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Effect of neem leaf meal and citric acid supplementation on hemato-biochemical parameters in broilers

Mayur Solanki, Kuldeep Verma, Rakesh Vora, Sajani Ustadiya and Sawan Rathva

Abstract
The present studied effect of supplementation of neem leaf meal (NLM) and citric acid (CA) on hemato-biochemical parameters in broilers. A total of 288 Vencobb-400 day old chicks were randomly assigned into 6 homogenous groups (4 replicates in each) based on their live body weight using RBD. All the birds were fed on standard diet (BIS, 2007) without any supplementation control (T0); and with supplementation of Neem leaf meal 2.5 g/kg of feed (T1); Citric acid 15g/kg of feed (T2); Citric acid 25g/kg of feed (T3); Neem leaf meal 2.5g/kg of feed + Citric acid 15g/kg of feed (T4); Neem leaf meal 2.5g/kg of feed + Citric acid 25g/kg of feed (T5). To study hemato-biochemical parameters, blood sample were collected randomly from 8 birds of each group (2 birds/replicate) at the end of experiment and serum was separated. The finding of the study indicated that the haematological (Hb, PCV and TLC) and biochemical (glucose, total protein, albumin, globulin, albumin-globulin ration, cholesterol and serum enzymes) parameters did not influence significantly by feeding of NLM and CA in broiler birds.

Keywords: Neem leaf meal, citric acid, hemato-biochemical, broiler

1. Introduction
Poultry industry is one of the fastest growing sectors in the country and has taken a quantum leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily achieved due to the exploitation of different modern growth promoting strategy and proper disease prevention measures (Angelakis *et al.,* 2013) [3]. Presently the demand for cheap and quality food is continuously rising around the globe which reflects the significance of the cost effective broiler production using appropriate growth promoters for efficient utilization of the nutrients (Huyghebaert *et al.,* 2011) [10]. The phytobiotics are utilized since years ago as preservatives as well as health promoting substances along with the characteristic to impart aroma and flavor to food. Presently there is an increasing interest in the use of natural phytogenic medicinal products as feed additives in poultry ration to boost the production potential of the poultry birds (Hashemi and Davoodi, 2011; Khan *et al.,* 2010, 2012) [9, 12, 13]. Among the medicinal plants neem (*Azadirachta indica*) is recognized as "sacred gift of nature". It is a plant native to south eastern Asia and distributed in India and neighboring countries (Kumar and Navaratnam, 2013) [14]. Neem holds a unique place in ethnoveterinary medicine due to its vast array of medicinal properties without showing any deleterious effects on health (Kale *et al.,* 2003) [11]. More than 140 compounds have been isolated from neem. The chief active principles found in neem leaves are nimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Mitra *et al.,* 2000) [16] Presently the use of organic acids in broiler feeding has been increased many times due their ability to improve the growth performance and reduce the harmful pathogenic microbes, specially intolerant to pH variations, providing the better gut health to maximize the nutrient availability by lowering the gut pH (Ao *et al.,* 2009) [5].

2. Materials and Methods
The present experiment was conducted to study the effect of supplementation of neem leaf meal and citric acid on hemato-biochemical parameters. The experiment was conducted for a period of 6 weeks during the month of December 2017 to February 2018 at Instructional Livestock Farm Complex (ILFC), College of Veterinary Science and Animal Husbandry, Navsari Agricultural University (NAU), Navsari, Gujarat.

2.1 Hemato-biochemical parameters

Blood samples were collected randomly from 8 birds of each group (2 birds/ replicate) at the end of 6th week during morning using vacutainer tube through wing vein puncture under aseptic conditions. For estimation of serum biochemicals, blood samples were collected into vacutainer tubes (*BD vacutainer SST II -Gel-5ml*) without anticoagulant and allowed to clot for 1 h at room temperature (25°C). Serum was separated from the blood sample after centrifugation at 2000 rpm for 15 min. Separated clear, non-haemolysed serum samples were stored in deep freeze (-20ºC) using clean, dry, Eppendorf tubes for future use. However, for estimating hematological observations fresh blood samples were collected in vacutainer tubes (*BD vacutainer K2 EDTA-3ml*) coated with anticoagulant (EDTA).

