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Original Article

Effect of *Moringa oleifera* feed supplementation on the serum biochemical profile of broilers challenged with very virulent infectious bursal disease virus

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ABSTRACT

Objective: This study was conducted to assess the effect of dietary *Moringa oleifera* leaf (MOL) feed supplementation on serum biochemical parameters of broilers challenged with very virulent infectious bursal disease virus (vvIBDV).
Materials and methods: Two hundred and forty day-old Ross 308 hybrid broiler chicks were randomly assigned into four groups (A, B, C and D) of 60 chicks each and raised in deep litter housing. Broiler starter (BS) and broiler finisher (BF) mash were formulated each with 5% MOL included as part of the feed ingredient for broilers in groups A and B while BS and BF for broilers in groups C and D were formulated without MOL. Broilers in groups A, B and C were challenged intraocularly at 35 days of age with with 0.05 mL of a live vvIBDV, while those in group D served as control. Blood was collected from 10 broilers in each group via the wing vein at 35, 38 and 42 days of age to determine their serum biochemical profile.
Results: The level of melondialdehyde (MDA) was observed to significantly decrease in groups A and C. There was a significant decrease in the level of AST in group A, B, C and D. The values of ALT significantly decreased in group A, B, C and D.
Conclusion: Supplementing broilers feed with MOL neither protect the liver from damage nor prevent lipid peroxidation.

KEYWORDS

ALT; AST; Broilers; IBD; MDA; *Moringa oleifera* leaf; Serum

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INTRODUCTION

The therapeutic effect of *Moringa oleifera* (MO) has been accredited to its possession of several antioxidants that are known to have suppressive effects on development of reactive oxygen species (ROS) and free radicals (Sofidiya et al., 2006; Ogbunugaforet al., 2011). The level of lipid peroxidation is frequently used as a pointer to reactive oxygen species (ROS) mediated injury (Kuun and Borchert, 2002) and the concentration of melondialdehyde (MDA) in blood and tissues are generally used as biomarkers of lipid peroxidation (Sehirliet al., 2008; Yousef et al., 2009). Medicinal values of MO such as the prevention of early liver injury and restoration of antioxidant status by leaf extract in mice fed with high fat diet has been reported (Das et al., 2012). Acetaminophen induced liver injury has been prevented by the leaf extract of MO through the restoration of glutathione level (Fakuraziet al., 2008).

Besides the severe clinical signs and high mortality rate that results from vvIBDV infection in susceptible chickens, it also cause many pathological changes that form part of the pathogenesis of the disease which could basically be explained in terms of the biochemical changes that occur in relation to the pathological effect of the virus in several organs such as the liver and kidneys (Tesfaheywet et al., 2012; Aliyu et al., 2016; Igwe et al., 2017).

The few studies that have attempted to determine biochemical changes associated with IBDV infection (Zain et al., 2014; Beenish et al., 2017), reported variable biochemical profiles. Changes in various antioxidant enzyme activities can be used to estimate the level of oxidative stress and total antioxidant status (Rayman, 2000). *Moringa oleifera* has been reported to possess an antioxidant known to have suppressive effects on formation of ROS and free radicals (Ogbunugaforet al., 2011; Sofidiyaet al., 2006). Feeding broilers with supplemented MOL in their feeds and challenging them with vvIBDV in order to determine biochemical changes that is associated with the disease has not been reported. Therefore, this study was aimed at assessing the effect of dietary *Moringa oleifera* leaf supplementation on serum biochemical parameters of broilers challenged with very virulent infectious bursal disease virus.

MATERIALS AND METHODS

The study was conducted at the Poultry Research Unit of the Faculty of Veterinary Medicine, Ahmadu Bello University Samaru, Zaria, Nigeria. Approval for this research was sort from the ethics committee of the Ahmadu Bello University, Zaria and guidelines for the care and humane handling of animals were strictly adhered to all through the study (FASS, 2010).

