MEDICINAL PLANTS LEAF MEAL SUPPLEMENTATION IN BROILER CHICKEN DIET: EFFECTS ON PERFORMANCE CHARACTERISTICS, SERUM METABOLITE AND ANTIOXIDANT STATUS

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ABSTRACT

This experiment was carried out to investigate the effect of medicinal plants leaf meal on performance characteristics, serum metabolites and antioxidant status of broiler chicken. The experiment employed a completely randomised design. All data generated were subjected to analysis of variance. A total of one hundred and forty four 4-week Abor-Acre broiler chickens were used in a finisher phase, the birds were fed with broiler finisher diets for 28 day feeding trial. The birds were assigned to 4 dietary treatments replicated three times with 12 birds per replicate. Diet I, the control diet (basal diet), Diet II contained 0.2 % bitter leaf meal (BLM), Diet III contained 0.2 % Moringa oleifera leaf meal (MOLM) and Diet IV contained 0.2 % of mixture of BLM and MOLM (1:1). The average final live-weight and daily weight gain increased (p<0.05) with the mixture of BLM and MOLM. The feed conversion ratio of birds on control diet and Diet IV were better at 1.91 and 2.03, respectively. The cholesterol levels of birds fed medicinal plant reduced significantly (p<0.05). Supplementation of diets with medicinal plants resulted in a significant (p<0.05) increase in creatinine and bilirubin. The enzyme activities test and total protein were not influenced (p>0.05) by experimental diets. The oxidative activities increased (p<0.05) in the dietary herbal supplement. It was concluded that supplementation of broiler chicken with herbs or mixture of herbs enhanced the growth performance and antioxidant capacity of birds without any deleterious effect on the health status of the birds.

Keywords: Bitter leaf meal, Moringa oleifera leaf meal, Serum, Anti-oxidant status

INTRODUCTION

Antibiotics have been used for more than a half of a century to improve animal performance and decreasing the pathogenic bacteria population. Unfortunately, the bacteria have established resistant strains by transferring resistance to other species between humans and animals which have resulted in serious problems in public health and livestock production (Thakare, 2004). This formed the basis for the ban of these antibiotics growth promoters by the European Union.

In recent years, extensive research has been performed on the use of phytochemicals such as bitter leaf meal and moringa leaf meal as alternative to antibiotic growth promoters in poultry diets (Mirzaei-Aghsaghali, 2012). Phytochemical screening of bitter leaf and moringa leaf meals revealed the presence of phenolic acids, flavonoids, tannins, cardiac glycosides, saponin and glucosinolates. These compounds are of great value in preventing the onset or progression of many human and animal diseases. The health promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (Muanda et al., 2011).

Medicinal plants like bitter leaf meal, moringa leaf meal contain some toxins that have multi-system effects such as kidney damage (Swanepoel et al., 2008). Serum
metabolites can also be affected by some medicinal plants; it can increase or decrease due to some damages or repairs taking place in the cells or organs (Oduola et al., 2007). Ewuola and Egbunike (2008) reported that some medicinal plants are basically used as feed supplements or medicinal thereby becoming involved in a cascade of physiological reactions that may lead to alteration of serum metabolites. This could result from the toxic substances present in the plant that causes lowering or elevating the serum metabolites. It could also act as non-toxic invaluable compounds that maintain the values within the expected reference ranges for chickens (Simaraks et al., 2004). This study was conducted to evaluate the role of dietary supplementation of bitter leaf meal, moringa leaf meal and the combination of bitter leaf meal and moringa leaf meal on performance characteristics, serum metabolites and antioxidant enzyme status of broiler chicken.

MATERIALS AND METHODS

Location and Duration of Feeding Trial: The feeding trial was carried out at the Poultry Unit of the Teaching and Research Farm, The Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria. The study was carried out between October to December, 2017.

Site Preparation: The poultry house was thoroughly washed and fumigated. It was allowed to stay for two weeks before the arrival of the experimental birds.

Experimental Birds: A total number of one hundred and forty four 4-week old broiler chicks of Arbo-Acre commercial breed were used in the experiment. Thirty-six birds distributed to four dietary treatments (12 birds per replicate, 36 birds per treatment) in a completely randomized design.

Preparation of Test Ingredients: The test ingredients bitter leaf meal (BLM) and Moringa oleifera leaf meal (MOLM) were harvested fresh from maturing stems. The fresh leaves were subjected to air-drying indoors. The air dried bitter leaf meal and moringa leaf meal were later milled to powder using a commercial feed milling machine.

