30, 0.45, and 0.60 % eucalyptus leaf powder, respectively, in a basal diet containing phytase @250 FTU/kg feed. Results showed a significant ($p < 0.05$) increase in egg production in the group T₂ compared to other treatment groups. There was no significant ($p \geq 0.05$) effect on feed intake, feed conversion ratio, and nutrient utilization among various treatment groups. The eggshell quality, egg albumen, and yolk traits parameters did not differ significantly among treatment groups except shell thickness. The hematological values, *i.e.*, hemoglobin, total erythrocyte count (TEC), and total leukocyte count (TLC) showed significant improvement ($p < 0.05$) in T₃ and T₄ as compared to the control and T₂ group, but no statistical differences were noted in packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). There were no significant differences in serum biochemical attributes among the treatment groups, but a considerable decrease in level of serum cholesterol and triglycerides was observed with an increasing level of eucalyptus leaf powder in diet. Similarly, glucose concentration differed significantly among treatment groups being lowest in T₃ group.

Keywords: Blood profile Eucalyptus leaf, Nutrient utilization, Phytase, Production performance, White Leghorn laying hens.

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INTRODUCTION

The poultry industry is one of the fastest-growing and most well-organized agricultural sectors in India. Poultry farmers are changing their focus to natural feed additives to increase their flock's health and production. Since 2006, when antibiotic growth promoters were completely banned or restricted, most producers have supplemented poultry rations with one or more natural additives as a safe and inexpensive alternative source in an acceptable way (Castanon, 2007). Plant-based feed additives have recently gained a lot of attention as a long-term alternative in poultry diets.

Eucalyptus (*Eucalyptus globulus*) is the most widely cultivated evergreen tree in the subtropical and Mediterranean regions. It contains several important chemicals, such as p-cymene, 1,8 cineole, phellandrene, spathulenol, cryptone aldehydes, cuminal, phellandral, which have antibacterial, anti-oxidative, and anti-inflammatory properties (Barra *et al.*, 2010). Phytate is an anti-nutritional ingredient, which exists in the form of a mixed salt of phytic acid in poultry feed ingredients, most of which come from plants. Therefore, adding microbial phytase to poultry diets can improve the hydrolysis of phytic acid and increase the availability of minerals combined with phytic acid, while reducing phosphorus pollution in the environment. However, the

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effect of eucalyptus bioactive qualities has been studied in humans, but information on the influence of its leaf powder along with phytase on growth performance and egg production of laying hen is very limited. Therefore, this study was planned to evaluate the potential effect of incorporating eucalyptus leaf powder in a diet containing phytase of laying hens on nutrient utilization, egg quality, and haemato-biochemical parameters.

MATERIALS AND METHODS

A total of 72, twenty-eight days old White Leghorn laying hens approved by the Institutional Animal Ethics Committee (IAEC) were randomly distributed to four treatments. Each treatment comprised of three replicates consisting 6 hens per replicate. The four dietary treatments were T₁ basal diet as per BIS (2007) and T₂, T₃, and T₄ with the incorporation of 0.30, 0.45, and 0.60% eucalyptus leaf powder, respectively in a basal diet containing phytase @ 250 FTU/kg feed. The feeding trial was carried out for a period of 12 weeks at Instructional Poultry Farm of GBPUAT, Pantnagar. Laying hens were reared in deep litter system using standard management and health care practice. The required quantity of eucalyptus (*Eucalyptus globulus*) leaves were obtained from CRC Pantnagar. It was air-dried in shade, followed by drying in a hot air oven at 60° C for 3-4 days, and then finely ground to powder using a laboratory mill and stored in a closed and dry container. It contains 7.88 % crude protein (CP), 7.88 % ether extract (EE), 15.78 % crude fibre (CF), 4.36 % total ash and 67.03 % nitrogen-free extract (NFE) on dry matter basis.