Collection and processing of *Moringa oleifera* leaf: *Moringa oleifera* leaf (MOL) was harvested (between the months of August and September) from an orchard at an early flowering stage. The stem and branches were cut from the Moringa trees and spread out to dry under shade at room temperature for five days. The MOL were then removed manually by hand and grounded into powder using a locally manufactured milling machine.

Mineral analysis: Mineral analysis of MOL was carried out according to the procedure of AOAC (1990) and calcium, phosphorus, magnesium, iron, sodium, zinc, copper, selenium, potassium, and manganese components was known (Balami et al., 2018) (**Table 1**).

Table 1: Mineral composition of *Moringa oleifera* leaf

Element	Concentration
Ca	2.26 %
P	0.35 %
Mg	0.45 %
K	1.9 %
Na	0.11 %
Zn	34 ppm
Cu	7.5 ppm
Mn	40.5 ppm
Fe	116.5 ppm
Se	0.85 ppm

ppm=parts per million (1 mg/kg=1 ppm)

Table 2: Phytochemical composition of *Moringa oleifera* leaf

Phytochemical	Concentration (%)
Phytates	2.57
Tannins	2.19
Saponins	1.06
Oxalates	0.45
Cyanides	0.1

Table 3: Proximate composition of *Moringa oleifera* leaf

Metabolite	% composition
Carbohydrate	55.14
Crude protein	25.9
Crude fibre	13.91
Moisture	7.94
Fat	5.85
Ash	3.72
Energy	2930.63 (KCal/Kg)

Table 4: Composition of experimental dietsof broilers starter and finisher per 100 kgfeed.

	Broiler starter (A and B) (%)	Broiler finisher (A and B) (%)	Broiler starter (C and D) (%)	Broiler finisher (C and D) (%)
Maize	50.14	52	50.14	52
Maize offal	9.2	10	9.2	10
Soyabean cake	11.695	8.4875	14.1925	10.18
Ground nut cake	11.69	13.9875	14.1925	17.295
MOLM	5	5	0	0
Fish meal	5	5	5	5
Salt	0.3	0.3	0.3	0.3
Lime stone	1.5	0.5	1.5	0.5
Bone meal	3.5	3.5	3.5	3.5
Lysine	0.85	0.5	0.85	0.5
Methionine	0.85	0.375	0.85	0.375
Premix (B/S, B/F)	0.25	0.25	0.25	0.25
Enzyme	0.025	0.1	0.025	0.1
Total:	100	100	100	100
Proximate analysis				
ME Kcal/Kg DM	2798.45	2752.55	2687.88	2664.83
Crude protein%	22.50	20.69	22.31	20.63
Crude fiber%	5.53	5.15	5.06	5.24
Ether extract%	16.45	16.69	16.01	15.93

MOLM: Moringa oleifera leaf meal

Premix used contained: Vitamin A – 15,000.00 IU, Vitamin D3 - 3, 000,000 IU, Vitamin E- 30,000 IU, Vitamin K- 3,000 mg Vitamin B1 3000 mg, Vitamin B2 6000 mg, Vitamin B6 5,000 mg, Vitamin B 40 mg, Biotin 200 mg, Niacin-40,000 mg, Pantothenicacid 15,000 mg, Folic acid 2,000 mg, Choline chloride 300,000 mg, Iron 60,000 mg, Manganese 80,000 mg, Copper 25,000 mg, Zinc 80,000 mg, Cobalt 150 mg, Iodine 500 mg, Selenium 310 mg, Antioxidant 20,000 mg.

Table 5: Malondialdehyde concentration (IU^{-1}) of broilers fed 5% *Moringa oleifera*leaf supplemented feed.