Experimental Diets: The compositions of the experimental diets are presented in Tables 1.

Table 1: Composition of experimental diets (g/100 g) for broiler finisher birds

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Diets</th>
<th>1 Control</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0 %</td>
<td>0.2 %</td>
<td>0.2 %</td>
<td>0.2 % BLM + MOLM</td>
</tr>
<tr>
<td>Maize</td>
<td></td>
<td>65.00</td>
<td>65.00</td>
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<td>65.00</td>
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<tr>
<td>Groundnut cake</td>
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<td>11.00</td>
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<td>11.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td></td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Bone meal</td>
<td></td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Oyster shell</td>
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<td>2.00</td>
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</tr>
<tr>
<td>NaCl</td>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
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<td>0.30</td>
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</tr>
<tr>
<td>Lysine</td>
<td></td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Premix</td>
<td></td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Total Calculated composition</td>
<td></td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Crude protein (g/100 g)</td>
<td></td>
<td>20.31</td>
<td>20.31</td>
<td>20.31</td>
<td>20.31</td>
</tr>
<tr>
<td>Crude fibre (g/100 g)</td>
<td></td>
<td>2.85</td>
<td>2.85</td>
<td>2.85</td>
<td>2.85</td>
</tr>
<tr>
<td>M/energy (Kcal/kg)</td>
<td></td>
<td>2984.7</td>
<td>2984.7</td>
<td>2984.7</td>
<td>2984.7</td>
</tr>
</tbody>
</table>

NaCl = Sodium chloride *Supplied per kg diet: Vit. A, 8×10⁶ IU; Vit. D₃, 1.2×10⁷ IU; Vit. E 7×10⁵ mg; Vit. 1.5×10⁷ mg; Vit.B₁, 2.00 mg; Niacin 15 g; Pantothenic acid, 5.5 g; Vit.B₂, 2 g; Vit.B₆, 10 mg; Folic acid 500 mg; Biotin 500 mg; Choline chloride 175 g; Cobalt 200 mg; Copper 3 g; Iodine 1 g; Fe 21 g; Mn 40 g; Selenium 200 mg; Zinc 31 g

The basal diets were formulated for the finisher phase (29 – 56 days of age) to meet NRC (1994) requirements for broilers. Thereafter, the basal diets were divided into four diets. The four diets were formulated to be iso-caloric and iso-nitrogenous. Diet I, control diet (diet without any supplementation), Diet II and Diet III contained 0.2 % of bitter leaf meal and moringa leaf meal, respectively. Diet IV contained 0.2 % mixture of bitter leaf meal and moringa leaf meal.
meal (1:1). After the broiler starter phase, birds were fed with broiler finisher diets for 28 days.

Data Collection: Data on growth performance of the experimental birds were obtained from weekly feed intake and body weight records. They were used to calculate the feed conversion ratio (ratio of feed intake to weight gain) at the end of the experiment.

At the end of the study, prior to blood collection, the birds were starved of feed, 3 birds per treatment were randomly selected and bled by severing the jugular vein. About 5 ml of blood samples were collected from each bird in a two different labelled vacutainer tubes without anticoagulants and were taken to the medical laboratory for serum biochemistry test and science laboratory for antioxidant enzyme determination. The serum biochemistry tested were cholesterol, creatinine, bilirubin, aspartate transaminase, alanine transaminase and total protein. The serum samples were kept in sterile vacutainer tubes and kept deep frozen prior to analysis to determine cholesterol as outlined by Roschlau et al. (1974), creatinine and bilirubin were assessed by the colometric method described by Newman and Price (1999). Aspartate transaminase and alanine transaminase levels were determined as described by Huang et al. (2006). The total protein was determined using the method described by Peters (1968). The antioxidant enzymes such as glutathione peroxidase (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) concentrations were determined using the methods of Beutler et al. (1963). Enzymatic activity of SOD in the serum was determined through xanthine oxidase method. Samples were taken to detect absorbance at 550 nm with spectrophotometer. GSH activity was measured by dithio-dinitrobenzic acid method at the absorbance of 412 nm and analysed according to Beutler et al. (1963). The MDA level was analysed with 2-thiobarbituric acid (TBA), monitoring the change of absorbance at 532 nm with a spectrophotometer (Jensen et al., 1997).

Statistical Analysis: All data collected in this study were subjected to Analysis of Variance (ANOVA) using SPSS statistical package (SPSS 17.0 for widows Inc. Chicago IL, USA). Duncan’s Multiple Range Test was used to separate significant mean differences. Significant differences were considered at 95 % level.

