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Immunoglobulin and Oxidative Status Activities of Breast Meat in Broilers Fed Different Levels of Sodium Butyrate and Rosemary (*Rosmarinus officinalis L*) Leaf Meal

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THE effect of sodium butyrate and rosemary leaf meal inclusion on immunoglobulin and oxidative status in breast meat of Arbor Acre broiler birds was investigated. The day-old chicks (n=320) were randomly allotted to 10 dietary treatments that had four replications of 8 birds each. The dietary treatments were allocated as follows : **T1**= basal diet (BD), **T2** = basal diet + 1g/kg Oxytetracycline , **T3**= BD +2g/kg Sodium butyrate (SB), **T4**= basal diet +4g SB/kg, **T5** = basal diet + 2.5g/kg rosemary leaf meal (RLM), **T6**= BD +5.0g/kg RLM , **T7**= BD +2g/kg SB + 2.5g/kg RLM, **T8**= BD + 2g/kg SB +5.0g/kg RLM, **T9**= BD + 4g/kg SB + 2.5g/kg RLM and **T10**= BD + 4g/kg SB + 5.0g/kg RLM. Results showed that birds fed T1 recorded the lowest ($p<0.05$) IgM and IgA values at both phases. The concentration of IgM and IgA for birds fed T4 and T6 were higher ($p<0.05$) compared with those in other groups. Birds fed T4 and T6 recorded the highest ($p<0.05$) values for superoxide dismutase (SOD) and catalase at both phases when compared with the control groups. At the starter phase, the values for glutathione peroxidase (GPx) was lowest ($p<0.05$) in the meat of birds in all the supplemental groups when compared with those of birds in the control groups. It was concluded that; 4 g/kg SB and 5 g/kg RLM supplementation improved the immunoglobulin markers and oxidative status of breast meat in broiler birds.

Keywords: Immunoglobulin, Oxidative status, Sodium butyrate, Rosemary leaf meal, Broilers

Introduction

Poultry industry is one of the most important sectors providing high quality protein for human consumption all over the world. However, efficient feed conversion at minimal production cost is the need of the modern broiler industry which to a certain extent could be achieved by the use of specific feed additives. The use of synthetic growth promoters (antibiotic feed additives) has been rampant over the years in a bid to improve feed efficiency, health status and productive performance of broilers [1]. Prior to their discovery, the incorporation of antibiotics in broilers feed has been for therapeutic purposes for combating diseases and as well for sub-therapeutic purposes to enhance the growth of the animals [2]. Based on these beneficial effects, the antibiotics seemed to be an essential additive for balancing and maintain the gut ecosystem and also help in improv-

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ing growth in broiler birds beyond 50 years [3]. Notwithstanding, considering the increase in the rate of resistance in livestock [4] and dwindling efficacy of antibiotics in humans consumers of livestock products [5], the European Union (EU) in 2006 restricted the incorporation of antibiotics in broiler feeds [6]. As a result of the ban, the attention of many researchers have been focused on finding alternative products; such as organic acids (for instance, sodium butyrate) and medicinal plants (e. g. rosemary) amongst others as alternative feed additives to replace antibiotics in poultry production. As already elucidated, these alternatives should posses antimicrobials properties and also be safe to the animal, man and environment [7]. To this end, sodium butyrate (SB) and rosemary leaf meal (RLM) are among the spectrum of alternative feed additives that are increasingly researched and utilized in nutrition of broilers for enhancing the immune status and growth performance [8]. Sodium butyrate (SB) brings into play its useful effects in broiler birds by enhancing the growth of beneficial bacterial by providing an optimal environment, reducing the pH of the gut and as well hindering the development of pathogenic populations [9]. The dietary inclusion of SB showed some remarkable benefits on weight gain, immune-stimulatory and anti- oxidative enzyme activities in broiler birds [10, 2]. Also, it has also been documented that in increasing the barrier of colon mucosa defense in the intestinal wall via the production of mucin glycoprotein is done through the vital role that sodium butyrate plays in the gut 11]. SB also reduces albumin to globuline ratio and increases serum globulin concentration [12]. The key enzymes that defend the cells against oxidative stress are the Superoxide dismutase (SOD) and catalase (CAT). Studies have shown that supplementing broiler diets with 0.1% sodium butyrate increased the serum (SOD) activities in birds. Shaaban et al. [13] reported that feeding 21-day; broiler birds with 0.1% sodium butyrate supplemented diet elevated the serum SOD and CAT levels and decreased the levels serum malondialdehyde (MDA). Similarly, 0.04% microencapsulated sodium butyrate supplementation hindered stress because of injection of corticosterone via enhanced catalase activities and as well reduced the MDA level in broiler's breast muscle [13]. These positive outcomes of sodium butyrate supplementation in broilers suggests that it has free radicals scavenging capacity as well as decreasing oxidative harm done to cells or tissues by these radicals [11].