	Group			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	1.61 ± 0.21^{a}	1.37 ± 0.23	1.76 ± 0.36^{a}	1.48 ± 0.28^{ab}
38	1.27 ± 0.27^{b}	1.51 ± 0.30^{b}	1.45 ± 0.28^{b}	1.65 ± 0.18^{ab}
42	1.34 ± 0.38	1.13 ± 0.35^{c}	1.23 ± 0.37^{c}	0.80 ± 0.18^{c}
F statistics	4.854	3.847	8.033	7.132
P-value	0.028	0.044	0.008	0.000

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean

Means having different superscripts alphabets within columns differ significantly at P<0.05

A= Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.

B= Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35days old with vvIBDV.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.

D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

Table 6: Urea level (IU^{-1}) of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Groups			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	2.94 ± 0.59	3.03 ± 0.45	2.94 ± 0.51	3.96 ± 0.23^{a}
38	3.16 ± 0.65	3.29 ± 0.88	3.21 ± 0.72	3.26 ± 0.61^{bc}
42	$3.\ 28\pm0.49$	3.58 ± 0.84	3.03 ± 0.49	3.32 ± 0.74^{bc}
F statistics	0.874	0.987	0.419	5.634
P-value	0.433	0.361	0.636	0.014

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean

Means having different superscripts alphabets within columns differ significantly at P<0.05

A= Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.

B= Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35 days old with vvIBDV.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.

D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

Table 7: Sodium concentration (mg/dl) in broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	140.60±2.76	139.70±2.67	141.60±2.46[a]	139.89±2.93
38	140.00±2.79	140.10±2.51	140.10±3.07	139.33±2.55
42	139.90±2.69	138.30±2.50	137.60±3.41[c]	139.56±3.57
F statistic	0.183	1.147	4.031	0.076
P-value	0.833	0.337	0.044	0.863

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean
Means having different superscripts alphabets within columns differ significantly at P<0.05
A=Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
B=Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35 days old with vvIBDV.
C=Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

Table 8: Creatinine kinase enzyme (IU L^{-1}) activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	150.80±10.32	139.80±2.39[a]	142.90±7.48	143.63±6.72
38	142.40±6.99	146.70±4.74[bc]	139.50±6.67	151.38±12.19
42	151.20±10.57	150.70±1.17[bc]	144.40±8.97	144.88±8.11
F statistics	2.910	7.035	1.083	1.200
P-value	0.095	0.018	0.351	0.329

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean
Means having different superscripts alphabets within columns differ significantly at P<0.05
A= Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
B= Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35 days old with vvIBDV.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

Table 9: Aspertate aminotransferase enzyme (IU L^{-1}) activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	39.90±3.96[ab]	39.80±3.68[ab]	38.40±3.20[a]	41.80±3.85
38	37.50±4.83[ab]	36.80±6.11[ab]	35.50±3.27[b]	37.40±4.43[b]
42	45.10±5.70[c]	48.50±4.22[c]	42.80±4.02[c]	45.30±5.64[c]
F statistics	5.762	14.161	9.265	5.460
P-value	0.028	0.001	0.005	0.029

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean
Means having different superscripts alphabets within columns differ significantly at P<0.05
A= Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
B= Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35 days old with vvIBDV.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

Table 10: Alanine aminotransferase enzyme(IU L^{-1}) activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	42.90±3.21[ab]	43.30±3.83[a]	42.70±4.80[ab]	44.20±4.52[ab]
38	41.00± 3.20[ab]	40.10±3.03[b]	40.30±2.53[ab]	40.40 ±1.54[ab]
42	49.60±3.56[c]	54.20 ±5.53[c]	48.60±4.45[c]	51.60±3.69[c]
F statistics	23.380	23.924	14.126	12.101
P-value	0.000	0.000	0.000	0.003

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean
Means having different superscripts alphabets within columns differ significantly at P<0.05
A= Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
B= Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35 days old with vvIBDV.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

