Nigerian J. Anim. Sci. 2023, 25 (2): 49-58

Performance, sperm production and ejaculate characteristics of Marshall broiler breeder cocks fed varying salt inclusions and supplemental acetylsalicylic acid

***Olofin, A.A., *Aro, S.O. *Oluwalade, O.R., Alade, I.T., *Osho, I.B., Abdulkadr, M.T. and Atoyebi, G.O.**

**Department of Animal Production and Health, School of Agriculture and Agricultural Technology*
The Federal University of Technology, Akure, Ondo State, Nigeria
340110, Ilesha-Akure Expressway, PMB 704, Akure, Ondo State Nigeria,

Corresponding Authors: *adeyemiolofin@gmail.com* ***Phone Number:*** *+2348164179905*;

Target Audience: *Animal Reproductive Physiologist, Poultry Farmers*

Abstract

Sixteen Marshall Broiler breeder cocks with an average body weight of 3.30 kg were used to determine the performance and ejaculate characteristics to varied levels of supplemental acetylsalicylic acid (ASA) and dietary salt (NaCl) inclusions. 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 treatments with four replicates in each treatment and fed for sixteen weeks. The semen was collected once a week in the last six weeks of the trial. The performance parameters did not vary significantly ($P>0.05$), however, there was a significant difference ($P<0.05$) in the feed conversion ratio with birds fed diet T_3 (11.66 ± 1.12) having the best conversion. The ejaculate parameters and hypo-osmotic swelling (HOS) did not vary significantly ($P>0.05$). Significant differences ($P<0.05$) were observed in the acrosome integrity where birds fed T_4 (78.50 ± 5.58%) had the highest mean value while T_3 had the lowest percentage of dead sperm cells. In conclusion, 0.750% NaCl worked synergistically with 0.050% ASA to improve the feed conversion ratio and sperm cell viability while 1.00% NaCl with 0.0750% ASA improved the acrosome integrity of breeder cocks.

Keywords: *Acetylsalicylic acid; performance; salt; Marshall Breed; ejaculate; acrosome integrity*

Description of Problem

Poultry production is one of the fastest growing sectors of livestock industry in developing countries (1). Environmental variation is one of the major factors that affect sustainability of livestock production systems in tropical climate (1). In addition, acute heat stress extremely decreases reproductive performance of hens and cocks due to alterations in acid-base balance and ion exchange mechanism (2). Salt (NaCl), also known as sodium chloride, is necessary for life because it assists the body in maintaining fluid balance in various compartments (3). Salt has been linked to livability and improved egg production in layers (4, 5), and as an immune booster (6). However, excess NaCl in livestock and poultry diets has been the major cause of wet litter, which has had a negative impact on the phenomenon of climate change and its associated negative carbon footprint (7).

Many authors advocate the use of acetylsalicylic acid (ASA) or aspirin as a

first-line treatment for heat stress (8), this is due to ASA physiological functions as an antipyretic, anti-inflammatory, and analgesic agent (9). This drug has been shown to improve egg production and livability in laying chickens, to prevent wet litter in poultry houses, (and lower livestock core body temperature (10, 11). Aspirin helps to keep this environmental stressor to a minimum. There is, however, a paucity of data on the effects of the combination of aspirin and NaCl on the reproductive performance of broiler breeders, particularly males. The broiler breeder stock's infertility issues are skewed toward males (12). This infertility problem is exacerbated further, particularly in the tropics, by perennial heat stress (13), which has been linked to decreased spermatogenesis (14). The combination of ASA and NaCl in the poultry diet has been shown to reduce wet litter (10) and lessen heat stress (8). The optimal combination of ASA and NaCl in poultry diets, particularly in Marshall Breeder broiler stock, has not yet been determined. Thus, the purpose of this study is to determine the best combination of ASA and NaCl in Marshall Broiler breeder cocks to reduce heat stress while improving the performance and reproductive indices of this particular breed of domestic chicken.