Rosemary (*Rosmarinus officinalis L)* is an essential phytogenic (i.e. plant based) feed additive with positive effects on health and growth of animals [15]. *R. officinalis* leaf meal is known to have some beneficial properties such as antioxidative, antiviral, antimicrobial and antifungal, and properties [16]. It also improves feed absorption and digestion that leads to rapid gain, improved immune status and better feed efficiency [17]. There is a recent interest in the use of rosemary leaf meal and sodium butyrate nutrition of animals [13]. However, there is limited literature on the comparative effects of sodium butyrate and rosemary leaf meal as growth-promoting feed additives in broilers. Therefore, this study was designed to investigate the effects of different inclusion levels of sodium butyrate and rosemary leaf meal on immunoglobulin and oxidative status of breast meat in broilers birds.

Materials and Methods

Study site and ethical consideration

The study was conducted at Animal Science Department (Poultry Unit), University of Nigeria Nsukka, Nigeria. The research was conducted on the basis of the provision allowed for animal use by the University of Nigeria, Nsukka ethical committee (MUC271SOYE01) for biomedical research. Nsukka climate is typically tropical with relative humidity within 65-80% with 26.8^{0}C daily temperature [17].

Oxyteracycline, Sodium butyrate and Rosemary leaf meal (RML) Characteristics

Oxyteratcycline

The Oxytetracycline is an antibiotic of synthetic nature that has been used to create more broad evaluation of its differences and similarities as regards to the use of sodium butyrate and rosemary meal powder effect. Each gram of oxytetracycline that was used for this study contains Oxytetracycline Hydrochloride BP 50mg in a soluble powder of 5% W/W [5]. The tested oxytetracycline is manufactured by Tetracin® Vetindia Pharmaceuticals limited India; ®African Representative, Global Organics limited No 81A, Lamido Road, Kano, Nigeria.

Sodium Butyrate

The sodium butyrate used consisted of 30% protected and 40% free sodium butyrate [5]. The sodium butyrate was secured from Barmagen Agro Nig Ltd, Ibadan; A subsidiary of Bar-Margen group Isreal.

Rosemary leaf meal

The tested rosemary leaf meal has the following

bioactive components; 24 diterpenoids (rosmanol and carnosol derivatives and carnosic acid), 3 lignans (medioresinol derivativs), 24 flavonoids (mainly flavones), 1 triterpenoid (betulinic acid) and 5 phenolic acids [5]. The rosemary leaf meal was purchased from the main market Onitsha, Anambra State, Nigeria.

Experimental birds and management

Total number of 320 "Arbor acre" day old chicks (DOC) was allotted randomly to 10 experimental treatments groups (T1-T10) and was replicated 4 times with each replicate having 8 birds each in a 2.6m width x 3 m length wire cage. General flock prophylactic management (supply of anti-stress vitamin supplement on arrival, monitoring and adjustments for dead birds etc.) was administered to the birds both on arrival and during the course of the feeding trial. Routine vaccination was also administered to the birds on days 1 (New castle disease (NCD) vaccine, intra ocular), 14 (Gumboro disease (GD) vaccine), 21 (Lasota vaccine against New castle disease) 28 (Gumboro disease vaccine), 35 (fowl-pox vaccine), and also a repeat of Lasota vaccine at week 6-8 to its prevalence in the farm area. Throughout the eight-week (56 days) of the feeding trial, fresh feed and clean water were made available *ad libitum* for the birds. A thermometer and 200v watt bulb were used to monitor the temperature of the room provide lighting, respectively.