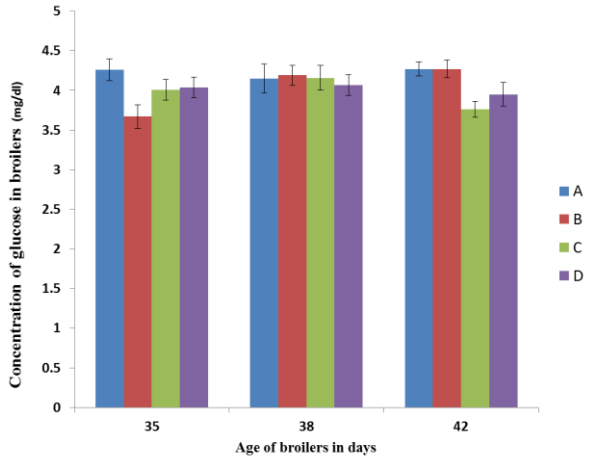

Figure 1: The glucose level of broilers fed 5% *Moringa oleifera* leaf supplemented feed. (A) Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (B) Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus. (C) Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (D) Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

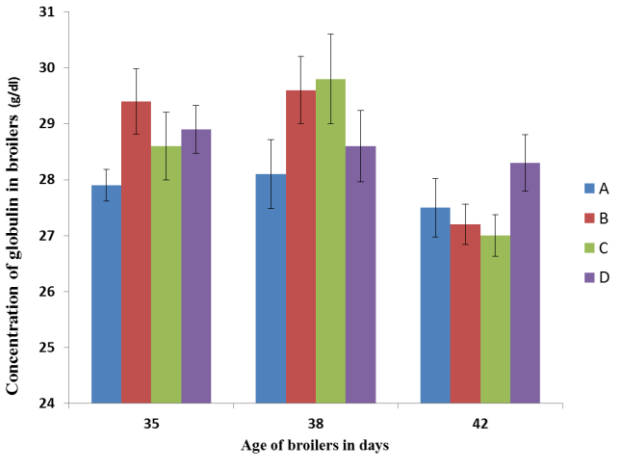

Figure 2: The globulin level of broilers fed 5% *Moringa oleifera* leaf supplemented feed. (A) Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (B) Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus. (C) Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (D) Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 11: Alkaline phosphatase enzyme (IU L^{-1}) activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
Age in days	**A (n=10)**	**B (n=10)**	**C (n=10)**	**D (n=10)**
35	82.60±6.31^{ab}	84.40±4.62^{ac}	83.80±7.18	81.50±5.40
38	79.20±6.61^{ab}	77.70±5.96^{b}	82.90±7.29	81.10±8.42
42	96.70±6.04^{c}	88.20±6.70^{ac}	84.90±6.67	78.70±5.14
F statistics	23.994	10.311	0.217	0.443
P-value	0.000	0.002	0.803	0.636

n=total number of birds sampled, all values are expressed as Mean±SD=standard deviation of the mean
Means having different superscripts alphabets within columns differ significantly at P<0.05
A= Broilers fed Moringa oleifera leaf supplemented feed, vaccinated with killed IBD vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
B= Broilers fed Moringa oleifera leaf supplemented feed, not vaccinated but challenged at 35 days old with vvIBDV.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with vvIBDV.
D=Broilers fed control feed, not vaccinated and not challenged at 35 days old with vvIBDV.

Phytochemical analysis: Qualitative and quantitative analysis of MOL was carried out, according to the method described by Sofowora (1993), and the presence of tannins, phytates, saponins, oxalates, cyanides, alkaloids, carbohydrates, flavonoids, steroids, terpenoids, phenols and phylobatanins was quantified (Balami et al., 2018) (**Table 2**).

Proximate analysis: The standard methods of the AOAC (1990) for the proximate analysis of the MOL were used and the percentage carbohydrates, crude protein (CP), fats, fibre, ash, moisture and metabolizabale energy was determined (Balami et al., 2018) (**Table 3**).