Materials and Methods

Experimental site

The experiment was conducted at the Poultry Unit of the Livestock Section of the Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria. The average annual rainfall is about 1500 mm usually between March and November. The average annual relative humidity is more than 75%, and the temperature is around 27^{o}c (15).

Procurement and management of experimental animals

Twenty Marshall Broiler breeder cocks were purchased from Vettinson Breeder's Farm in Oyo State, Nigeria. The birds were twenty weeks old when they arrived, and they were stabilized for two weeks before the experiment began. Sixteen birds with an average live weight of 2.3kg were randomly assigned to four dietary treatments, each with four replicates. Four treatment diets were developed, 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 gross composition of the experimental diets is presented in Table 1.

Performance assessment of Marshall Broiler breeder cock

All cocks were weighed at the beginning of the experiment and weekly to determine the average daily weight change. The average daily feed intake and feed conversion (g fed/g gained) rate were recorded. The amount of feed offered as well as feed left over were daily weighed and recorded for each animal during the experimental period to calculate average daily feed intake. Any sign of mortality was monitored and recorded per treatment. The birds were fed 180g/day of broiler breeder mash for Marshall Breed of cock while water was given *ad libitum.*

Data were taken on daily Feed Intake (FI) as the difference in weight between the feed given and the leftover feed per day; these were added up to obtain the feed intake per week in (g) for the sixteen-week study.

Weight change = Final weight – Initial weight

Feed conversion ratio (FCR) = Feed intake (g) ÷ Weight gain (g)

Table 1: Gross composition (g/100kg) of the experimental diets

Ingredients	T_1 (kg)	T_2 (kg)	T_3 (kg)	T_4 (kg)
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
Breeder's premix	0.25	0.25	0.25	0.25
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	2700.93	2700.93	2700.93
Crude protein (%)	16.01	16.01	16.01	16.01

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; DCP = Dicalcium Phosphate. Breeder premix: Supplied the following /kg of feed; Vit. A, 10,000 IU; Vit. D3 2,800 IU; Vit. E, 35,000 IU; Vit. K, 1,900mg; Vit. B12, 19mg; Riboflavin, 7,000mg; Pyridoxine, 3,800mg; Thiamine, 2,200mg; Pantothenic acid, 11,000mg; Nicotinic acid, 45,000mg; Folic acid, 1,400mg; Biotin, 113mg; Cu, 8,000mg; Manganese, 64,000mg; Zn, 40,000mg; Fe, 32,000mg; Se, 160mg; I, 800mg, Co, 400mg; Choline, 475,000mg; Methionine, 50,000mg; Spiramycin, 5,000mg; BHT, 5,00mg.

Semen collection and evaluation

Semen collection, through the abdominal massage method as described by (16) commenced at the 30th week of age till the 36th week of age and was carried out once weekly between 6.00 hours and 8.00 hours. Semen evaluation was done at the diagnostic laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure.

Determination of qualitative semen analysis

Semen analysis was done at the diagnostic laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure. Ejaculate volume, spermatocrit, seminal plasma, sperm concentration, total number of cells/ejaculate and percentage abnormal and dead sperm cells were determined as described by (17) and (18). Sample bottles were used for semen collection from which semen or ejaculate volume for each bird was taken and recorded. The percentages of live/dead and abnormal/normal spermatozoa were evaluated as described by (18). Eosin/nigrosin stains were used on smears for live/dead and abnormal/normal sperm cell counts. The smears were dried on a warm slide and observed immediately with a light microscope at high power magnification (×100) (18). Capillary tubes were used to draw up fresh semen and were placed alternatively in the centrifuge machine. The centrifuge machine was set at 3000rpm. After centrifugation, the sperm cell in the capillary tubes were separated into seminal plasma and the spermatocrit. The capillary tubes that contained centrifuged seminal plasmas and spermatocrits were placed inside haematocrit reader to measure the seminal plasma and the spermatocrit in percentage (%).