Parameters Studied

Eggs were collected twice a day and recorded replicate-wise for a period of 12 weeks. Further, egg production percentage, feed intake, and feed conversion ratio (FCR) for each treatment group were recorded. In the last week of the experiment, three eggs were collected from each replicate (12 eggs per treatment) in all groups to evaluate egg quality parameters, viz., eggshell, albumen, and egg yolk quality. Blood samples were taken at the end of the experiment for haemato-biochemical analysis. To assess nutrition utilization,

a metabolic experiment was conducted for 7 days on two hens randomly selected from each replicate.

Statistical Methods

The data obtained in this study was statistically analyzed using the general linear model method of SPSS software (Snedecor and Cochran, 1994). Duncan's multiple range test is used to compare differences between treatment means.

RESULTS AND DISCUSSION

Production Performance

The average production performance of White Leghorn layers in terms of egg production, feed intake, feed conversion ratio (kg/dozen eggs and kg/kg eggs) of different groups during the feeding trial are presented in Table 1.

The average egg production of the feeding trial was significantly ($p < 0.05$) higher in the layers of T₂ group compared to other treatment groups, and T₄ had the lowest egg production rate. There were no significant differences ($p \geq 0.05$) among the treatments groups in average feed intake (gm/day) and average FCR (kg feed /dozen eggs and kg feed /kg eggs) during the feeding trial, yet values were lowest in T₂ (Table 1). These results were in corroboration with the findings of Motaal *et al.* (2008), who reported significantly higher egg production in laying hens on adding eucalyptus leaf powder in the diet. Similarly, Hassan *et al.* (2011) reported significant increases in egg number on adding eucalyptus leaf powder in the diet of Japanese quail. Supplementation of eucalyptus leaves did not affect the feed intake and FCR of Japanese quail (Fathi *et al.*, 2020). Mashayekhi *et al.* (2018) and Hassan *et al.* (2011) reported significant improvement in FCR by incorporating 0.5 % eucalyptus leaf powder in the diet of broiler chickens and Japanese quail, respectively.

Nutrient Utilization

The average values of nutrient utilization of White Leghorn layers in terms of dry matter, crude protein, ether extract and organic matter on adding eucalyptus leaf powder in diet during the metabolic trial is presented in Table 2. There were insignificant variations in the utilization pattern of dry matter, crude protein, ether extract, and organic matter

Table 1: Average values of production performance of White Leghorn laying hens fed a diet supplemented with Eucalyptus leaf powder during the experimental period

	Treatments/Groups			
	T ₁	T ₂	T ₃	T ₄
Performance parameters (28-40 weeks)	Basal diet (control)	Basal diet + 0.3% Eucalyptus leaf powder	Basal diet + 0.45% Eucalyptus leaf powder	Basal diet + 0.6% Eucalyptus leaf powder
Feed intake (gm)	110.04 ± 0.13	107.16 ± 1.60	107.75 ± 0.30	109.15 ± 0.42
Egg production (%)	66.82 ± 0.73 ^b	72.00 ± 1.03 ^a	66.20 ± 0.95 ^b	63.39 ± 1.70 ^b
FCR (kg/dozen egg)	1.87 ± 0.006	1.7 ± 0.18	1.84 ± 0.02	1.97 ± 0.06
FCR (per kg egg mass)	2.92 ± 0.015	2.82 ± 0.05	2.85 ± 0.02	2.93 ± 0.03

Mean values bearing different superscripts in a row differ significantly ($p \leq 0.05$).

among different groups. The results were in close agreement with Ahmed *et al.* (2005) and Safaa (1999), reported that the digestibility coefficients of crude protein and crude fiber did not change significantly ($p \geq 0.05$) when rabbits and sheep were given a eucalyptus added in the diet compared to control diet.

Egg Quality Traits

The results for egg quality parameters due to the effect of eucalyptus leaf powder supplementation are presented in Table 3 and 4:

The egg weight and shell quality parameters in terms of shape index and shell weight (g or %) did not significantly change. However, significantly higher shell thickness was observed in T_4 (0.37 mm) as compared to T_1 (0.33 mm) and T_2 (0.35 mm). The findings were in accordance with Motaal *et al.* (2008), Chen *et al.* (2017), and Fathi *et al.* (2020), who also found no significant difference among treated groups for all egg quality traits and egg weight, except shell thickness. Hassan *et al.* (2011) observed improvement in egg weight, egg mass and egg quality by incorporating eucalyptus leaf in diet of quail.

