SEMEN ANTIOXIDANT PROPERTIES OF BROILER BREEDER COCKS FED SUPPLEMENTAL ACETYLSALICYLIC ACID AND DIETARY INCLUSION OF SALT

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ABSTRACT
The semen antioxidant properties of breeder cocks fed varied supplemental levels of acetylsalicylic acid (ASA) and different inclusion levels of dietary salt (NaCl) was investigated in a sixteen-week study. A total of twenty Marshall Broiler Breeder cocks were used for the study. The cocks were randomly assigned into four dietary treatment in a completely randomized design and replicated four times where one cock served as a replicate of its treatment diet. The experimental diets were prepared as dietary inclusion of salt (NaCl) at 0.25%, 0.50%, 0.75%, and 1.00% with acetylsalicylic acid (ASA) supplemented in the diets at 0.00%, 0.025%, 0.050%, and 0.075% in T1, T2, T3 and T4 respectively. Semen samples was collected per replicate on the sixteenth week of the experiment for antioxidant analysis. The result of the semen antioxidant analysis revealed significant differences ($p<0.05$) among treatments except for the reduced glutathione. Birds on T3 recorded the lowest (4.46E-06 $M^{-1}Cm^{-1}$) lipid peroxidase value and the highest superoxide dismutase (88.2±5.29%) and catalase (4.56±0.69KU) values, an increase in glutathione peroxidase was observed with increasing ASA supplementation and NaCl inclusion. The correlation coefficients between these semen antioxidants revealed that lipid peroxidase was 82.90% and 70.20% ($p<0.01$) negatively correlated with superoxide dismutase and catalase respectively, while a positive and significant correlation was observed between superoxide dismutase and catalase (0.678). 0.050% ASA supplementation and 0.75% NaCl inclusion enhanced the semen antioxidant properties of the breeder cocks which is evident from the lower lipid peroxidase value recorded at this level.
Keywords: Acetylsalicylic acid, dietary salt, antioxidants, semen, breeder cocks.

Introduction
Antioxidants are molecules that shield cells from other molecules' oxidation. Oxidation is a chemical reaction in which electrons are transferred from one molecule to an oxidizing agent. Although oxidation reactions are necessary for life, they can also be harmful. Reactive oxygen species (ROS) play an important role in male reproductive function, and these active molecules are needed in small amounts for normal sperm function, whereas large amounts can reduce sperm quality and impair sperm fertilization capability (Kefer *et al.*, 2009). High proportions of polyunsaturated fatty acids (PUFAs) in avian spermatozoa are indicative of the species' susceptibility to reactive oxygen species (ROS) and lipid peroxidation. Recent developments in avian reproduction have concentrated on ROS's potential to be one of the main mediators of infertility. Although spermatozoa's physiological processes involve many ROS, an excessive amount of ROS production can lead to oxidative stress. An efficient scavenging system is necessary to prevent the production of ROS because it is amplified under adverse environmental and stressful conditions (Khan *et al.*, 2011).

Superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water; catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water; and glutathione peroxidase (GPx), which facilitates the destruction of both hydrogen peroxide and organic peroxides, have been identified as enzymes with important antioxidant functions. Reduced glutathione (GSH), a tri-peptide thiol, is both an important antioxidant and a co-factor for various antioxidant enzymes (Kidd, 1997). There is a paucity of information on the effect of ASA and NaCl combination on the semen antioxidants of breeder cocks, however, studies have shown that aspirin may shield biological targets from hydroxyl radical (•OH)-induced cellular damage by scavenging this radical because its chemical structure is similar to salicylic acid's (Logan *et al.*, 1993). This experiment was therefore conducted to determine the effect these two feed additives could have on the antioxidant properties of breeder cocks semen and to determine the optimal levels at which they could be combined in the diet to improve the fertility of breeder cocks.