Table 2: Performance parameters of Marshall Broiler breeder cocks fed varied levels of ASA-supplementation and dietary NaCl-inclusions

Parameter	T1	T2	T3	T4	P-value
IW (kg)	2.17 ± 0.07	2.20 ± 0.05	2.10 ± 0.04	2.13 ± 0.06	0.277
FW(kg)	3.17 ± 0.12	3.17 ± 0.09	3.25 ± 0.9	3.23 ± 0.09	0.687
WC (kg)	1.00 ± 0.06	0.97 ± 0.33	1.15 ± 0.06	1.1 ± 0.06	0.065
TFI (kg)	12.58 ± 0.79	13.50 ± 0.29	13.56 ± 0.43	13.59 ± 0.24	0.176
FCR	12.64 ± 0.50^{ab}	13.90 ± 0.27^{a}	11.88 ± 0.61^{b}	12.43 ± 0.44^{ab}	0.008
MORT (%)	0.25 ± 0.25	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.372
LIV (%)	75.00 ± 25.00	75.00 ± 25.00	100.00 ± 0.00	100.00 ± 0.00	0.372

a, b = Means on the same rows but different superscripts are statistically ($P<0.05$) significant. IW= Initial Weight; FW = Final Weight; WC = Weight Change; TFI = Total Feed Intake; FCR = Feed Conversion Ratio; 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.

Table 3: Ejaculate parameters of Marshall Broiler breeder cocks fed varied levels of ASA-supplementation ASA and dietary NaCl-inclusions

Parameters	T1	T2	T3	T4	P-VALUE
TEV (ml)	0.51 ± 0.18	0.43 ± 0.12	0.38 ± 0.28	0.37 ± 0.10	0.61
SP (%)	88.00 ± 0.25	89.83 ± 1.07	91.17 ± 1.14	87.41 ± 3.76	0.64
ST (%)	13.37 ± 0.03	12.62 ± 0.76	10.55 ± 1.09	13.21 ± 0.97	0.35
MT (%)	91.75 ± 4.25	95.25 ± 4.25	97.75 ± 0.00	90.75 ± 8.00	0.76
SC (×$10^9$$ml^{-}$)	2.22 ± 0.01	1.80 ± 0.02	1.66 ± 0.02	1.63 ± 0.02	0.66
ASC (%)	18.41 ± 0.50	17.70 ± 1.29	16.31 ± 0.77	19.46 ± 1.07	0.54
NSC (%)	81.00 ± 0.44	83.11 ± 0.72	83.22 ± 1.10	80.50 ± 3.47	0.68
DSC (%)	29.95 ± 0.83	27.89 ± 1.36	26.76 ± 1.18	28.43 ± 1.38	0.90
LSC (%)	70.61 ± 1.07	72.19 ± 1.36	73.26 ± 0.83	70.76 ± 1.18	0.15

TEV = Total Ejaculate Volume; SP = Seminal Plasma; ST= Spermatocrit; MT= Motility; SC = Sperm Cell Concentration; ASC = Abnormal Sperm Cell; NSC = Normal Sperm Cell; DS = Dead Sperm Cell and LSC = Live Sperm Cell; 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.

An improved Neubauer haemocytometer and red blood cell pipette were used to determine sperm concentration. Sperm count was done as described by (19) with a light microscope. Sperm concentration was calculated by multiplying the number of sperm cells counted by the dilution factor of the semen. Total sperm count/ejaculate was then calculated by multiplying sperm concentration with semen volume.

Determination of acrosome integrity of breeder cocks

Semen samples of 50µl were added to 500µl of formalin citrate solution (96ml of 2.9% sodium citrate with 4ml of 37% of formaldehyde). A drop of each of the mixtures was placed on microscope slides and a count of 200 sperm cells from different microscopic fields with normal apical ridges versus those with abnormal apical ridges was made and expressed in percentage as described by (20).