2.1.1 Hematological parameters

About 2 ml of whole blood in EDTA vial was used for hematological assay of experimental birds. The hematological parameters *viz*. haemoglobin (Hb), haematocrit value/packed cell volume (PCV) and total leucocyte count (TLC) were estimated in the laboratory using manual methods. Haemoglobin was estimated by acid haematin method using Sahli's haemoglobinometer whereas; Microhaematocrit method was utilized to determine PCV. The white blood cells in the whole blood were counted in a hemocytometer counting chamber under the microscope.

2.1.2 Serum bio-chemicals

The serum concentration of glucose, total protein, albumin, cholesterol, and serum enzymes *viz.* alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated by using their respective commercial analytical kits (Diatek Healthcare Pvt. Ltd, Kolkata). The assay was carried out as per the prescribed protocol by manufacturer's leaflet by using *Merck Microlab 300 Biochemistry analyzer*. Estimation of serum glucose was done by GOD/PAP, total protein done by biuret method, Serum albumin done by Bromocresol Green (BCG), Serum globulin was estimated by subtracting the albumin from total protein in serum samples and expressed in g/dL, Serum cholesterol was done as per CHOD-POD method using assay kit. And Estimation of serum enzymes ALT, AST, was done by UV kinetic method using assay kit. Estimation of serum ALP was done by International Federation of Clinical Chemistry (IFCC) method using assay kit.

3. Results

3.1 Hematological profile

The perusal of Table 1 revealed that values of serum concentration of haemoglobin (g/dL) and packed cell volume (%) did not show any significant change among different groups. The mean values of Hb (g/dl) and PCV (%) were 8.43±0.31, 8.81±0.44, 9.43±0.19, 9.12±0.39, 8.50±0.31, 9.68±0.24 and 33.75±1.25, 35.75±1.38, 37.75±0.75, 37.00±1.47, 34.50±1.32, 36.25±2.25 in T0, T1, T2, T3, T4 and T5 groups respectively. The mean values of TLC were also comparable to various dietary treatments. The mean values of TLC (10^3/µL) were 22.02±1.50, 22.19±2.35, 22.79±1.43, 21.71±2.20, 23.38±2.68 and 23.85±2.02 in T0, T1, T2, T3, T4 and T5 groups respectively.

3.2 Metabolic profile

The mean concentration of glucose did not differ significantly among the dietary treatments. The mean values of glucose (mg/dL) were 219.07±10.71, 256.31±4.41, 234.12±14.76, 243.72±23.79, 246.10±33.67 and 240.50±13.28 for T0, T1, T2, T3, T4 and T5 groups respectively.

The serum concentration of total proteins and their fractions viz. albumin, globulin and A/G ratio were comparable (P>0.05) among various dietary treatments. The mean values (g/dL) of total protein for T0, T1, T2, T3, T4 and T5 groups were 3.21±0.15, 3.24±0.11, 3.32±0.20, 3.29±0.09, 3.24±0.29 and 3.47±0.16, respectively while that of albumin were 1.97±0.24, 1.85±0.23, 1.93±0.12, 1.82±0.24, 2.04±0.30 and 2.27±0.29, respectively. Similarly, mean values (mg/dL) of globulin and A/G ratio were 1.24±0.19, 1.39±0.18, 1.38±0.32, 1.47±0.19, 1.19±0.13, 1.20±0.16 and 1.77±0.44, 1.47±0.37, 1.70±0.47, 1.36±0.33, 1.81±0.39, 2.05±0.44 across the different group respectively.

