EFFECTS OF DIETARY SUPPLEMENTATIONS OF ACETYLSALICYLIC ACID AND SALT ON SERUM BIOCHEMISTRY AND EXPRESSION OF SELECTED CYTOKINES IN BROILER BREEDER COCKS

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ABSTRACT
Heat stress is an environmental stressor limiting the performance and production of poultry birds. This study therefore, carried out to investigate optimum combination of aspirin (ASA) and salt (NaCl) that will combat heat stress in Marshall Breeder cock and synergistically improve serum biochemistry and suppress the expression of cytokines. Sixteen Marshall breeder cocks were used in this experiment. Four diets were formulated and labelled T_1 (the control 0.25% NaCl and 0.00% ASA), T_2 (0.50% NaCl and 0.025% ASA), T_3 (0.75% NaCl and 0.050% ASA) and T_4 (1.00% NaCl and 0.075% ASA). The cocks were randomly distributed into four treatment diets with four replicates in each treatment and fed for 16 weeks. Total protein (7.91 ± 1.11mg/dL), globulin (3.40 ± 1.12mgldL) and creatinine (451.69 ± 50.76mg/dL) were significantly ($P<0.05$) higher in the control diet while diet T_4 had the highest percentage of albumin (4.90 ± 0.06mg/dL) and aspartate aminotransferase (67.70 ± 7.66mg/dL). T_3 (178.5) and T_4 (230) had the lowest expression of tumour necrosis factor alpha gene in the liver and heart respectively while the control diet T_1 (244.5) and T_4 (155) had lowest expression of interleukin-1gene in the liver and heart respectively. In conclusion, 0.750% NaCl worked synergistically with 0.050% ASA to suppress the expression of tumour necrosis factor alpha gene in the liver while 1.00% NaCl with 0.0750% ASA could be used to reduce urea concentration in the blood and also suppress the expression of tumour necrosis factor alpha gene and interleukin-1 gene in the heart of breeder cocks.
Keywords: Cytokines, serum biochemistry, Marshall Breeder cocks, acetylsalicylic acid and Salt.

INTRODUCTION
Heat stress has been identified as the most ubiquitous environmental stressor militating against the intensive livestock production industry in the tropics (Aro *et al*., 2020). It is known to hamper the functionalities of the immune system with a concomitant reduction in livestock performance (Aro *et al.,* 2019). Different approaches have been employed to mitigate the harmful effects of heat stress on the performance of poultry namely, with the use of ascorbic acid (Cier, *et al.,*1992), sodium chloride (Smith, 1994), potassium chloride (Ahmad, *et al.,* 2008) and acetylsalicylic acid (Aro, *et al.,* 2017). Acetylsalicylic acid (ASA) i.e. Aspirin, works as a blood thinner and acts as an analgesic, anti-inflammatory as well as an anti-pyretic agent (Aro, *et al.,* 2017). Sodium the principal cation of extracellular fluid, is involved in numerous functions including the regulation of extracellular fluid volume, acid base balance, cell membrane potential, nerve function, absorption of glucose and amino acids (Mongin *et al.,* 1980). The poultry birds are among the most vulnerable livestock species when it comes to exposure to heat stress. The right combination of both ASA and NaCl in poultry diet especially in breeder cocks has not been established. This research thus seeks to unravel the optimum combination of ASA and NaCl in Marshall Broiler breeder cock that will synergistically reduce heat stress while improving carcass parameters serum and gene expression of some selected cytokines of this particular breed of domestic chicken.

METHODOLOGY
Experimental Area
This experiment was carried out at the Poultry Unit of the Livestock Section of the Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria.
Experimental Materials
Twenty broiler breeder cocks of the Marshall Breed were acquired from Vettinson Breeders' Farms, Oyo State, Nigeria. Sixteen birds with an average live weight of 2.3kg were randomly aassigned to

four dietary treatments with four replicates in each treatment. Four treatment diets were formulated in which ASA was supplemented at 0.00, 0.025, 0.050 and 0.075% of the diets and graded salt levels at 0.25, 0.50, 0.75 and 1.00% of the diets. The diets were labelled as T_1, T_2, T_3 and T_4 respectively in which T_1 served as the control diet. The birds were fed with these diets throughout the 16 weeks of the experiment. Table 1 shows the gross composition of the Marshall Broiler Breeder cocks' diets.