Determination of hypo-osmotic swelling test (HOST) of breeder cocks

HOST solution was prepared a day before the laboratory analysis in which 0.735g of sodium citrate dehydrate and 1.35g of fructose were added to 100ml of distilled water and stirred. 0.1ml of fresh semen was well-mixed with 1ml of HOST solution (21).

The mixture was suspended gently and drawing sample in and out of the pipette for proper mixture. The samples were placed into an incubator for 60 minutes to incubate. After incubation, a drop of the sample mixture was placed on a glass slide and pass over a cover slip. It was then observed under 40x phase contrast lens. Two-dimensional counter, differentiate 100 spermatozoa by counting spermatozoa with tail swelling versus non-swelling tails as described by (18).

Statistical analysis

The data obtained from the experiment were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences, (22), version 25. Significant means were separated using the Duncan Multiple range test of the same statistical package. Also, data were subjected to a correlation analysis using the same software to measure the statistical relationship between them.

Table 4: Acrosome integrity test parameters of Marshall Broiler breeder cocks fed varied levels of ASA-supplementation and dietary NaCl-inclusions

Parameters	T1	T2	T3	T4	P-Value
TEV (ml)	0.30 ± 0.18	0.60 ± 0.20	0.30 ± 0.11	0.40 ± 0.21	0.27
MT (%)	91.75 ± 8.25	100.00 ± 0.00	100.00 ± 0.00	87.50 ± 7.25	0.62
DSC (%)	30.67 ± 4.91^{a}	28.33 ± 4.33^{a}	13.00 ± 0.71^{b}	31.00 ± 3.70^{a}	0.00
LSC (%)	69.33 ± 4.90^{b}	71.67 ± 4.33^{b}	87.00 ± 0.71^{a}	69.00 ± 3.70^{b}	0.00
AI (%)	75.33 ± 4.26^{a}	69.25 ± 4.64^{b}	74.67 ± 5.78^{a}	78.50 ± 5.58^{a}	0.00

a, b = Means on the same rows but different superscripts are statistically ($P<0.05$) significant. 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. TEV = Total Ejaculate Volume; MT = Motility; DSC = Dead Sperm Cell; LSC = Live Sperm Cell and AI = Acrosome Integrity.

Table 5: Hypo-osmotic swelling test parameters of Marshall Broiler breeder cocks fed varied levels of ASA-supplementation and dietary NaCl-inclusions

Parameters	T1	T2	T3	T4	P-VALUE
TEV (ml)	0.43 ± 0.88	0.33 ± 0.89	0.38 ± 0.54	0.38 ± 0.11	0.35
Motility (%)	90.75 ±9.25	91.75 ±7.25	100.00±0.00	91.75 ±7.25	0.40
HOST (%)	69.33 ±0.93	71.67±2.60	71.80±3.07	71.75±2.29	0.54

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. HOST = Hypo-Osmotic Swelling Test and TEV = Total Ejaculate Volume.

Results

Performance parameters of Marshall Broiler breeder cock fed supplemental ASA and dietary salt inclusion

Table 2 shows the growth performance of Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and dietary salt inclusions. In this current study, the performance parameters; final weight, weight change, total feed intake, livability and mortality were not significant ($P>0.05$). However, the feed conversion ratio values varied significantly ($P<0.05$) among treatments, with birds fed diet T_3 (11.88 ± 0.61) having the lowest value.

Ejaculate parameters of Marshall Broiler breeder cocks fed dietary supplementation of acetylsalicylic acid (ASA) and dietary salt inclusions

Table 3 shows the ejaculate parameters of Marshall Broiler breeder cocks fed varied

levels of ASA and NaCl. The result of the ejaculate parameters did not vary significantly (P>0.05) with respect to the treatments applied, however, total ejaculate volume, spermatocrit, sperm concentration, dead sperm cell and abnormal sperm cell of T_1 (control diet) were the highest while seminal plasma, sperm motility, normal sperm cell, and live sperm cell of T_3 were the highest.

