The present experiment was conducted to study the effect of supplementation of Neem leaf meal (NLM) and citric acid (CA) on hemato-biochemical parameters in broiler birds. 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 serum biochemical parameters (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 a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system. The enormous growth in poultry industry has been primarily a leap in past decades, emerging from an unscientific farming practice to commercial production system.
2.1 Hematopoietic 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 vacuum 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 (-20°C) 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; Microhaemocrit 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-PDOH 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 (10³/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

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Dietary groups</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>8.43±0.31</td>
<td>8.81±0.44</td>
<td>9.43±0.19</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>33.75±1.25</td>
<td>35.75±1.38</td>
<td>37.75±0.75</td>
</tr>
<tr>
<td>TLC (10³/µL)</td>
<td>22.02±1.50</td>
<td>22.19±2.35</td>
<td>22.79±1.43</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Dietary groups</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>219.07±10.71</td>
<td>256.31±4.41</td>
<td>234.12±14.76</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>3.21±0.15</td>
<td>3.24±0.11</td>
<td>3.32±0.20</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>1.97±0.24</td>
<td>1.85±0.23</td>
<td>1.93±0.12</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>1.24±0.19</td>
<td>1.39±0.18</td>
<td>1.38±0.32</td>
</tr>
<tr>
<td>A:G</td>
<td>1.77±0.44</td>
<td>1.47±0.37</td>
<td>1.70±0.47</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>174.75±4.42</td>
<td>146.25±2.98</td>
<td>150.37±9.75</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>2.99±0.30</td>
<td>2.81±0.21</td>
<td>2.78±0.38</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>341.87±9.43</td>
<td>319.30±32.57</td>
<td>324.37±17.09</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>1671.11±89.27</td>
<td>1662.75±83.59</td>
<td>1664.18±59.34</td>
</tr>
</tbody>
</table>

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...
aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (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 1671.14±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) [10], Alam et al. (2015) [11] 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) [18] 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) [10] 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) [22] 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) [8] 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) [22] 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.

6. Acknowledgement

I would be pleased to mention the names of Dr. B. P. Brahmkshtri (Head of department) and other supporting staff members of ILFC farm, Navsari for the invaluable cooperation rendered directly or indirectly during my research work. The technical assistance of and the guidance of Dr. K. K. Verma and Dr. R. R. Singh in the initial stages of the study are also acknowledged.

7. References

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