Feed formulation and analysis: The dried MOL was milled with a hammer mill and sieved with 3 mm mesh siever to obtain *Moringa oleifera* leaf meal. Broiler starter (22% CP) and finisher (20% CP) were formulated with 5% MOL inclusion as described by the methods of Olugbemi et al. (2010) using pearson square. The feed was subjected to proximate and mineral analysis based on the method described by the AOAC (1990) and the level

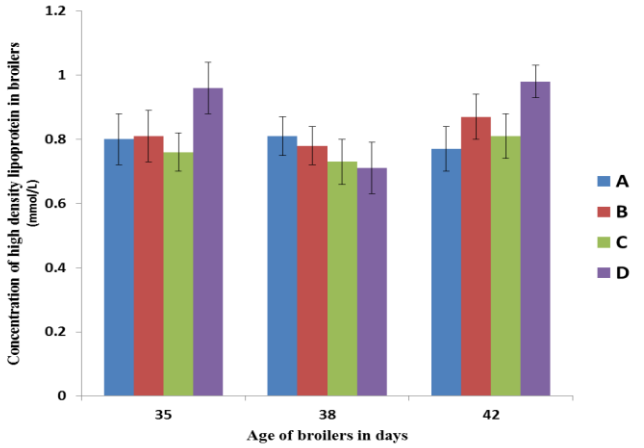

Figure 3: High density lipoprotein level of broilers fed 5% *Moringa oleifera* leaf supplemented feed. (A) Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (B) Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus. (C) Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (D) Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

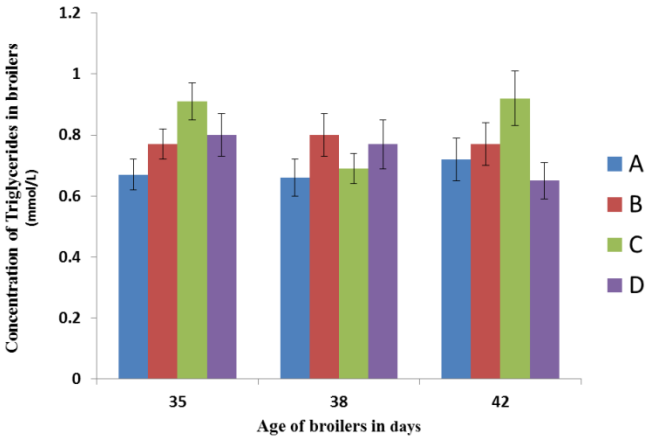

Figure 4: Triglycerides' level of broilers fed 5% *Moringa oleifera* leaf supplemented feed. (A) Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (B) Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus. (C) Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus. (D) Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

of metabolizable energy, crude protein, crude fibre, moisture, ash content, and dry matter was determine (Balami et al., 2018).

Experimental chicks and housing: A total of 240 day old Ross 308 hybrid broiler chicks of both sexeswere obtained from a commercial hatchery located in Yola, Nigeria. The chicks were brooded in a deep litter house which was properly cleaned and disinfected before the arrival of the chicks with wood shavings as litter material and feeders and drinkers were provided. The chicks were individually weighed and assigned in a complete randomised design into four different groups A, B, C and D of 60 chicks each. A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding.

Feeds and feeding: All the broilers were fed with broiler starter for 28 days (from 0 to 4 weeks of age) and broiler finisher for 21 days (from 5 weeks to 7 weeks). Feed and water were provided *ad libitum* (**Table 4**).

Experimental design: Groups A and B were fed with broiler starter and finisher diets each containing 5% MOL, while groups C and D were fed with broiler starter and finisher feed without MOL. Groups A, B and C were challenged at 35 days of age with a vvIBDV. All the groups were fed for 49 days (7 weeks).