Experimental diets

The ten experimental starter diets were fed to the birds for the first 28 days of life (Table 1), and the finisher diets from 29-56 days (BD) (Table 2). The diets were formulated to meet the dietary nutritional requirements of the birds according to the recommendation of the National Research Council [18]. **T1**= Diet with no additive, **T2** =Diet containing 1gram of Oxytetracycline per kilogram of feed (Positive control), **T3**= Diet containing 2grams SB per kilogram of feed, **T4**= Diet containing 4grams SB per kilogram of feed, **T5** Diet containing 2.5grams of RLM per kilogram of feed, **T6=** Diet containing 5.0grams of RLM per kilogram of feed, **T7=** Diet containing 2grams of SB and 2.5grams RLM per kilogram of feed, **T8**= Diet containing 2grams SB and 5.0grams RLM per kilogram of feed, **T9**= Diet containing 4grams SB and 2.5grams RLM per kilogram of feed and **T10**= Diet containing 4grams SB and 5.0grams RLM per kilogram of feed diet. The starter and finisher experimental diet was subjected for proximate composition according to the method of [19,5].

Data collection

Determination of immunoglobulin

At day 28 and 56 which were the end feeding trail of each phase, 4 broilers were selected randomly per replicate for blood collection. In an attempt to allow various plasma constitutes stabilization; feed was withdrawn from the bids for 4 hours prior to collection of blood. Using a sterile needles and syringe, blood samples (3ml) were collected. The samples of the blood were emptied into a labeled sterilized bottle for an immunoglobuline study. The Humoral immune response of chicken was sampled based on ELISA method (IDEXX Laboratories, B.V., The Netherlands), according to the manufacturer's instruction.

Slaughter and collection of samples for antioxidant activity assays

At day 28 and 56 which were the end of feeding trail of each phase, 3 broilers per replicate were selected randomly for and feed- fasted for a period of 8 hours. Manual plucking was used to de-feather the birds after cutting off the vein at the neck region and for 1 minute was allowed to scald in warm water. Following the slaughter, breast meat samples were obtained for lipid peroxidation and antioxidant enzyme activity assays and stored at 4^{o}C until the analysis was done [20].

Oxidative status

From each of the 4 replicates, 3 birds were selected randomly. 10g of the breast meat that was taken from each slaughtered bird per replicate was used for antioxidant enzyme activities determination such as glutathione peroxidase, catalase and superoxide dismutase (GPx, CAT, SOD), respectively. As described by AOAC [20], the superoxide dismutase activity was assayed in the tissue homogenates at 560 nm. As described by Larrainzar et al.[21] the activities of the catalase were determined at room temperature, also the samples absorbance was determined in UV spectrophotometer at 240 nm. The glutathione peroxidase homogenates concentration was measured, as described by Chunmei et al.[22]. Following the standard described by Gao et al. [23] using the albumin serum of bovine; the protein of the tissue was estimated and the activities of the enzymes were expressed as protein per mg. As described by Mansouri et al.[24], using a spectrophotometer to monitor the absorbance

TABLE 1. Percentage (%) composition of broiler starter experimental diets

Ingredients (%)	**Diets**									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Maize	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	44.00
Wheat offal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	5.00
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	14.00
Groundnut cake	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	24.00
Palm kernel cake	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	5.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit-min premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
SB	0.00	0.00	0.20	0.40	0.00	0.00	0.20	0.20	0.40	0.40
RLM	0.00	0.00	0.00	0.00	0.25	0.50	0.25	0.50	0.25	0.50
Oxytetracycline	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calculated Composition										
Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Energy(Mcal/kg ME)	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Crude fibre(%)	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99
Chemical Composition (%)										
Dry matter	89.61	90.00	89.40	90.80	91.40	91.60	90.40	91.60	91.70	92.15
Crude protein	22.85	22.45	21.96	22.18	22.79	22.85	22.85	22.28	21.95	22.96
Ether extract	4.00	4.00	3.00	5.00	3.00	3.00	4.00	5.00	4.00	3.00
Crude Fibre	4.75	4.69	4.73	4.59	4.71	4.67	4.66	4.74	4.05	4.53
Ash	5.15	7.21	6.38	8.25	5.13	7.21	6.33	6.12	7.41	7.32
NFE	52.86	51.65	53.33	50.73	55.77	53.87	52.56	53.46	54.29	54.34
CHO	51.8	53.40	52.44	46.02	54.71	58.25	54.55	58.52	54.60	54.66