The egg albumen quality (albumen height, albumen weight, and Haugh unit) did not differ significantly ($p < 0.05$) among T_1 , T_2 , T_3 and T_4 groups. The results were consistent with Motaal *et al.* (2008) and Fathi *et al.* (2020), who observed no effect on egg albumen quality on adding different levels of eucalyptus in diet of laying hen and quail, respectively.

Similarly, egg yolk quality parameters *viz.*, yolk weight

(g or %), yolk index, yolk color, yolk cholesterol, and yolk triglyceride did not differ significantly ($p > 0.05$) among different treatment groups. Chen *et al.* (2017) showed no statistical differences in yolk percentage by incorporating polyphenol of eucalyptus in the laying hen diet, but yolk cholesterol decreased significantly. Fathi *et al.* (2020) reported a significantly decreased yolk index by incorporating eucalyptus leaf powder in the quail's diet. Still, values of yolk percent and yolk color were statistically similar among treatment groups.

Hematological Parameters

The hematological parameters in terms of hemoglobin, PCV, TEC, TLC, MCV, MCH, and MCHC in laying hens fed diets incorporated with eucalyptus leaf powder are summarized in Table 5.

The hematological values, namely hemoglobin, total erythrocyte counts (TEC), total leucocyte count (TLC) were significantly ($p \leq 0.05$) different among the treatment groups. The hemoglobin content of the T_3 group, TEC of T_4 group, and TLC of both the groups was significantly higher as compared to other groups. These results were in agreement of the reports of Bello (2015), Dakheel *et al.* (2017). Waly *et al.* (2019), also observed significantly ($p < 0.05$) increased hemoglobin concentration, TEC, and TLC on adding different concentrations of leaves or extract of eucalyptus in quail, Wistar rats and growing rabbits than controls. On the contrary, Motaal *et al.* (2008) and Mashyehki *et al.* (2018) reported no significant effect on hematological indices

Table 2: Average values of nutrient utilization (%) in White Leghorn Laying hens fed diets supplemented with Eucalyptus leaf powder during metabolic trial

Particulars	Treatments/Groups			
	T_1 Basal diet (control)	T_2 Basal diet + 0.3% Eucalyptus leaf powder	T_3 Basal diet + 0.45% Eucalyptus leaf powder	T_4 Basal diet + 0.6% Eucalyptus leaf powder
Dry matter	68.49 ± 1.89	70.51 ± 0.61	70.39 ± 0.53	69.82 ± 0.52
Crude protein	80.11 ± 0.72	80.40 ± 0.56	80.30 ± 0.78	80.26 ± 0.95
Ether extract	82.47 ± 0.93	82.95 ± 1.38	82.93 ± 1.19	81.97 ± 1.4
Organic matter	73.84 ± 1.07	74.32 ± 0.72	73.71 ± 1.14	73.72 ± 0.85

Table 3: Average values of egg weight, shape index, and shell quality of White Leghorn laying hens fed diet supplemented with Eucalyptus leaf powder during the experimental period.

Parameters	Treatments/Groups			
	T_1 Basal diet (control)	T_2 Basal diet + 0.3% Eucalyptus leaf powder	T_3 Basal diet + 0.45% Eucalyptus leaf powder	T_4 Basal diet + 0.6% Eucalyptus leaf powder
Egg wt. (g)	54.29 ± 0.12	54.50 ± 0.15	54.67 ± 0.02	54.47 ± 0.09
Shape index(%)	76.62 ± 0.64	76.00 ± 1.12	75.18 ± 0.24	76.05 ± 0.55
Shell wt. (g)	5.74 ± 0.11	5.75 ± 0.09	5.92 ± 0.06	5.96 ± 0.09
Shell wt. (%)	10.57 ± 0.11	10.72 ± 0.24	10.09 ± 0.30	10.20 ± 0.07
Shell thickness (mm)	0.33 ± 0.006 ^c	0.35 ± 0.006 ^b	0.36 ± 0.003 ^{ab}	0.37 ± 0.006 ^a

Mean values bearing different superscripts in a row differ significantly ($p \leq 0.05$)



Table 4: Average values of albumen quality, Haugh unit, and yolk quality parameters of White Leghorn Laying hens fed a diet supplemented with Eucalyptus leaf powder during the experimental period.