RESULTS AND DISCUSSION

The results of proximate and phytochemical compositions of bitter leaf meal (BLM) are shown in Table 2.

Table 2: Proximate analysis and phytochemicals in bitter leaf meal (BLM)

<table>
<thead>
<tr>
<th>Proximate Composition (%)</th>
<th>Moisture content</th>
<th>10.78 ± 1.58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>15.60 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Crude fibre</td>
<td>10.58 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.00 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>5.57 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>52.12 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phytochemicals (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
</tr>
<tr>
<td>Phytate</td>
</tr>
<tr>
<td>Oxalate</td>
</tr>
<tr>
<td>Phenol</td>
</tr>
</tbody>
</table>

Source: Daramola et al. (2018a)

The moisture content of BLM was 10.78 ± 1.05 %. The value was slightly higher than that reported 10.02 % of Asaolu et al. (2012). The variation may be due to soil nutrients and environmental factors which have impact on the nutrient availabilities for plants. The crude protein content (15.60 ± 1.58 %) was higher than that reported by Umit et al. (2011) 5.53 % for rosemary leaf. The fat content (5.00 ± 0.10 %) indicated the presence of oil in bitter leaf. The value recorded was higher than that reported by Udosen (1995). The crude fibre content was found to be 10.58 ± 0.18 %, this value fell within the range (8.50 – 20.90 %) for some vegetables from Niger Republic (Freiberger et al., 1998). The ash content (5.57 ± 0.21 %) was lower than that reported by
Asaolu et al. (2012) for bitter leaf (9.56 %). The presence of ash content in bitter leaf is a confirmation of availability of mineral elements. The nitrogen free extract content (52.12 ± 0.47 %) was higher than that reported by Asaolu et al. (2012) for the same plant (8.56 %) the variation may be due to soil nutrients and environmental factors which have effects on the nutrients availability for plants (Adewole et al. 2015). Phytochemicals are of benefit to health and play an active role in the management of some diseases. The flavonoid content was 4.84 ± 0.16 mg/100g, phytate 17.53 ± 0.15 mg/100mg, oxalate was 3.76 ± 0.05 mg/100mg and phenol was 1.90 ± 0.17 mg/100g. Flavonoids have been found useful in drug preparation, in food, feed and beverages. Flavonoids from bitter leaf including phenolic acids had inhibitory activity against bacteria (Farombi and Owoeye, 2011).

The result of proximate analysis in Table 3 showed that MOLM had an appreciable crude protein (CP) content (27.95 ± 1.62 %), crude fibre (9.47 ± 0.27 %), ash (8.07 ± 1.63 %), nitrogen free extract (NFE) (46.82 ± 0.87 %) but low content of ether extract (EE) (4.96 ± 1.24 %).

The CP value of MOLM obtained in this study was lower than the value reported by Ojo and Adetoyi (2017) which was 28.43 % although Mutayoba et al. (2011) recorded a higher CP (30.65 %) value. The EE and ash values 9.47 and 8.07 % observed in this study were higher than the values 2.11 and 7.93 % reported by Ogbe and Affiku (2011).

Crude fibre value of 9.47 % reported in this study was higher than 5.43 % reported in the study conducted by Sodamade et al. (2013). Differences in proximate values of MOLM observed in the previous studies may be due to differences in the soil type, climatic conditions, stage of maturity and the genetic make-up.

The performance characteristics of broiler chickens fed the experimental diets, indicated that the final live-weight and average daily weight gain for birds fed diets 1 and 4 were similar (p>0.05) but significantly higher (p<0.05) than birds fed diets 2 and 3 (Table 4). The FCR of birds fed Diet II were significantly higher (p<0.05) than the FCR of birds fed other diets. The lowest FCR value was recorded for birds fed the control diet (1.91 ± 0.01), while the highest FCR was recorded for birds fed Diet II (2.41 ± 0.02). Inclusion of medicinal plant extracts in poultry diets impacted the metabolism by reducing stress and microbial activity (Sanjyal and Sapkota, 2011). The general improvement by equal mixture of BLM and MOLM on final live-weight and daily weight gain in this study revealed that the mixture of herbs (BLM and MOLM), enhanced growth performance of broiler birds. Apart from the control diets, birds fed Diet IV performed better, the combination of herbs in broiler chicken diet might have improved the production and activities of digestive enzymes. The improved performance recorded for birds on Diet IV may be attributed to the increased secretion of digestive enzyme and enhanced nutrition utilization in liver (Khan et al., 2012). The antibacterial action of essential component of the combination of medicinal plant may suppress the growth of pathogenic bacteria on one hand and promote the growth probiotic bacteria in the gut (Barreto et al., 2008). These indicated why medicinal plants may be used as alternative to antibiotic growth promoters because they exhibit antimicrobial properties and thus can form integral part of poultry nutrition (Onibie et al., 2009). The excellent performance of birds on diets supplemented with the mixture of those leaves suggested the