***** Vitamin A: 10,000.00 IU., Vitamin D3: 2,000 IU., Vitamin B1: 0.75g., Vitamin B2: 5g., Nicotinic acid:25g., Vitamin B1: 2-0.015g., K3: 2.5g., E: 25g., Biotin: 0.050g., Folic acid: 1g., Calcium pantothenate: 12.5g., Choline chloride: 250g. Manganese: 64g., Cobalt: 0.8g., Copper: 8g., Manganese: 64g., Iron: 32g., Zn: 40g., Iodine: 0.8g., Selenium 0.6g., Flavomycin: 100g., Spiramycin: 5g., DL-methionie-50g, Lysine 120g. Sodium butyrate, RLM,= Rosemary leaf meal, NFE= Nitrogen free extract, **T1**= Basal diet (BD: Negative control) , **T2** = BD + 1g/Kg diet of Oxytetracycline (Positive control), **T3**= BD +2g SB/kg diet, **T4**= BD + 4g SB/Kg diet, **T5** BD + 2.5g RLM/kg diet, **T6**=BD + 5.0g RLM/kg diet, **T7**= BD +2g SB + 2.5g RLM/kg diet, **T8**= BD + 2g SB +5.0g RLM/kg diet, **T9**= BD + 4g SB + 2.5g RLM/kg diet and **T10**= BD + 4g SB + 5.0g RLM/kg diet.

TABLE 2. Percentage (%) composition of broiler finisher experimental diets

Ingredients (%)	Diets									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Maize	55.00	55.00	55.00	55.00	55.00	55.00	55.00	55.00	55.00	55.00
Wheat	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95
Soybean meal	12.55	12.55	12.55	12.55	12.55	12.55	12.55	12.55	12.55	12.55
Groundnut cake	15.50	15.50	15.50	15.50	15.50	15.50	15.50	15.50	15.50	15.50
Palm kernel cake	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin + mineral premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Butyrate	0.00	0.00	0.20	0.40	0.00	0.00	0.20	0.20	0.40	0.40
Rosemary	0.00	0.00	0.00	0.00	0.25	0.50	0.25	0.50	0.25	0.50
Oxytetracycline	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calculated Composition										
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Metabolizable energy(Mcal/kg)	2800	2800	2800	2800	2800	2800	2800	2800	2800	2800
Crude fibre(%)	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Phosphorus	0.81	0.81	10.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
Calcium	1.71	1.71	1.71	1.71	1.71	1.71	1.71	1.71	1.71	1.71
Chemical Composition(%)										
Dry matter	89.45	91.35	90.03	90.75	89.97	90.15	89.67	90.26	90.52	89.29
Crude protein	19.12	19.67	18.99	19.95	19.25	18.95	19.44	18.97	19.61	19.34
Ether extract	4.12	4.03	4.00	3.75	4.10	4.84	4.57	5.01	5.17	5.08
Crude Fibre	4.98	4.98	5.00	5.97	4.99	5.05	5.00	5.03	5.04	4.91
Ash	5.21	7.34	6.81	6.11	5.21	5.15	4.20	7.12	6.32	5.55
NFE	56.02	55.33	55.23	54.97	56.42	5.16	56.46	54.13	54.38	54.41
CHO	63.48	61.68	62.87	65.10	62.60	63.70	62.20	64.10	58.51	63.06