Table 1: Mean±SEM of hematological profile in broilers supplemented with neem leaf meal and citric acid

Attributes	Dietary groups						F Value	P Value
	T0	T1	T2	T3	T4	T5		
Haemoglobin (g/dL)	8.43±0.31	8.81±0.44	9.43±0.19	9.12±0.39	8.50±0.31	9.68±0.24	2.452	0.082
Packed cell volume (%)	33.75±1.25	35.75±1.38	37.75±0.75	37.00±1.47	34.50±1.32	36.25±2.25	0.993	0.455
TLC (10^3/µL)	22.02±1.50	22.19±2.35	22.79±1.43	21.71±2.20	23.38±2.68	23.85±2.02	0.287	0.913

Table 2: Mean±SEM of serum metabolic profile of broilers supplemented with neem leaf meal and citric acid

Attributes	Dietary groups						F Value	P Value
	T0	T1	T2	T3	T4	T5		
Glucose (mg/dL)	219.07±10.71	256.31±4.41	234.12±14.76	243.72±23.79	246.10±33.67	240.50±13.28	0.366	0.864
Total protein (g/dL)	3.21±0.15	3.24±0.11	3.32±0.20	3.29±0.09	3.24±0.29	3.47±0.16	0.258	0.929
Albumin (g/dL)	1.97±0.24	1.85±0.23	1.93±0.12	1.82±0.24	2.04±0.30	2.27±0.29	0.382	0.853
Globulin (g/dL)	1.24±0.19	1.39±0.18	1.38±0.32	1.47±0.19	1.19±0.13	1.20±0.16	0.330	0.887
A:G	1.77±0.44	1.47±0.37	1.70±0.47	1.36±0.33	1.81±0.39	2.05±0.44	0.334	0.885
Cholesterol (mg/dL)	174.75±4.62	146.25±2.98	150.37±9.75	157.87±5.80	156.00±5.87	160.25±21.64	0.918	0.496
ALT (U/L)	2.99±0.30	2.81±0.21	2.78±0.38	2.70±0.26	2.77±0.24	2.87±0.24	0.179	0.966
AST (U/L)	341.87±9.43	319.50±32.57	324.37±17.09	345.50±21.00	328.87±10.48	337.50±17.50	0.231	0.943
ALP (U/L)	1671.11±89.27	1662.75±63.59	1684.18±98.54	1657.67±26.48	1649.92±145.53	1635.08±40.25	0.054	0.998

The perusal of Table 2 revealed that numerically lower value of cholesterol concentration was observed in T1 group but difference was statistically non significant. The mean values of cholesterol (mg/dL) ranged from 174.75±4.62, 146.25±2.98, 150.37±9.75, 157.87±5.80, 156.00±5.87, and 160.25±21.64 for T0, T1, T2, T3, T4 and T5 groups respectively.

The serum activity of three hepatic enzymes viz. alanine

amnimotransferase (ALT), aspartate aminotransferase (AST) and alkaline phospshatase (ALP) did not show any significant variation among the different groups. The mean values (U/L) of ALT and AST were 2.99±0.30, 2.81±0.21, 2.78±0.38, 2.70±0.26, 2.77±0.24, 2.87±0.24 and 341.87±9.43, 319.50±32.57, 324.37±17.09, 345.50±21.00, 328.87±10.48, 337.50±17.50 for different dietary groups T0, T1, T2, T3, T4 and T5, respectively. Similarly the mean values (U/L) of ALP were 1671.11±89.27, 1662.75±63.59, 1684.18±98.54, 1657.67±26.48, 1649.92±145.53, 1635.08±40.25, respectively for the T0, T1, T2, T3, T4 and T5 groups, respectively.

4. Discussion

4.1 Hemato-biochemical parameters

Hematology is an integral part of health management in the poultry. Any alteration in hematological profile from their normal physiological range is an indication of ill health and hence, can be utilized as health indicator. The results of present study showed that supplementation of NLM, CA and combination did not affect the hematological indices viz. Hb, PCV and TLC in broiler chickens. Further, the values of hematological parameters are found in their normal ranges indicated that dietary supplementation of NLM and CA has no deleterious effects on the internal physiology of broilers.

