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The Effects of Blue Gum (*Eucalyptus Globulus.*) Leaves as Feed Additive on Hematological Traits of Japanese quail

Alhassan U. Bello

University Farm Research, Yobe State University, Damaturu, Nigeria

Abstract: **A study was conducted to investigate the effects of supplementing the diet with different levels of *Eucalyptus globulus* leaves on hematological traits of Japanese quails. A total number of one hundred and twenty-eight (128) quails, three weeks old, were used in this experiment. The birds were placed on four treatment groups consisted of thirty-two (32) quails each. Treatment groups were: Control diet (C) (free from eucalyptus), R1: Control diet + 4g/day eucalyptus, R2: Control diet + 8g/day eucalyptus; R3: Control diet + 12g/day eucalyptus. The birds were kept for four weeks. At the end of the trial, blood samples were obtained from all quails from the brachial vein by venipuncture. Hematological traits measured were red blood cells count (RBC), hemoglobin concentration (Hgb), packed cell volume (PCV), mean cell volume (MCV), white blood cells count (WBC), mean corpuscular hemoglobin (MCH), percent lymphocytes, mean corpuscular hemoglobin concentration (MCHC), thrombocyte count, heterophils/lymphocytes (H/L) ratio, percent heterophils, monocytes, basophils, eosinophils. Results revealed that supplementing the diet of quails with different levels of eucalyptus (R1, R2 and R3) caused significant increased (P<0.05) in Hgb, PCV, MCV, MCH, MCHC, thrombocytes, and H/L ratio as compared to group C, while WBC and lymphocytes significantly decreased (P<0.05) in control group. But, there were no significant difference (P>0.05) among all experimental groups as regards heterophils, monocytes, and basophils. In conclusion, supplementing ration of quails with *Eucalyptus globulus* leaves led to significant improvement in most of the blood traits.**

Keywords: **Diet, Eucalyptus *globulus*, hematological traits, Japanese quails.**

1. INTRODUCTION

Health management in poultry depends on many factors in which diet plays a pivotal role in health maintenance and prevention of many diseases [7]. Feed additives are essential materials that increase the efficiency of feed utilization and performance in birds [21, 22]. Many efforts in the field of animal nutrition have been made to attain an increase in productive animal performance thereby maximize profit [25]. The world health organization (WHO) encourage producers to use medicinal plants to reduce the use of chemicals via the global trend to go back to nature [1].

Eucalyptus globulus is an evergreen tree 40 to 70 m tall extensively planted in the tropics, sub-tropics, and Mediterranean [16, 8, 12]. Its leaves, roots, stem, and seed has been commonly used in the traditional medicine in several parts of West Africa countries [20]. The fresh plant leaves are occasionally consumed as a vegetable while the dry leaves are frequently smoked for the treatment of asthma [4].

Blue Gum seeds and fruits have been used as a diuretic and the hypoglycemic activity [24]. The blue gum flowers are considered a good source of nectar and pollen for bees as compose of ascorbic acid, flavonoids, carotenoids, coumarins, apiole, phenylpropanoids, phathalides, furanocoumarins, tocopherol, and various terpenoic compounds [26]. The components of fresh blue gum scavenge superoxide anion in-vitro, and the methanol extracts of blue gum scavenge hydroxyl radical in addition to protection against ascorbic acid induced membrane oxidation [5]. Supplementation of the diets with fresh blue gum leaf can significantly increase antioxidant capacity [22]. However, the effects of *Eucalyptus globulus* on hematological traits in quails are not documented. This experiment was conducted to determine the effect of dietary *Eucalyptus globulus* supplementation on hematological characteristics of Japanese quails.

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2. MATERIALS AND METHODS

A total of one hundred and twenty-eight (128) quails, three weeks old were used in this experiment. The birds were housed in four separate floor pens under artificial lighting program of 12L:12D consisted of thirty-two (32) quails per each group. Birds were kept for four weeks. All quails were fed commercial ration containing 2800 Kcal/ kg metabolizable energy and 28% crude protein (Table 1). *Eucalyptus globulus* fresh dried leave powder was supplemented into the treatment rations. Treatments were as following:

Control diet (free from eucalyptus; C)

R1: Control diet + 4g/day eucalyptus

R2: Control diet + 8g/day eucalyptus

R3: Control diet + 12g/day eucalyptus

At the end of the experiment (seventh week), blood samples were obtained from each quail by venipuncture from the brachial vein; using disposable needles (5G, 0.5x30MM-J-Type) fitted with a plastic syringe, and was cautiously transferred to collecting tubes containing EDTA (1.5 g/ml). The blood collecting tubes were kept ice and transported to the laboratory where cell counting and hematological analyzes were conducted. In the laboratory, aliquots were diluted 200 times for red blood cell (RBC), and white blood cell (WBC) counts in hematological pipette with a Natt-Herrick solution [18]. Total RBC and WBC counts were determined according to Mitchell and Johns [17]. Hemoglobin concentration (Hgb) was determined by a cyanmethemoglobin method following lysate centrifugation [28].