| Table 3: Proximate analysis and phytochemicals in Moringa oleifera leaf meal (MOLM) |
|-----------------|-----------------|
| Proximate Composition (%) | Phytochemicals (mg/100 g) |
| Moisture content | 7.42 ± 2.15 |
| Crude protein | 27.95 ± 1.62 |
| Crude fibre | 9.47 ± 0.27 |
| Ether extract | 4.96 ± 1.24 |
| Ash | 8.07 ± 1.63 |
| Nitrogen free extract | 46.82 ± 0.87 |
| Flavonoid | 3.72 ± 0.15 |
| Phytate | 2.34 ± 0.02 |
| Oxalate | 0.65 ± 0.03 |
| Phenol | 2.74 ± 0.25 |
Medicinal plants leaf meal supplementation in broiler chicken diet

Table 4: Effect of herbal supplementation on growth performance of broiler finisher phase

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diets</th>
<th>Herbal inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Average initial weight (g)</td>
<td>751.39 ± 0.02</td>
<td>745.90 ± 0.22</td>
</tr>
<tr>
<td>Average final Live-weight (g)</td>
<td>2045.00 ± 34.54bc</td>
<td>1658.67 ± 41.93c</td>
</tr>
<tr>
<td>Average daily weight gain (g)</td>
<td>73.04 ± 2.00a</td>
<td>59.24 ± 1.25c</td>
</tr>
<tr>
<td>Total feed intake (g)</td>
<td>3906.84 ± 40.56b</td>
<td>3991.96 ± 48.23b</td>
</tr>
<tr>
<td>Average daily feed intake</td>
<td>139.53 ± 0.42bc</td>
<td>2.41 ± 0.02a</td>
</tr>
</tbody>
</table>

Means with different superscript on the same row differ significantly (p<0.05); BLM = bitter leaf meal, MOLM = Moringa oleifera leaf meal.

Table 5: Serum metabolites of broiler finisher birds fed experimental diets

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diets</th>
<th>Herbal inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>92.73 ± 9.18a</td>
<td>63.53 ± 10.56c</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.38 ± 0.03bc</td>
<td>0.98 ± 0.06a</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>2.17 ± 0.13bc</td>
<td>2.74 ± 0.09a</td>
</tr>
<tr>
<td>Aspartate transaminase (IU/L)</td>
<td>44.17 ± 0.97ac</td>
<td>40.73 ± 0.89abc</td>
</tr>
<tr>
<td>Alanine transaminase (IU/L)</td>
<td>24.99 ± 0.12</td>
<td>24.93 ± 0.10</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>4.10 ± 0.02</td>
<td>4.08 ± 0.01</td>
</tr>
</tbody>
</table>

Means with different superscript on the same row differ significantly (p<0.05); BLM = bitter leaf meal, MOLM = Moringa oleifera leaf meal.

The serum concentration of creatinine and bilirubin of birds on diets 2, 3 and 4 were similar (p>0.05) but significantly higher (p<0.05) than birds fed the control diet. The aspartate transaminase concentration of birds fed the control diet was significantly higher (p<0.05) than for birds fed Diet IV. Alanine transaminase and total protein of birds were not significantly influenced (p>0.05) by the experimental diets.

The low creatinine value recorded for birds fed the control diet was clinically non-significant (Saleh et al., 2018). The bilirubin value recorded for birds fed the control diet was lower than the values recorded for other birds fed experimental diets. The increased levels of bilirubin could be due to an acute haemolytic disorders or lipaemia (Saleh et al., 2018). The aspartate transaminase, alanine transaminase and total protein were not influenced by experimental diets.

Hyperlipidaemia is one of the risk factor for cardiovascular disease, while cholesterol is the major lipid constituent of atherosclerotic plaque (Daramola et al., 2017). The creatinine values recorded for birds fed diets 2, 3 and 4 were within the range of chemical component in serum of chicken (0.90 – 1.85 mg/dl) (Mitruka and Rawnsley, 1977).