	Treatments/Groups			
	T ₁	T ₂	T ₃	T ₄
<i>Egg quality parameters (internal)</i>	(Basal diet)	(Basal diet + 0.3% Eucalyptus leaf powder)	(Basal diet + 0.45% Eucalyptus leaf powder)	(Basal diet + 0.6% Eucalyptus leaf powder)
Albumen height (mm)	6.57 ± 0.22	6.52 ± 0.03	6.67 ± 0.26	6.52 ± 0.09
Albumen weight (gm)	33.78 ± 0.63	33.33 ± 0.22	33.70 ± 0.65	34.00 ± 0.44
Albumen weight (%)	60.93 ± 0.43	60.14 ± 0.38	60.83 ± 0.76	61.49 ± 0.49
Haugh unit	82.41 ± 0.35	83.75 ± 1.76	82.77 ± 1.72	81.61 ± 0.67
Yolk weight (gm)	15.94 ± 0.01	15.99 ± 0.25	16.02 ± 0.07	15.71 ± 0.24
Yolk weight (%)	28.76 ± 0.33	28.89 ± 0.33	29.11 ± 0.26	28.41 ± 0.36
Yolk index (%)	43.54 ± 0.46	43.21 ± 1.05	43.16 ± 0.205	42.91 ± 0.85
Yolk colour score	4.83 ± 0.67	5.17 ± 0.33	5.33 ± 0.44	5.33 ± 0.44
Yolk cholesterol (mg/g)	14.66 ± 0.43	13.97 ± 0.12	13.89 ± 0.23	14.36 ± 0.41
Yolk triglyceride (mg/g)	203.86 ± 0.38	196.68 ± 4.27	197.79 ± 1.07	198.31 ± 1.53

Table 5: Average values of hematological parameters of White Leghorn Laying hens fed a diet supplemented with Eucalyptus leaf powder

	Treatments/Groups			
	T ₁	T ₂	T ₃	T ₄
<i>Hematological parameters</i>	Basal diet (control)	Basal diet + 0.3% Eucalyptus leaf powder	Basal diet + 0.45% Eucalyptus leaf powder	Basal diet + 0.6% Eucalyptus leaf powder
Haemoglobin (gm/dL)	9.65 ± 0.25 ^b	9.71 ± 0.32 ^b	10.94 ± 0.26 ^a	10.09 ± 0.21 ^{ab}
Packed cell volume (%)	29.82 ± 0.48	30.71 ± 0.98	31.59 ± 0.94	31.85 ± 0.15
Total erythrocyte count (×10 ⁶ / μL)	3.00 ± 0.12 ^b	3.08 ± 0.06 ^b	3.28 ± 0.07 ^{ab}	3.57 ± 0.13 ^a
Total leukocyte count (×10 ³ / μL)	25.64 ± 0.32 ^b	26.65 ± 0.31 ^{ab}	27.78 ± 0.40 ^a	28.06 ± 0.33 ^a
MCV(fl)	99.61 ± 5.33	99.74 ± 4.69	96.48 ± 4.49	90.05 ± 2.69
MCH(pg)	32.26 ± 1.98	31.55 ± 1.54	33.37 ± 0.40	28.33 ± 1.50
MCHC (g/dL)	32.39 ± 1.09	31.57 ± 1.60	34.87 ± 1.77	31.66 ± 0.59

Mean values bearing different superscripts in a row differ significantly ($p \leq 0.05$)

with supplementation of eucalyptus leaf powder in the diet of laying hens and broilers, respectively. Also, Fathi *et al.* (2019 and 2020) reported the same observation in rabbits and Japanese quail. Other parameters *viz.*, PCV, MCV, MCH, and MCHC did not differ significantly due to incorporating eucalyptus leaf powder in the hens' diet. These findings corroborate with the reports of Duskaev *et al.* (2020) and Waly *et al.* (2019) in broiler chicken and rabbit, respectively. Motaal *et al.* (2008) and Fathi *et al.* (2019) observed no differences in dietary supplementation of eucalyptus leaf powder in White Leghorn laying hens and rabbits, respectively.