***** Vitamin A – 10,000.00 IU., Vitamin D_3-2,000 IU., Vitamin B_1-0.75g., Vitamin B_2-5g., Nicotinic acid; 25g., Vitamin $B_1$2-0.015g., K_3-2.5g., E-25g., Biotin – 0.050g., Folic acid:1g., Calcium pantothenate 12.5g., Choline chloride 250g, Manganese 64g., Cobalt-0.8g., Copper 8g., Manganese 64g., Iron 2g., Selenium 0.6g., Zn-40g., Iodine-0.8g., Flavomycin-100g., Spiramycin 5g., Dl-methionie-50g, Lysine 120g. SB= sodium butyrate, RLM= Rosemary leaf meal, NFE, Nitrogen free extract, CHO= Carbohydrate, f meal, **T1**= Basal diet (BD: Negative control) , **T2** = BD + 1g/Kg diet of Oxytetracycline (Positive control), **T3**= BD +2g SB/kg diet, **T4**= BD + 4g SB/Kg diet, **T5** BD + 2.5g RLM/kg diet, **T6=** BD + 5.0g RLM/kg diet , **T7=** BD +2g SB + 2.5g RLM/kg diet, **T8**= BD + 2g SB +5.0g RLM/kg diet, **T9=** BD + 4g SB + 2.5g RLM/kg diet and **T10**= BD + 4g SB + 5.0g RLM/kg diet.

change at 532 nm with 2-TBA, the malonaldehyde (MDA) level was analyzed.

Statistical analysis

Obtained data from the study on immunoglobulin, lipid oxidation, and anti-oxidative enzymes activities of broiler breast meat were analyzed according to SAS [25] general linear model procedure, as described for completely randomized design (CRD) in analysis of variance (One way ANOVA).

The model of the statistics used was as follows:

$Y_{ij} = \mu + A_i + \Sigma_{ij}$

Where: Y_{ij} : observed value of a dependent variable

μ: overall mean; A_i: sodium butyrate and rosemary leaf meal

Σ_{ij}: residual error.

At $p<0.05$, the significance differences between means were tested using least significant difference (LSD) range test.

Results

Immunoglobulin of broiler birds at starter and finisher phases

The result on immunoglobuline broiler birds fed with varying dietary and combination levels of SB and RML are shown in Table 3. For both phases, the concentration of immunoglobulin M (IgM) was highest ($p<0.05$) in the meat of birds on T4 (4 g/kg SB) and T6 (5 g/kg RLM) when compared with samples from other dietary groups. At the starter phase, the concentration of immunoglobulin A (IgA) was higher in T4 and T6 meats when compared with the meat of other treatment groups except T2 (1 g/kg oxytetracylcine; positive control). At this phase, similar $p>0.05$ IgA values seen for T2, T4 as well as T6 birds. At finisher phase, while IgA values were similar ($p>0.05$) for T3 (2 g/kg SB), T4 and T6 meat samples, T4 and T6 meats had highest ($p<0.05$) values when compared meat samples from birds fed other dietary treatments.

Oxidative status of broiler breast meat

The results on lipid oxidative status of broiler breast meat at both phases (28: starter, 56: finisher) are shown in Table 4. At starter phase, T1 and T2 samples had higher malonaldehyde (MDA) values while the lowest MDA values were recorded in T4, T5, T6 as well as T8 diets had lowest ($p<0.05$) MDA values at starter phase. For the finisher phase, meat of birds on T1 and T2 had higher ($p<0.05$) MDA values when compared with meat samples from birds in the SB and RLP supplemented groups.

The result on the anti-oxidative enzyme activity in breast meat of birds fed SB and RLM at different inclusion levels are shown in Table 4. Parameters of all the anti-oxidative enzyme activities studied were affected significantly ($p<0.05$) by treatment diets. Higher ($p<0.05$)SOD and CAT values were consistently recorded in T4 and T6 meats compared with meat from the T1 and T2 groups for both periods of the feeding trial. At the starter phase, meat samples obtained from T1 and T2 birds had least ($p<0.05$) GPx values when compared with meat samples of birds in other dietary treatments. At day 56 (finisher phase), GPx

TABLE 3. Sodium butyrate and rosemary leaf meal supplementation on immunoglobulin of broiler birds (n = 32 per replicate).