MATERIALS AND METHOD

Experimental Site: The study was conducted at the poultry unit of the Federal University of Technology's Teaching and Research Farm (Livestock Section) in Akure, Nigeria. Akure is situated on Latitude 7º 15'N and Longitude 5º 12' E in the humid rainforest zone of western Nigeria which is characterized by two rainfall peaks and high humidity during the rainy season (Agbelade and Akindele, 2013).

Experimental Birds and their Management: Twenty Marshall Breeder cocks at 20 weeks of age were used for the experiment. Four experimental diets were formulated to meet the nutrient requirement of the cocks., diets were prepared as dietary inclusion of salt (NaCl) at 0.25%, 0.50%, 0.75%, and 1.00% with acetylsalicylic acid (ASA) supplemented in the diets at 0.00%, 0.025%, 0.050%, and 0.075% in T1, T2, T3 and T4 respectively, treatment (T1) and four birds were randomly assigned to each treatment diet, which was replicated four times, with each bird serving as a replicate of its treatment diet. The experimental diets' composition is shown in Table 1. The experiment was carried out over a 16-week period. Until the end of the experiment, the birds were fed experimental diets based on their treatments.

Table 1: Gross Composition (g/100g) of the Experimental Diets

Ingredient	**T1**	**T2**	**T3**	**T4**
Maize	47.55	47.55	47.55	47.55
Wheat offal	16.00	16.00	16.00	16.00
Soybean meal	18.00	18.00	18.00	18.00
Rice bran	15.00	14.75	14.50	14.25
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
DCP	1.30	1.30	1.30	1.30
Limestone	1.20	1.20	1.20	1.20
Premix	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.50	0.75	1.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
ASA	0.00	0.025	0.050	0.075

T1 = Treatment with 0.00% of ASA and 0.25% NaCl; T2 = Treatment with 0.025% ASA and 0.50% NaCl; T3 = Treatment with 0.050% ASA and 0.75% NaCl; T4 = Treatment with 0.075% ASA and 1.00% NaCl; ASA = Acetylsalicylic acid

Semen collection and analysis: On the 16th week of the experiment, semen samples per replicate were collected in EDTA free bottles through the abdominal massage method between 6.00hrs and 7.00hrs for analysis of semen antioxidant properties. Assay of reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidase (LPO) was done at the postgraduate laboratory of the Department of Biochemistry, Federal University of Technology, Akure. The activity of GSH, GPx, SOD, CAT, and LPO in the semen samples were determined according to the methods of Beutler *et al.* (1963), Rotruck *et al.* (1973), Misra and Fridovich (1972), Hadwan (2016) and Varshney and Kale (1990) respectively.

Data analysis: All data collected were subjected to analysis of variance using the statistical package for social science (SPSS) version 25.0 (2017) and the Duncan Multiple Range Test of the software was used for mean separation. Significance was accepted at a 5% confidence level ($p<0.05$). Also, data were subjected to a correlation analysis using the same software to measure the statistical relationship between them.

RESULTS AND DISCUSSION

Table 2 shows the semen antioxidant properties of Marshall Broiler breeder cocks fed different supplemental levels of acetylsalicylic acid and dietary inclusion of salt. The mean values recorded for T1 and T2 on LPO were statistically similar and significantly higher ($P<0.05$) than those of T3 and T4. Birds on ASA-supplemented and dietary salt inclusion diets (T2, T3, and T4) recorded low lipid peroxidase values when compared to those fed the control diet (T1). The lipid composition of chicken semen is considered to be an important determinant of its quality (Cerolini *et al.*, 1997). Avian spermatozoa are characterized by high concentrations of polyunsaturated fatty acids, predisposing them to oxidative damage within spermatozoa membranes causing male subfertility (Surai, 2019). The lower levels of lipid peroxidase in the semen of these birds is therefore an indication of the ability of ASA to work in synergy with NaCl to achieve this. An increase in glutathione peroxidase (GPx)

was observed with increasing ASA supplementation and NaCl inclusion, GPx plays an important role against the oxidation of lipids, protects the cell membrane, and takes part in hydrogen peroxide (H_2O_2) and lipids' hydroperoxide metabolism (Sikora *et al.*, 2008). Birds on T3 recorded the highest superoxide dismutase (SOD) (88.2±5.29%) and catalase (CAT) (4.56±0.69KU) values. SOD has been identified to remove the superoxide radicals and to function in the repair of body cells damaged by free radicals. Catalase on the other hand plays an important role in the tolerance acquisitions to oxidative stress during the adaptive response of cells to high oxygen tensions (Bindoli *et al.*, 2008)