The results of present study are in accordance with Nnenna and okey (2013) [18]; Alam *et al*. (2015) [1] and Nodu *et al.* (2016) [19] who reported that supplementation of neem in broilers did not affect the hematological indices. In contrast to our findings Ansari *et al.* (2012) [4] and Nayaka *et al*. (2013) [17] observed higher values while, significant reduction in hematological parameters were confirmed by Obikaonu *et al.* (2011) [23] and Bonsu *et al.* (2012) [7] in birds supplemented with neem leaf extract in their diets. The findings of this study had shown non significant effect of CA supplementation in broilers which is in agreement with Lala *et al.* (2016) [15] and Ogunwole *et al.* (2017) [24] who reported the non responsive effect of acidifier in broilers. However, contradictory findings were observed by Al-Saad *et al.* (2014) [2] who reported significant increase in number of WBC in birds of organic acids group than control one.

The results of present study had shown that serum metabolites in different groups were found in their normal physiological range. Though, the values did not differ significantly among the dietary groups. Non significant effect of NLM on serum total protein, albumin and globulin in present investigation is supported by Nnenna and okey (2013) [18] who reported that different dietary levels of neem leaf extract did not show any significant (P>0.05) impact on the serum protein, albumin and globulin in broilers. In contrast to our findings Ansari *et al.* (2012) [4] and Shihab *et al.* (2017) [25] observed higher serum total protein in birds fed with neem leaf powder than in control group. Similar to our results Nourmohammadi *et al.* (2011) [20] and Nourmohammadi and Khosravinia (2015) [22] confirmed non significant effect of CA on serum total proteins in broilers while, contradictory results were observed by Ghazalah *et al.* (2011) [8] who reported a significant (P<0.01) increase in total protein in birds supplemented with 2% CA as compared to control group.

In the present study numerically lower values of serum cholesterol were observed in birds treated with NLM and CA but difference was statistically non significant among the groups. In contrast to present findings Ansari *et al*. (2012) [4] and Shihab *et al.* (2017) [25] found significant reduction in cholesterol in birds supplemented with neem leaf meal. Similarly Nourmohammadi and Khosravinia (2015) [22] also confirmed the depression in the serum cholesterol concentration in CA supplemented broilers than control one.

The different serum enzymes viz. AST, ALT are generally found in higher concentration in heart, liver, kidneys and muscles, while the site of ALP is cell lining of the biliary ducts, gut epithelium, kidney, bone and liver. It has been stated that any kind of liver, kidney and muscle injury results in rising of serum level of these enzymes. Therefore, serum levels of AST, ALT and ALP can be utilized as biomarkers of liver, kidney and muscle injury. The findings of this study indicated that supplementation of NLM and CA in broilers did not affect the serum concentration of AST, ALT and ALP among the groups. Moreover, the values of these enzymes have been observed very much in their normal physiological range, indicated that broilers under different dietary treatments did not suffer from any hepatic, renal and muscle injury. In contrast to our findings Obikaonu *et al.* (2011) [23] and Ansari *et al*. (2012) [4] confirmed that birds supplemented with NLM found with reduced activity of serum AST, ALT and ALP. Our findings are in agreement with Nourmohammadi *et al*. (2011) [20] who reported non responsive impact of citric acid on serum level of AST ALT in broilers. In contrast to our results Nourmohammadi and Khosravinia (2015) [22] reported the increased serum activity of AST and drop in serum ALP level in broilers supplemented with citric acid than those without any supplementation.

5. Conclusions

The hematological profile viz. Haemoglobin, Packed cell volume and TLC did not show any significant change among different groups. Similarly the values of serum metabolites viz. glucose, total protein, albumin, globulins, cholesterol and serum enzymes (AST, ALT and ALP) revealed the non responsive effect of NLM and CA in the broilers.

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