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
Starter											
IgM (Ug/ml)	119.44^{d}	124.57^{c}	148.28^{b}	157.14^{a}	144.57^{b}	156.86^{a}	147.65^{d}	144.57^{b}	143.95^{b}	143.88^{b}	1.51
IgA (Ug/ml)	37.78^{d}	55.55^{ab}	53.33^{c}	57.61^{a}	54.03^{bc}	56.98^{a}	52.87^{c}	52.95^{c}	53.01^{c}	53.35^{c}	0.86
Finisher											
IgM (Ug/ml)	113.14^{ef}	124.59^{c}	149.64^{b}	155.86^{a}	146.72^{b}	157.25^{a}	149.62^{b}	140.96^{c}	143.06^{c}	144.01^{c}	1.74
IgA (Ug/ml)	41.48^{c}	54.93^{b}	55.61^{ab}	58.74^{a}	55.07^{b}	59.01^{a}	53.67^{b}	53.59^{b}	54.11^{b}	54.08^{b}	0.81

^{abcef}Rows means with different superscripts differ significantly ($p<0.05$). SEM: Standard error of the mean, **T1**= Basal diet (BD: Negative control) **T2** = BD + 1g/Kg diet of Oxytetracycline (Positive control), **T3**= BD + 2g SB/kg diet, **T4**= BD + 4g SB/Kg diet, **T5** = BD + 2.5g RLM/kg diet, **T6=** BD + 5.0g RLM/kg diet , **T7=** BD + 2g SB + 2.5g RLM/kg diet, **T8**= BD + 2g SB +5.0g RLM/kg diet, **T9=** 4g SB + 2.5g RLM/kg diet and **T10**= BD + 4g SB + 5.0g RLM/kg diet. SEM: standard error of the means, IgM: Immunoglobulin M, IgA: Immunoglobu

was higher ($p<0.05$) in the meat samples obtained from birds on T5 and T6 diets and lower ($p<0.05$) in the meat of T1 birds. The GPx values of T5 and T6 samples were statistically similar ($p>0.05$) with the meats of those birds that received T2, T3, T4, T7, T8, T9 and T10 diets.

Discussion

Immunoglobulin

Immunoglobulins are glycoprotein molecules which are produced plasma cells and are as well called antibodies [26]. These antibodies operate as a vital part of the immune response by particularly recognizing and binding to specific antigens like, viruses or bacteria and helping in their destruction [27]. It is well known that immunoglobulin M and A (IgM and IgA) plays considerable part in birds' immunity [28]. The first and largest antibody to appear in the initial exposure to an antigen response and it is one of the antibody several isotypes [29]. IgA on the other hand provides localized antibody protection on mucosal surface. IgA works by hindering the microorganism from clinging and entering the epithelial lining mucosal. The outcome of present study indicates that broiler birds fed with 4 g/kg SB and those that received 5 g/kg RLM supplemented diets had higher IgM and IgA values in their breast muscles. Mohamed et al.[30] opined that high immunoglobulin level signifies a better immune response against diseases. The study of Mahmoud et al. [31] showed that broiler birds fed with organic acid based diets had greater percentage of immunoglobulins compared with birds fed the control diet. The improvement of immune response linked with dietary acidification might be because of their restrictive effects against harmful microorganism all through the gastrointestinal tract. Inadequate environmental conditions can lead to poor performance by affecting the immune response directly and hence reduce the feed intake and weight gain [32]. Similarly, the increase in the IgA and IgM indicates the effective role of rosemary leaf powder in generating immunity in the birds. This enhanced immunity is due to the role of rosemary leaf powder in protecting cells and inhibiting non-enzymatic oxidation [33]. Ahsan et al. [27] had earlier reported an increase in the antibody of broilers fed butyric acid and acetic acid based diet respectively. The findings of our study on immunoglobulins are also in accordance with the works of Mohamed et al. [30] which revealed that feeding different dietary levels of rosemary and peppermint improved the immune status of broilers, as reflected by the Elisa titer compared with that of the control birds. In contradiction, Yang et al. [34] reported that the inclusion of 4.8g/kg rosemary and peppermint did not display any significant effect on the amount of antibody against some diseases like influenza and Newcastle virus at day 18 and 28. Mohamed et al. [30] that most herbs can aid the stimulation of the immune response and provide protection against bacteria. SB has also helps in the moderation of the immune response of broilers challenged with *Escherichia coli.* Immunoglobuline level has been used as a source of antibody production and also in immune response indication.