Table 2: Semen Antioxidant Properties of Broiler Breeder Cocks Fed Different Supplemental Levels of Acetylsalicylic Acid and Dietary Inclusion of Salt

Treatments	LPO ($M^{-1}Cm^{-1}$)	GSH (µg/ml)	GPx (µg/ml)	SOD (%)	CAT (KU)
T1	1.27E-05^{a}	83.05	233.93^{c}	39.87^{c}	0.48^{b}
T2	1.13E-05^{a}	82.13	237.90^{ab}	58.17^{b}	0.58^{b}
T3	4.46E-06^{c}	82.23	237.01^{b}	88.24^{a}	4.56^{a}
T4	7.75E-06^{b}	83.99	239.56^{a}	58.82^{b}	0.99^{b}
±SEM	0.94E-06	0.36	0.62	5.29	0.69
P VALUES	0.00E+00	0.20	0.00	0.00	0.05

a-c = Means in the same column but with different superscripts are statistically (P<0.05) significant. T1 = Treatment with 0.00% of ASA and 0.25% NaCl; T2 = Treatment with 0.025% ASA and 0.50% NaCl; T3 = Treatment with 0.050% ASA and 0.75% NaCl; T4 = Treatment with 0.075% ASA and 1.00% NaCl; ASA = Acetylsalicylic acid; LPO= Lipid peroxidase; GSH= Reduced Glutathione; GPx= Glutathione peroxidase; SOD= Superoxide dismutase; CAT= Catalase.

Table 3: Correlation Coefficients between Semen Antioxidant Properties of Broiler Breeder Cocks Fed Different Supplemental Levels of Acetylsalicylic Acid and Dietary Inclusion of Salt

	LPO	GSH	GPx	SOD	CAT
LPO	1				
GSH	-0.107	1			
GPX	-0.291	0.087	1		
SOD	-0.829**	-0.283	0.302	1	
CAT	-0.702**	-0.130	-0.057	0.678**	1

**. Correlation is significant at the 0.01 level (2-tailed). LPO= Lipid peroxidase; GSH= Reduced Glutathione; GPx= Glutathione peroxidase; SOD= Superoxide dismutase; CAT= Catalase

Table 3 shows the correlation coefficients between semen antioxidant properties of Marshall Broiler breeder cocks fed different supplemental levels of acetylsalicylic acid and dietary inclusion of salt. GSH, GPx, SOD, and CAT were negatively correlated to LPO, also a negative correlation was observed between SOD versus GSH and between CAT versus GPx. while a positive and significant correlation was observed between SOD versus CAT. Kidd (1997) identified superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as enzymes with important antioxidant functions, and reduced glutathione (GSH) as an important antioxidant and a co-factor for various antioxidant enzymes. Therefore, the negative correlations recorded between these antioxidants and the lipid peroxidase show that their activities were better improved with these additives (ASA and NaCl) in the diet of the breeder cocks, and thus were able to protect the sperm cells from oxidative damage.

CONCLUSION AND RECOMMENDATION

Enhancement of the semen antioxidant properties of the cocks as revealed by the results of the experiment indicated that ASA worked in synergy with NaCl to inhibit excessive amount of ROS production that aggravates oxidative stress, reduce sperm quality and impair sperm fertilization capability of breeder cocks. 0.050% ASA supplementation and 0.750% NaCl inclusion in the diet of breeder cocks is capable of reducing the effect of free radicals that affect semen quality and can thus enhance spermatozoa's physiological processes.

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