Packed cell volume (PCV) was determined by micro-hematocrit centrifugation. Mean corpuscular hemoglobin (MCH), mean cell volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard methods as reported by Mitchell and Johns [17]. Commercial Giemsa stain was used for the blood smears, fixed in methanol at a dilution of 1: 4 (v/v) in phosphate buffer for one hour at pH 6.80. Counting and Identification of leucocytes and thrombocytes were carried out under a light microscope with the aid of oil immersion lens (× 100). Average of 200 leucocytes were counted in each sample to establish cell ratio [15]. The number of thrombocytes was obtained by an estimation method that consists of counting the number of thrombocytes in five fields submerged and applying the following formula [17].

Estimated number of thrombocytes (thrombocytes/µl) = (mean number of thrombocytes in five fields × 3,500,000) × 10^{-3}.

3. STATISTICAL ANALYSIS

The data was measured by analysis of variance using the General Linear Model method (SAS, 2002). The test of significance difference ($P<0.05$) between treatments was done by Duncan multiple range test [6].

Table 1: The feed ingredient and composition of experimental diets

Ingredients	Composition (%)
Yellow Maize	63
Soybean cake	25
Wheat bran	03
Protein concentrate*	05
Oil	02
Oyster shell	02
Dicalcium phosphate	1.02
Vitamins-minerals premix**	0.5
Salt	0.48
Calculated content***	100
Crude protein (%)	28.0
ME (Kcal/Kg)	2,800
Total calcium	2.45
Available phosphorus	0.351
Methionine	0.205
Lysine	1.055

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*Protein concentrate supplemented per kg: 2918 ME / kg; 20% crude protein; 2% crude fiber; 5% crude fat; 2.6% calcium; 1.330% lysine; 0.4% available phosphorus; 0.720% methionine + cystine; 1.25 methionine.
**Vitamins and minerals premix supplemented per kg: 8,000,000 I.U. Vit. A; 500 mg Vit. B1; 500 MG Vit. B2; 200mg Vit. B6; 8 mg Vit. B12; 1,500,000 I.U. Vit. D3; 1100 I. U. Vit E; 2000 mg Vit. K3; 4000 mg Pantothenic acid; 6000mg Nicotinmide; 110 mg Folic acid; 0.50 gm Mn sulphate; 0.16 gm Zinc sulphate; 0.50 gm Fe sulphate; 0.04 gm Cu sulphate; 0.01 gm cobalt chloride. ***as recommended by NRC [19].

4. RESULTS

Table 2 data showed that quails fed different levels of fresh dried eucalyptus leaves (R1, R2 and R3) recorded the higher values (P<0.05) of Hgb, PCV, MCV, MCH, MCHC and thrombocytes as compared to group C. However, R3 group surpassed other groups (C, R1 and R2) as regards to RBC count, while there were no significant differences among C, R1 and R2 with relation to RBC count in spite that there was increasing in the trend in R1 and R2 groups than group C. Furthermore, there were no significant differences (P>0.05) between R2 and R3 groups in the mean values of Hgb, PCV, MCV, MCH, MCHC and thrombocytes (Table 2). The current results revealed that dietary supplementation with different levels of eucalyptus leaves (R1, R2 and R3) caused significant (P<0.05) increase in WBC count and percentage of lymphocytes when compared to group C (Table 3). Moreover, it was observed that (Table 2) control group (C) recorded the maximum means of H/L ratio and percentage of eosinophils as compared with eucalyptus treated groups (R1, R2, and R3). Moreover, there was no significant (P>0.05) effect on percentages of heterophils, monocytes, and basophils.

Table 2: Effect of dietary eucalyptus supplementation on blood traits of Japanese quails