Vaccines and vaccination: Inactivated killed vaccine against IBD (inactivated intermediate strain, Virsin 122, oil emulsion, Biovac Limited, Isreal, Batch 1- 382222) and inactivated killed vaccine against Newcastle disease (ND) (oil emulsion Komorov strain, Biovac Limited, Isreal, Batch 1-422222) were obtained from a Veterinary Pharmaceutical store in Jos, Nigeria. Broilers in groups A and C were vaccinated through the thigh muscles intramuscularly with 0.5 mL of the killed IBD vaccine at 14 and 21 days of age, while vaccination against ND was done with the killed ND vaccine (0.5 ml) through the thigh muscles intramuscularly at 18 days of age.

Challenge infectious bursal disease virus: At 35 days of age, all the broilers in groups A, B and C were challenged intra ocularly with 0.05 mL of a live vvIBD virus. The IBD virus used for the challenge was a field strain of vvIBDV obtained from previously vaccinated layers that died of natural outbreak of IBD. Sixty five per cent of commercial cockerels inoculated at 30 days of age with 50 µL of bursal suspension (v/w) in PBS (pH 7.4) died. One millilitre of bursal suspension (v/w) in PBS (pH 7.4) contained 10^{-976} CID_{50} of IBDV.

Blood sample collection: Blood samples for serum biochemical studies were collected when the broilers were 35, 38 and 42 days of age. On each blood collection day, 10 birds from each group were randomly selected and blood sample collected via the wing vein using a 25 gauge sterile needle on a 5 mL syringe. Two millilitres of blood was collected from each of the 10 selected broilers and emptied into a plain (without anticoagulant) test tube and allowed to coagulate to produce sera according to the methods described by Okeudo et al. (2003). The serum was separated from the blood by centrifugation at 447.2 x g for 10 min and stored at -20°C until analysed. A 10 µL each of the serum was immediately used to assay for glucose. Each of the sample bottles were properly labelled using a permanent marker.

Biochemical analyses: After thawing of the serum, creatinine kinase, blood urea and sodium were assayed by means of an Audiocomb Serum Auto-analyser (Bayer Express Plus, Bayer Germany, Serial Number 15950). The globulin fraction was calculated by subtracting the albumin fraction from the total protein. The levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine kinase (CK) was also determined by means of an auto-analyzer (Audiocomb Serum Auto-analyser, Bayer Express Plus, Bayer Germany, Serial Number 15950). The quantity of thiobarbituric acid reactive substance (TBA), melondialdehyde (MDA), as an indicator of lipid peroxidation was evaluated in the serum base on the double heating method of Draper and Hadley (1990) as modified by Yavuz et al. (2004). The concentration of MDA in the sera were calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 105/cm/M and expressed as IU^{-1} of protein.

Serum cholesterol and triglyceride assay: Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-cholesterol) and triglyceride (TG) was determined in the sera by colorimetric methods of Allain et al. (1974), Burstein et al. (1970) and Trinder (Trinder, 1969), respectively, using enzymatic diagnostic kits (AGAPPE Diagnostic Switzerland Gmbh). Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated according to the formula by Friedewald et al. (1972).

Data analyses: Data obtained from the results of the biochemical analyses were expressed as means (± standard deviation). They were further subjected to repeated measure one way analysis of variance (ANOVA), followed by tukeys post-hoc test for multiple comparism. Values of $P<0.05$ were considered significant using Statistical Package for Social Science (SPSS) version 20 for windows.

RESUTS

The level of MDA was observed to significantly decrease between 35 and 38 days of age in group A (P=0.028), and between 35 and 42 days of age in group C (P=0.008). A significant increase was observed in the level of MDA among broilers in group B (P=0.044) and D (P=0.000) at 38 days of age and a subsequent significant decrease in the same groups (B and D) when the broilers were 42 days of age (**Table 5**).