TABLE 4. sodium butyrate and rosemary leaf powder effect on supplementation on oxidative status of broiler birds (n = 32 per replicate).

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
Starter											
MDA(mmol/mg)	6.48^{a}	6.10^{ab}	5.26^{bc}	4.36^{c}	4.81^{c}	4.11^{c}	5.10^{bc}	4.76^{c}	5.09^{bc}	4.95^{bc}	0.15
SOD (u/mg protein)	32.89^{d}	38.61^{c}	42.64^{ab}	44.37^{a}	42.62^{ab}	45.30^{a}	40.78^{bc}	40.92^{bc}	41.15^{bc}	40.78^{bc}	0.58
Catalase (u/mg protein)	9.67^{c}	11.13^{bc}	12.70^{ab}	13.96^{a}	13.68^{a}	14.04^{a}	12.61^{ab}	12.81^{ab}	12.26^{ab}	12.41^{ab}	0.27
GPx(u/mg protein)	19.52^{b}	20.27^{b}	22.10^{a}	22.91^{a}	22.87^{a}	23.08^{a}	22.16^{a}	22.24^{a}	21.98^{a}	22.34^{a}	0.24
Finisher											
MDA(mmol/mg)	7.29^{a}	6.82^{ab}	5.78^{bc}	4.62^{cd}	4.93^{cd}	4.25^{d}	5.36^{cd}	5.11^{cd}	5.36^{cd}	5.09^{cd}	0.19
SOD (u/mg protein)	33.61^{e}	37.94^{d}	43.08^{abc}	45.07^{a}	43.86^{abc}	45.91^{a}	41.01^{cd}	41.17^{cd}	40.95^{cd}	41.88^{bc}	0.64
Catalase (u/mg protein)	9.55^{c}	11.67^{bc}	13.71^{ab}	14.25^{ab}	14.36^{ab}	15.27^{a}	13.59^{ab}	13.75^{ab}	13.11^{ab}	13.55^{ab}	0.37
GPx (u/mg protein)	21.44^{b}	22.46^{ab}	23.81^{ab}	24.24^{ab}	24.89^{a}	25.01^{a}	23.76^{ab}	23.92^{ab}	23.76^{ab}	23.64^{ab}	0.30

^{abcef}Rows means with different superscripts differ significantly. SEM: Standard error of the mean, **T1**= Basal diet (BD: Negative control) **T2** = BD + 1g/Kg diet of Oxytetracycline (Positive control), **T3**= BD + 2g SB/kg diet, **T4**= BD + 4g SB/Kg diet, **T5** = BD + 2.5g RLM/kg diet, **T6**= BD + 5.0g RLM/kg diet , **T7**= BD + 2g SB + 2.5g RLM/kg diet, **T8**= BD + 2g SB +5.0g RLM/kg diet, **T9**= 4g SB + 2.5g RLM/kg diet and **T10**= BD + 4g SB + 5.0g RLM/kg diet. SEM: standard error of the means, MDA: Malondiadehyde; SOD: Superoxide dismutase, GPx: Glutathione peroxide.

Lipid oxidative status of broiler breast meat

Extending the shelf life of meat is a crucial aspect of the meat industry and this is done by avoiding or delaying lipid oxidation to preserve the quality [35]. The process of oxidation during the meat's shelf life can reduce the nutritional and sensory values like taste, colour and tenderness, [11]. To this end, there is rise in demand for natural feed additives with potential to delay the onset of peroxidation of lipids in the meat of the broilers and livestock postmortem. Sodium butyrate and rosemary leaf powder have been found useful in this regard. In this study, the lipid peroxidation status of broiler breast meat was determined using the thiobarbituric reactive acid substances test (TBARS). Usually, malonaldehyde (MDA) is one of the many compounds resulting from the breakdown of lipid peroxidation products [36]. Hence, TBARS assay is the oldest and standard marker used to measure MDA [37]. It was observed that supplementation of SB at 4 g/kg diet (T4) and RLM at 5 g/kg diet (T6), respectively, reduced the MDA values in broiler breast meat. As reported by Mahmoud et al.[13], 21-day broiler birds fed with 0.1% SB supplemented diets had lower MDA values. Similarly, microencapsulated SB supplementation at 0.04% inhibited stress by decreasing the MDA levels in the breast meat samples of broilers Shaaban et al. [13]. According to Qaisrani et al. [11] feeding 21-d-old broilers with 800 mg/kg SB diet also decreased the MDA concentration of the birds. The plant secondary metabolites like phenolic, carotenoid and flavonoids compounds in RLM has also been reported to be potent in retarding oxidation of lipids in both cooked and raw meat by decreasing the formation of MDA and this shows that RLM is an efficient antioxidant in broiler meat [16,38]. RLM is among the useful antioxidants which are possibly transferred from animal feed to tissue [16, 39]. The obtained result the study is the same with the outcome obtained by Shaaban, [40] and Nieto et al. [16] who stated that rosemary supplementation in feed successful in reducing the MDA concentration in broilers. One of the main active constituents of RLM is the presence of phenolic acid which has positive effects in antioxidant defense system enhancement [37]. The ability of inactivating free radicals which are produced during the process of auto-oxidation shows its anti-oxidative properties or effect [41].