Table 3.1: Gross composition (g/100kg) of the experimental diets

Ingredients (kg)	T_1	T_2	T_3	T_4
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
Dicalcium phosphate	1.30	1.30	1.30	1.30
Limestone	1.20	1.20	1.20	1.20
Breeder's premix	0.25	0.25	0.25	0.25
Common salt	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
ME (Kcal/kg)	2700.93	2701.18	2701.43	2701.68
Crude protein (%)	16.01	16.12	16.23	16.34

T 1 = Diet with 0.25% NaCl and 0.00% ASA; T 2 = Diet with 0.50% NaCl and 0.025% ASA; T 3 = Diet with 0.75% NaCl and 0.050% ASA; T 4 = Diet with 1.00% NaCl and 0.075% ASA; ASA = Acetylsalicylic Acid; NaCl = Sodium Chloride; ME = Metabolizable Energy DCP = Dicalcium Phosphate and Breeder premix.

Biochemical analysis

Blood was collected by cutting the jugular veins of four birds per treatment in a plane red top venipuncture tubes .The blood was allowed to clot for serum analysis. The blood was centrifuged for five minutes to separate serum from blood cells. Total protein, albumin, globulin, creatinine, urea, alkaline phosphatase, aspartate amino-transferase and alanine amino-transferase were analysed for.

Gene Expression of Some Selected Cytokines

Organs were obtained from two randomly selected birds per treatment and gene expression were evaluated for cytokines levels through analysing for Tumour Necrosis Factor (TNF) alpha gene and interleukin 1 gene. Total RNA was extracted from the cerebral cortex and hippocampus with trizol®. The amount of RNA in the samples were measured with a Nanodrop2000™, visualized in a 1.5% agarose gel and treated with DNase 1 (Invitrogen). 1 µg of cDNA was synthesized using iScript™ cDNA synthesized kit.

Data Analysis

General linear model (GLM) of SPSS version 25 was used to analyze the data. The differences between the mean values were separated by Duncan's multiple range tests.

Result and Discussion

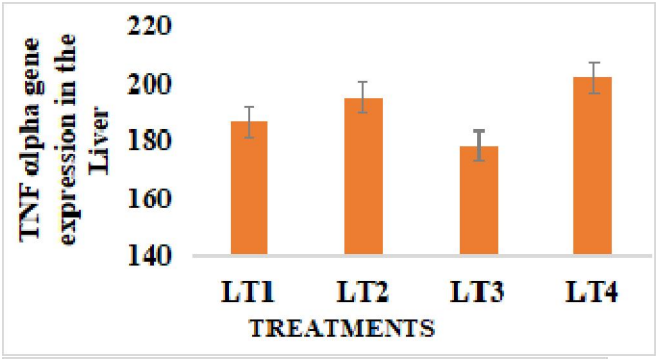
The blood serum analysis in table 2 showed that the control diet T1 had higher concentration of globulin (3.40 ± 1.12), total protein (7.91 ± 1.11) and creatinine (451.69 ± 50.76) whereas birds on diet T4 was highest in the albumin (4.90 ± 0.06), aspartate aminotransferase (67.70 ± 7.66), and alanine aminotransferase (38.50 ± 2.35) and lowest in urea (26.41 ± 1.13). Diets T_4 treated with supplemented acetylsalicylic acid and dietary salt elevated serum of albumin, aspartate amino transferase, and alanine amino transferase reflecting the toxic effects on administration. The globulin and total protein (mg/dl) were higher in the control diet T_1 and this is in similar to the findings of Mohammad *et al.* (2022) the total proteins, globulins and albumin levels were increased ($P<0.05$) in ASA at the dose of 600 and 1200 mg/L as compared to the control group after 21 days post-treatment. Among all the experimental treatments, birds on diet T_3 (178.5) had the lowest expression of tumor

necrosis factor alpha gene in the liver while T4 had the higherexpression of tumor necrosis factor alpha gene (202.5) on the liver of the birds. This showed that the combination of both ASA and NaCl suppressed the inflammation in the body through this gene in the liver. This findings in this study is in agreement with the finding of Shackelford *et al*. (1997) who reported that the aspirin inhibits the tumour necrosis factor alpha gene expression in murine tissue macrophages Birds on diet T_2 (250.25) was observed to have the most expression of tumor necrosis alpha gene in the heart followed by the control diet T_1 (240), diet T_3 (235) and lowerin diet T_4 (230). The report of this study is in accord with the report of Patrignani and Patrono (2016) who stated that aspirin fed to breast cancer patient inhibit the TNF-α-mediated cell survival pathway. The expression of interleukin-1 alpha gene in the liver of the birds was higher in treatment T_2 (252.50) and lower in control diet T_1 (244.50). Birds fed diet T3 (177.50) had the higher expression of interleukin-1 alpha gene in the heart followed by diet T_2 (170.00), the control diet T_1 (166.25) and lower in birds fed diet T_4 (155.00). Ignatios *et al*. (1999) reported that aspirin reduced the expression of the interleukin-1 and interleukin-T6 gene expression in the heart of patients with chronic Stable angina.