A significant decrease and a subsequent significant decrease was observed in the level of urea in group D at 38 (P=0.008) and 42 (P=0.031) days of age, respectively (**Table 6**). A significant decrease (P=0.028) was also observed in the values of sodium at 42 days of age in group C (**Table 7**). Glucose was observed to significantly increase (P=0.004) at 42 days of age in group B (**Figure 1**). A significant decrease was observed in the values of globulin in group B (P=0.016) and C (P=0.024) at 42 days of age (**Figure 2**).

A significant increase in the level of creatinine kinase was observed between 35 and 42 days of age in group B (P=0.018) (**Table 8**). There was a significant decrease and increase in the level of AST between 38 and 42 days of age in group A (P=0.028), B (p=0.001), C (P=0.005) and D (P=0.029) (**Table 9**). The values of ALT significantly decreased and increased between 38 and 42 days of age in group A (P=0.000), B (P=0.000), C (P=0.000) and D (P=0.000) (**Table 10**). The level of ALP significantly increased and decreased between 38 and 42 days of age in group A (P=0.000) and B (P=0.002) (**Table 11**).

High density lipoprotein cholesterol was observed to significantly decreased (P=0.033) at 38 days of age and increased (P=0.011) at 42 days of age in group D (**Figure 3**). Triglycerides were however observed to significantly decreased (P=0.002) at 38 days of age and increased (P=0.043) at 42 days of age in group C (**Figure 4**).

DISCUSSION

Malondialdehyde (MDA) is an indicator of lipid peroxidation which usually occurs in birds as a result of high oxidative stress. The significant MDA decrease among the broilers of groups A and C at 38 days of age indicates that the IBDV vaccine administered to them was able to prevent lipid peroxidation due to oxidative stress that is associated with vvIBDV. The significant

increase in the level of MDA in group B at 38 days of age showed that the MOL could not prevent lipid peroxidation that might have taken place as a result of oxidative stress following challenge with vvIBDV. Comparison between groups showed that, at 38 days of age, broilers in group A significantly had lower levels of MDA than broilers in groups B, C and D.

The finding (above) implies that supplementing broilers feed with MOL without vaccination against IBDV may not prevent lipid peroxidation in broilers following infection with vvIBDV. The decrease in MDA level observed in broilers of group A when compared with broilers in groups B, C and D could be associated with the amount of Zn (34 ppm) contained in the MOL that was used in supplementing the diet fed to broilers of groups A and B. This is because Zn has been reported to induce the production of metallothionein, which is said to be effective in scavenging hydroxyl radical (Sahin et al., 2009). In other studies, inclusion of Zn in the diet of broilers has been shown to result in decrease in the level of MDA (Tawfeek et al., 2014).

The significant decrease in Na^+ concentration (hyponatermia) observed in group C could be as a result of the dehydration, anorexia, diarrhoea, reduced water intake that is associated with IBD infection. These signs were observed in the present study commencing at 2 dpi in groups A, B and C. The decrease in the concentration of Na^+ observed in this study is in agreement with the findings of Tesfaheywet et al. (2012), who reported a decrease in the Na^+ concentration in the serum of broilers at 5 and 7 dpi with vvIBDV. The results of this experiment could as well imply that the quantity of Na^+ (0.11%) contained in the MOL used for this study may have been responsible for the maintainance of Na^+ concentration of broilers in groups A and B following challenge with vvIBDV.

The increase in the blood urea concentration observed in group D could be as a result of an impaired kidney function which may be due to an immune-mediated glomerulonephritis compatible with immune-complexemia (Ley et al., 1983). Renal function in chickens is indicated by serum uric acid concentration. This is because the uric acid is the major nitrogenous end product of chickens excreted through the renal tubules into the urine (Sturkie, 1986).