Acrosome integrity test parameters of Marshall Broiler breeder cock fed supplemental ASA and dietary salt inclusions

The result of the acrosome integrity of Marshall Broiler breeder cocks fed supplemental ASA and dietary salt levels is presented in Table 4. No statistical difference (P>0.05) was observed in the total ejaculate volume along the treatment groups, however, birds on diet T_2 (0.60 ± 0.20ml) was observed to be numerically higher in the ejaculate volume. Generally, the cocks in the current study had a high percentage of sperm motility and were statistically (P>0.05) significant. Percentage dead sperm cells ranged from 13.00 ± 0.71% in T_3 to 31.00 ± 3.70% in T_4. These values were statistically (P<0.05) significant. The percentage live sperm cells were statistically (P<0.05) significant among the four dietary treatments with the highest (87.00 ± 0.71%) recorded in T_3 and the lowest (69.00 ± 3.70%) in T_4. Percentage acrosome integrity ranged from 69.25 ± 4.64% in T_2 to 78.50 ± 5.58% in T_4 with significant differences observed among the treatments.

Hypo-osmotic swelling test (HOST) Parameters of Marshall Broiler breeder cock fed supplemental ASA and dietary salt inclusions

The results of the HOST of Marshall Broiler breeder's cocks fed supplemental ASA and dietary NaCl were given in Table 5. Generally, the cocks in the current study had a very high number of sperm cells that responded to HOST analysis, though it was similar among the experimental animals and statistically (P>0.05) not significant.

Discussion

Performance parameters

No statistical difference was observed in the mean values recorded for the performance parameters across the treatment groups except for FCR where a significant difference (P<0.05) was observed among the birds (Table 2). (4) found that feeding laying hens with dietary supplementations of salts had no significant effect on the weight change and feed intake. According to this current study, birds fed control diet had the highest value in feed conversion ratio and this suggest lower feed utilization or efficiency. Diet supplemented with ASA and NaCl inclusion i.e., diet T_2, T_3 and T_4 were significantly lower in the values of feed conversion ratio. This showed that birds fed those diets, ensured efficient feed utilization because NaCl and ASA in the diets might have worked together synergistically to improve health and aid beneficiary gut microbiota (8). The finding in this current study is similar to the report of (11) who recorded the highest FCR in Isa White cocks fed 0.75% NaCl inclusion and 0.10% ASA supplemented diets. The implication of this is that ASA supplementation and dietary salt inclusion synergistically worked together to reduce heat and environmental stress and as well improved the performance parameters of the birds in this current experiment.

Ejaculate parameters

Sperm motility, spermatocrit, seminal plasma volume, viability, morphology, total ejaculate volume, and sperm concentration are important indicators of male fertility potential, with lower sperm quality associated with a lower fertility rate (18).