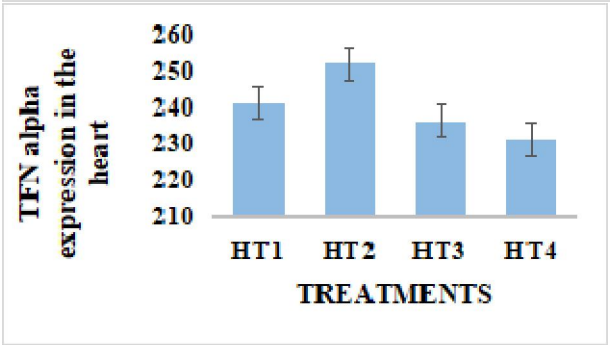
Table 4.5: Serum parameters of Marshall Broiler breeder cocks fed ASA-supplementation and dietary NaCl- inclusions

Parameters	T1	T2	T3	T4	P-value
ALB (mg/dL)	4.51 ± 0.15^{b}	4.75 ± 0.09^{ab}	4.60 ± 0.04^{ab}	4.90 ± 0.06^{a}	0.05
GLB (mg/dL)	3.40 ± 1.12^{a}	1.23 ± 1.10^{b}	1.34 ± 0.61^{b}	1.10 ± 0.15^{b}	0.01
T.P (mg/dL)	7.91 ± 1.11^{a}	5.98 ± 1.18^{b}	5.94 ± 0.59^{b}	6.00 ± 0.30^{b}	0.01
AST (U/L)	29.33 ± 1.59^{c}	42.00 ± 1.80^{bc}	53.03 ± 2.47^{b}	67.70 ± 7.66^{a}	0.01
ALT (U/L)	13.33 ± 0.13^{d}	20.73 ± 1.05^{c}	31.05 ± 2.90^{b}	38.50 ± 2.35^{a}	0.01
URE (mg/dL)	30.92 ± 2.18^{b}	38.56 ± 1.17^{a}	33.86 ± 0.24^{b}	26.41 ± 1.13^{c}	0.01
CRE (mg/dL)	451.69 ± 50.76^{a}	313.17 ± 12.85^{b}	243.27± 13.22^{bc}	174.22 ±18.57^{c}	0.01
ALP (U/L)	0.129 ± 0.05	0.102 ± 0.04	0.127 ± 0.01	0.133 ± 0.03	0.528

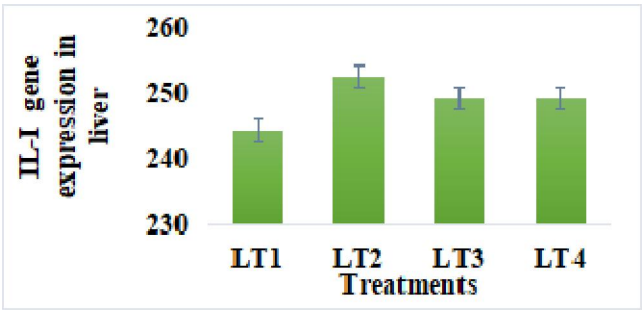
a, b, c and d = Means on the same rows but different superscripts are statistically significant (P<0.05). ALB = Albumin; T.P = Total Protein; AST = Aspartate Amino Transferase; ALT = Alanine Amino Transferase; CRE = Creatinine; ALP = Alkaline Amino Phosphatase; GLB = Globulin and URE = Urea

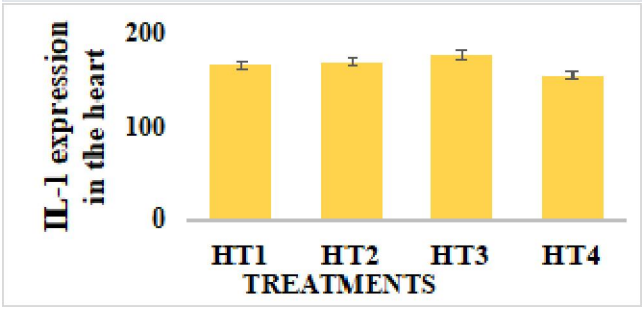


L = liver; T = Treatment



L = liver; H = Heart





L = liver; T = Treatment L = liver; H = Heart

T1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA; ASA = Acetylsalicylic Acid.

CONCLUSION

In conclusion, 0.750% NaCl worked synergistically with 0.050% ASA to suppress the expression of tumour necrosis factor alpha gene in the liver while 1.00% NaCl with 0.0750% ASA could be used to reduce urea concentration in the blood and also suppress the expression of tumour necrosis factor alpha gene and interleukin-1 alpha gene in the heart of breeder cocks.

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