Significant increase observed in the values of globulin in groups B and C at 3 dpi shows that vvIBDV has not affected the concentrations of globulin. This finding agrees with that of Afaleq (1998), and Panigraphy et al. (1986) following infection with vvIBDV. Significant difference was not observed in the values of total proteins and albumin within the group between 38 and 42 days of age. However, total protein and albumin significantly increased in group C when compared to groups A, B and D at 35 days of age. This finding contradicts that of Afaleq (1998) and Panigraphy et al. (1986) who reported a reduction in total proteins and albumin, respectively in the serum of birds following challenge with vvIBDV. The finding of this study also imply that MOL supplementation in the diet of groups A and B did not significantly increased their serum total proteins, which is contrary to the findings of Onu and Aniebo (2011), who reported a significant increase in total proteins when broilers were fed with MOL at 5% inclusion rate.

The significant increase in glucose level observed in group B following challenge with vvIBDV may be associated with the high available energy contained in the MOL that was used in the supplementation of the diet used for this study. In broilers, CK is believed to be released into circulation following changes in the permeability of the sarcolemma (muscle membrane) in response to various pathologies or physiological changes in the body (Mitchell and Sandercock, 1995; Mitchellet al., 1992). The significant increase in CK observed in broilers of groups B and D could not be the consequence of the challenge with vvIBDV, because IBD infection is not associated with muscle damage (Holland et al., 1980), but could either be as a result of the increase in the metabolic activity of the liver or due to the significant development of the muscle that occur at this age. This is evident in the significant weight gain earlier observed in all the groups in the course of this study. This finding agrees with an observation made by Szabo et al. (2005) in turkeys of commercial strain.

The significant increase in serum concentrations of AST and ALT at 42 days of age in groups A, B and C is suggestive of pathology involving the liver and kidneys, respectively which is common sequelae in IBDV infection, especially following secondary viraemia (Hair-Bejo et al., 2004; Roosevien et al., 2006). Liver and kidney injuries are postulated to result from hypoxic state caused by aplastic bone marrow following IBDV infection (Nunoyaet al., 1992). The result of this study agrees with the findings of Tesfaheywet et al. (2012) who also reported an increase in AST, ALT and ALP at 3, 5 and 7 dpi with vvIBDV in 32 day old broilers. The finding of this study therefore implied that MOL did not protect the liver and kidneys from the pathological damage caused by vvIBDV.

The significant decrease observed in the values of TG in group C at 38 days of age could be associated with the anorexia and diarrhoea that usually accompanied IBDV infection. This condition will cause a reduced availability and absorption of fatty acid (Dhawale, 2007). Similar finding was reported by Tesfaheywet et al. (2012) when 32 day old broilers were challenged with a vvIBDV. The significant decrease and increase observed in HDL-cholesterol in group D could be due to the high energy demand at this stage of their growth due to high body development (Almeida et al., 2006).

High density lipoproteins are sets of proteins of different sizes (from 8 to 11 nm in diameter). Lipoproteins aids in transportation of fatty acids and cholesterol from the tissues to the liver. Lipoproteins are known as 'good' cholesterol because they prevent the accumulation of cholesterol by taking away excess cholesterol from the body (Blake et al., 2002). The findings of this study therefore showed that, MOL supplementation in the diet of broilers had no influence on the lipid profile of broiler chickens. This agrees with the result of Zanu et al. (2012) and Gakuya et al. (2014) who during their separate studies reported that MOL had no significant influence on the lipid profile of broiler chickens. However, the findings of this study is contrary to the reports of Olugbemi et al. (2010) who noted that MOL possesses hypocholesterolemic properties in broilers, and that of Ashong and Brown (2011) who reported MOL to have significantly decreased the levels of cholesterol and triglycerides in White Leghorn.

CONCLUSION

Supplementing broilers feed with MOL could not protect the liver from pathological damage as evident by increase in the liver enzymes activity nor prevent lipid peroxidation following challenge with vIBDV.

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CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

The design of the study was done by BAG, APA, WAM and AT. The experiment was performed by BAG; the laboratory analysis was performed by BAG and ESJ and the paper was written by BAG and APA with input from all the authors mentioned above.

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