The antioxidant properties of herbs need special attention because undesirable oxidation produces changes in odour, colour and other undesirable effects. The effect of supplementing medicinal plant on antioxidant parameters such as serum activity of...
glutathione (GSH), catalase (CAT) superoxide
dismutase (SOD) and malondialdehyde (MDA) of
broiler birds are shown in Table 6.

The GSH concentration of birds fed
diets 2, 3 and 4 were similar (p>0.05) but
significantly higher (p<0.05) than GSH
concentration of birds fed the control diet. The
catalase concentration of birds fed diets 3 and 4
were significantly higher (p<0.05) at 491.26 ±
5.34 µM⁻¹ and 489.51 ± 7.05 µM⁻¹, respectively
than catalase concentration of birds fed the
control diet and Diet II at 431.48 ± 6.54 µM⁻¹
and 462.94 ± 5.73 µM⁻¹ respectively.

Table 6: Effect of herbal supplementation on antioxidant status of broiler finisher birds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diets</th>
<th>Herbal inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Glutathione</td>
<td>88.71 ± 1.37</td>
<td>97.33 ± 1.05</td>
</tr>
<tr>
<td>Catalase</td>
<td>431.48 ± 6.54</td>
<td>462.94 ± 5.73</td>
</tr>
<tr>
<td>SOD</td>
<td>94.40 ± 0.36</td>
<td>96.21 ± 0.39</td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td>13.56 ± 0.47</td>
<td>10.14 ± 0.52</td>
</tr>
</tbody>
</table>

Means with different superscript on the same row differ significantly (P<0.05); BLM = bitter leaf meal, MOLM = Moringa oleifera leaf meal, SOD = Superoxide dismutase

Serum SOD activity was significantly increased
(p<0.05) in birds fed Diet III and reached a
maximum (98.19 ± 0.44 µM⁻¹) with MOLM
supplemented diet, while the least activity was
recorded for birds fed control diet (94.40 ± 0.36
µM⁻¹). The lipid peroxidation concentration of
birds fed control diet was significantly higher
(p<0.05) than lipid peroxidation concentration
of birds fed diets 2, 3 and 4 (10.14 ± 0.52
nmol/ml, 9.7 ± 0.45 nmol/ml and 8.74 ± 0.54
nmol/ml) respectively.

These results conformed with the report
of Daramola et al. (2018 a, b) that the intake of
herbs or medicinal plants or their contents
resulted in increased serum antioxidant enzyme
such as GSH, catalase and SOD and a decreased
lipid peroxidation concentration. Elevated levels
of antioxidant enzymes may improve the steady
state of the system of broiler birds. The results
of this study demonstrated that phytochemicals
such as flavonoid, quercetin and phenol were
identified as the most potent antioxidants in
moringa leaves and bitter leaf (Atawodi et al.,
2010). Adewole et al. (2015) reported that BLM
and MOLM possess some antioxidative
properties with high nutritive values; the leaves
are rich in mineral and other essential
phytochemicals. The antioxidants may be used
as defense system to prevent free radicals from
damaging the cells and organs of the body, and
against infections and degenerative diseases

(Sreelatha and Padma, 2009). An over
production of reactive system can cause the
imbalance of defense system therefore,
antioxidants are needed which focus on natural
compounds from natural sources. From this
study, supplementation with mixture of BLM and
MOLM increased the activities of GSH and
catalase but decreased the MDA concentration
in the serum of broiler chickens. The higher the
activities of GSH, catalase in diets 3 and 4 the
higher the capacity of broiler chicken to clear
out the oxygen free radicals i.e. reactive oxygen
species (ROS) and lower MDA concentration.
The increase in the GSH, catalase and SOD may
be due to the enhanced antioxidant status by
MOLM and mixture of BLM and MOLM which is
likely attributed to the antioxidant compounds.

**Conclusion:** It can therefore be concluded that
supplementation of broiler finisher birds with
herbs or the mixture of herbs enhanced the
antioxidant capacity of broiler chicken without
any deleterious effect on the serum
biochemistry of the experimental birds. Further
studies can be done to determine the use of
BLM and MOLM in broiler chickens.

**ACKNOWLEDGEMENTS**

I want to appreciate Dr. O. D. Oloruntola and
the unanimous peer reviewers for taking their
time to correct this manuscript. I also want to appreciate Janet Lawal, Grace Daramola and Emmanuel Ani for their assistance during the brooding phase of the study.

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