The most common enzymetic antioxidants used to measure the oxidative status of living cells are (SOD), (CAT) and (GPx) [42]. These endogenous enzymatic proteins are reputed for their defensive role in protecting the animal's body from reactive oxygen species effects and free radical damaging effects [42, 43]. However, when there is an imbalance between the free radicals production and endogenous enzymatic protection, the resultant effect is termed oxidative stress and this causes cell damages [37, 42]. SOD acts as a first enzymatic defense system against free radicals damage [44], whereas, CAT and GPx are anti-oxidative enzymes primarily involved in the hydrogen peroxide catalytic decomposition to water and oxygen [42].

The antioxidant property of herbs (rosemary leaf meal) and sodium butyrate needs to be looked into because undesirable oxidation produces undesirable changes in odour, flavor colour and other quality factors of the mea [39]. The antioxidant properties in sodium butyrate and rosemary leaf meal (phenolic, carotenoid and flavonoids) increased the SOD and GPx of broilers meat studied. The obtained result from the study agrees with many investigators results [45, 13], who stated that supplementing broiler diets with 1000 mg/kg sodium butyrate led to an increase in SOD and CAT activity. This observation suggests that SB enhanced the free radical scavenging capacity and decreased cells or tissues damages [13]. SB also enhances the ability of CAT to rapidly detoxify H_2O_2 to yield H_2O and O_2 which makes it the key defense system against oxidative stress Kalyanaraman; Shabaan et al. [46, 13]. Also, Farag et al. [39] reported that the inclusion of rosemary at 6g/kg diet increased the SOD of laying birds. The increased antioxidant enzymes levels (SOD, catalase and GPx) could possibly advance the steady state of the antioxidant system of the broiler birds. From the report of this study, it can be seen that SB and RLM inclusion in the broiler diet was helpful in improving the antioxidant ability of the birds. It is also good to note that RLM is a rich source of beneficial rosmarinic and carnosol acids, and phenolic compounds with strong anti-inflammatory, antioxidant and anti-cancer activities [47, 48]. Medicinal plants have a special value and importance in biological, veterinary and medical sciences in terms of prevention and treatment of diseases [49-53]. In recent years, the use of medicinal plants in poultry nutrition has increased significantly and the most important reasons are to prove the beneficial effects of these drugs, cheapness, no side effects and also compatible with the environment [54]. Large-

scale and intensive industrial maintenance of livestock and poultry has increased the possibility of diseases, which to reduce the incidence of these diseases and also help increase growth and improve production traits of various chemicals, including antibiotics in poultry farms are used. Today, the use of medicinal plants due to its antimicrobial and antioxidant effects can be used to treat diseases [55-61].

Conclusion

From the results obtained in this study, it can be concluded that 4 g/kg sodium butyrate and 5 g/kg rosemary leaf meal supplementation improved the immunoglobulin markers and lipid oxidative status in broiler birds. The inclusion of rosemary leaf meal improved the antioxidant ability of the birds. It is due to its rich source of beneficial rosmarinic and carnosol acids, and phenolic compounds.

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Conflict of interests

The authors declare that there are no existing conflicts of interest.

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