Serum Biochemical Parameters

The average values of serum biochemical parameters, namely serum glucose, total serum protein, serum albumen, serum globulin, serum cholesterol, serum triglycerides, serum calcium, and phosphorus, are summarized in Table 6.

The serum biochemical constituents in terms of total protein, albumen, globulin, calcium, phosphorus, and

enzyme activities, SGOT and SGPT of laying hens were statistically similar among dietary treatments. There was a significant decrease in glucose, cholesterol, and triglyceride in groups T₃ and T₄. Our observations on glucose corroborate with the reports of Ismail (2007) and Arise *et al.* (2009).. According to Gray and Flatt (1998), eucalyptus water extract can increase the peripheral glucose utilization in mouse abdominal muscle and gradually increase insulin secretion of the cloned pancreatic β-cell line. Hence, the insulin release effect of *Eucalyptus globulus* water extract may be the reason for the anti-hyperglycemic effect.

In the present study, a significant ($p \leq 0.05$) decrease in serum cholesterol and triglycerides concentration (mg/dl) was noted in T₃ as compared to T₁ and T₂ group, which concurred with results of Mashayekhi *et al.* (2018), Duskaev *et al.* (2020) and Namamian *et al.* (2020) in broiler chicken. Similar results were obtained by Waly *et al.* (2019) and Hassan *et al.* (2011) in growing New Zealand white rabbits and Japanese quail, respectively, on adding eucalyptus leaves in diet. The active components of plants, which function as natural antioxidants,

Table 6: Average values of serum biochemical constituents in White Leghorn Laying hens fed diet supplemented with Eucalyptus leaf powder

Serum biochemical parameters	Treatments/Groups			
	T ₁ Basal diet (control)	T ₂ Basal diet + 0.3% Eucalyptus leaf powder	T ₃ Basal diet + 0.45% Eucalyptus leaf powder	T ₄ Basal diet + 0.6% Eucalyptus leaf powder
Blood glucose (mg/dL)	188.50 ± 5.64 ^a	178.38 ± 1.28 ^{ab}	165.74 ± 1.45 ^c	170.33 ± 2.19 ^{bc}
Total protein (g/dL)	4.49 ± 0.206	4.50 ± 0.08	4.69 ± 0.22	4.63 ± 0.20
Albumen (g/dL)	1.47 ± 0.08	1.51 ± 0.06	1.45 ± 0.05	1.47 ± 0.08
Globulin (g/dL)	3.02 ± 0.17	3.00 ± 0.13	3.23 ± 0.26	3.16 ± 0.28
Cholesterol (mg/dL)	157.15 ± 0.57 ^a	153.16 ± 4.52 ^a	139.80 ± 0.81 ^b	143.24 ± 2.16 ^b
Triglyceride (mg/dL)	216.46 ± 0.96 ^a	203.67 ± 2.69 ^b	197.88 ± 3.23 ^b	195.70 ± 3.01 ^b
Calcium (mg/dL)	5.47 ± 0.08	5.50 ± 0.50	5.53 ± 0.68	5.42 ± 0.52
Phosphorus (mg/dL)	21.06 ± 0.58	21.61 ± 0.02	20.95 ± 0.59	21.03 ± 0.29
SGPT(U/l)	23.64 ± 0.32	23.65 ± 0.67	23.83 ± 0.15	23.69 ± 0.36
SGOT(U/l)	60.59 ± 0.38	60.65 ± 0.45	60.37 ± 1.01	60.36 ± 0.62

Mean values are bearing different superscripts in a row differ significantly ($p \leq 0.05$).

may have a role in the overall hypocholesterolemic effect of herbal plant supplementation.

CONCLUSION

The present study concluded that adding eucalyptus leaf powder to diet can reduce serum cholesterol, triglycerides, and glucose, increase hemoglobin content, RBCs, and WBCs, and ameliorate egg production at 0.3% eucalyptus leaf powder in diet containing phytase in laying hens.

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