Traits	Treatments			
	C	R1	R2	R3
RBC (× 106/µl)	4.61 ± 0.27^{b}	5.20 ± 0.7 ^{b}	5.42 ± 0.19^{b}	5.93 ± 0.61^{a}
Hb (g /dL)	9.27 ± 0.99^{c}	11.14 ± 0.79^{b}	13.00 ± 0.82^{a}	13.53 ± 1.00^{a}
PCV (%)	39.30 ± 1.99^{c}	43.12 ± 0.77^{b}	44.33 ± 1.22^{a}	44.92 ± 0.29^{a}
MCV (fL)	92.1 ± 1.07^{c}	98.3 ± 2.22^{b}	99.5 ± 1.49^{a}	98.7 ± 2.36^{a}
MCH (Pg)	23.21 ± 1.11^{c}	27.33 ± 0.91^{b}	30.23 ± 0.88^{a}	31.63 ± 0.99^{b}
MCHC (g /dL)	22.98 ± 0.34^{c}	26.60 ± 0.33^{b}	29.14 ± 0.50^{a}	30.82 ± 0.44^{a}
Thrombocytes (× 103 /µl)	19.13 ± 0.77^{c}	21.25 ± 1.01^{b}	23.34 ± 0.69^{a}	23.55 ± 0.71^{a}

C=Control group; R1, R2 and R3=Diet supplemented with 4g, 8g, and 12 g/day eucalyptus, respectively; $^{a-c}$ Values within rows followed by different letters differ significantly (P< 0.05)

Table 3: Effect of dietary eucalyptus supplementation on blood characteristics of Japanese quails

Traits	Treatments			
	C	R1	R2	R3
WBC (×103/µl)	8.79 ± 0.81^{c}	11.26 ± 0.91^{b}	13.14 ± 0.44^{a}	13.66 ± 0.11^{a}
Heterophils (%)	25.10 ± 1.79	24.83 ± 1.93	24.35 ± 1.75	25.00 ± 1.89
Lymphocytes (%)	56.12 ± 1.11^{c}	61.99 ± 0.99^{b}	62.87 ± 0.91^{a}	62.06 ± 0.88^{a}
H/L ratio	0.69 ± 0.09^{a}	0.48 ± 0.05^{b}	0.45 ± 0.01 ^{c}	0.44 ± 0.04^{c}
Monocytes (%)	7.12 ± 0.89	7.18 ± 0.71	6.97 ± 0.97	7.02 ± 0.98
Basophils (%)	5.10 ± 0.83	5.03 ± 0.71	4.95 ± 0.57	5.01 ± 0.60
Eisonophils (%)	1.05 ± 0.20^{a}	1.10 ± 0.20^{b}	0.90 ± 0.10 ^{c}	0.98 ± 0.10^{c}

C: Control group; R1, R2 and R3: Diet supplemented with 4g, 8g, and 12g/day eucalyptus, respectively $^{a-c}$ Values within rows followed by different letters differ significantly (P< 0.05)

5. DISCUSSION

In the current study, eucalyptus treated groups (R1, R2, and R3) were recorded the highest means for WBC count and percentage of lymphocytes as compared to group C (Table 3). It was observed that H/L ratio was significantly (P>0.05) higher in group C when compared with eucalyptus treated groups (R1, R2, and R3) as shown in Table 3. The current finding is in agreement with a report of Isa *et al*. [10] in which they stated that eucalyptus enhances immunity in chicks because it contains high vitamin C, beta carotene, B12, chlorophyll and essential fatty acid. Moreover, Hassan *et al*. [9] state that multivitamin contains blue gum improves immune response in broiler chickens.

Adding fresh, dried eucalyptus leaves to the quails diet resulted in significant improvement in the various hematological traits involved in this research. This improvement can be attributed to the fact that eucalyptus contains and serves as a good source of iron, beta-carotene and vitamin C, useful for enhancement of general health status [27].

There was no significant difference (P>0.05) between R2 and R3 regarding WBC count, H/L ratio, the percentage of lymphocytes and eosinophils. This can be attributed to the physiological effect of eucalyptus with an ability for reducing stress in birds. The result is agreement with finding of Richmond and Mackley [23] that eucalyptus alleviate stress in birds by enhancing the general health status and immunity. According to Al-Daraji *et al*., [2] bird under stress have higher H/L ratio due to the release of corticosterone. It is resultant effect of improving heterophils that reduces lymphocyte concentrations [3]. The protective role of eucalyptus may be ascribed to its higher content of flavonoids that scavenge free radicals.

The current findings showed that dietary supplementation with different levels of eucalyptus globulus leaves (R1, R2, and R3) caused significantly increase (P<0.05) in WBC count and percentage of lymphocytes when compared to group C (Table 3). It has been reported that natural chlorinated compound in eucalyptus significantly influences biological activities such as antibiotic, antiviral, antibacterial, anti-inflammatory, antihepatotoxic, antioxidant to enhanced the general health condition of the body [11, 13].

6. CONCLUSION

Ultimately it was found that supplementing the diet of quails with different levels of fresh, dried eucalyptus leaves (4g, 8g or 12g/day) resulted in significant improvement in most hematological characteristics included in this research. Moreover, leaves of *Eucalyptus globulus* could be used as an efficient feed additive for enhancement general physiological status of quails.

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