According to this current study, the higher values recorded for sperm motility, normal sperm cells, and live sperm cells (Table 3) may be due to the Na^+ in the salt providing the energy needed for sperm motility, as they play a role in the process of ATP synthesis, which is the primary source of energy for sperm movement (23). Furthermore, Na^+ is involved in the regulation of fluid balance within sperm cells, which is essential for maintaining structural integrity and protecting it from damage (24). In addition, aspirin, known for its anti-stress and anti-inflammatory activity, could have reduced any oxidative stress within the seminal plasma, avoided inflammation, and thus contributed to the improvement of those ejaculate parameters (25, 26). (27) also noted improvement in sperm viability of male Sprague-Dawley Rat fed 8% of NaCl and (28) noted that aspirin at 50mg increased the number of dead sperm cells to half of the total when fed to male albino rats which eventually led to complete infertility. This is an attestation to the synergy between ASA and dietary NaCl in this current study at modulating these reproductive indices because the two authors above did not combine ASA and NaCl Furthermore, birds fed diet T_4 had the lowest total ejaculate volume, spermatocrit, and sperm cell concentration values across the treatment groups and this is similar to the report of (29) on rams fed (12% NaCl) that had lower ejaculate volume and sperm concentration when compared with the control group of 0.5% NaCl. Similarly (30) and Aro (6) reported that rams and Broiler cocks had lower Ejaculate volume and spermatocrit respectively. The decrease in the total ejaculate volume might be due age of avian species of livestock (31). The reduction in the sperm concentration values of the birds fed supplemented ASA and dietary salt inclusion diets is in agreement with (30) who noted a reduction in sperm concentration of rats due to aspirin, causing androgen depletion in the target organ, particularly in the caudal epididymis of rat, thereby affecting physiological maturation of the sperm; and (27) also reported a significant decrease in epididymal sperm concentration of albino rats fed high salt content.

Acrosome integrity test parameters

Acrosome integrity is one of the determining factors for fertility because, for successful fertilization, sperm must have an intact acrosome and must react on time when they reach the site of fertilization (32). The significant ($P<0.05$) and highest percentage of acrosome integrity of the Marshall Breeder cocks (Table 4) in birds fed T_4, showed that NaCl is involved in the regulation of the fluid balance within the sperm cells which is critical for maintaining its structural integrity and protecting it from damage (24). Also, aspirin known for its anti-stress and anti-inflammation activity (8, 9) might have reduced any form of oxidative stress within the seminal plasma, and avoided inflammation (25), thereby contributing to the improvement of those ejaculate parameters. (33). In addition, (5) also suggested that 0.75% NaCl seemed the optimum level for the enhancement of egg production and livability in laying chickens at their late phase of egg production. The use of ASA-supplementation and dietary salt inclusion in the Marshall Broiler breeder cocks' diets is still beneficial to their acrosome integrity at 0.075% ASA and 1.00% NaCl inclusion level (i.e., diet T_4).

Hypo-osmotic swelling test (HOST) Parameters

The HOST is widely used to assess the functional integrity of the sperm plasma membranes of various domestic animals including poultry, cattle, horses, and swine (34). Higher percentage of HOST was

recorded in ASA-supplemented and dietary NaCl-inclusion diets and this negate the report made by (35) who reported that aspirin (14.3mg/kg day^{-1}) caused a slight reduction in the percentage of swollen gamete in the sperm cell of mouse may be due to Na^{+} involvement in regulating the fluid balance within sperm cells, which is essential for maintaining structural integrity and protecting it from damage (23). In addition, aspirin, known for its anti-stress and anti-inflammation activity, could have reduced any oxidative stress within the seminal plasma, avoided inflammation, and thus contributed to the normal functioning of the membrane integrity (25, 26).

Conclusions and Application

1. The feed conversion ratio per gain was lowest in cocks fed the 0.050% ASA-supplemented with 0.750% NaCl-inclusion.
2. The 0.05% ASA-supplemented with 0.750% NaCl-inclusion seemed the optimum level to improve live sperm cells and reduce dead sperm cells of Marshall Broiler breeder cocks.
3. The acrosome integrity of Marshall Broiler breeder cocks are best improved at 0.075% ASA-supplemented with 1.00% NaCl-inclusion.
4. The use of ASA and NaCl in broiler breeder cocks' diets is therefore most beneficial to their growth performance and reproductive capacity at 0.05% ASA-supplemented with 0.750% NaCl-inclusion.
5. Beyond this level of supplementation, the growth performance and reproductive capacity of broiler breeder cocks could be compromised.

References

1. Sinha, R., Lone, S.A., Ranjan, A., Rahim, A., Devi, I and Tiwari, S (2017). The impact of climate change on livestock production and reproduction: ameliorative management. *Intentional Journal of Livestock. Research,* 7(6): 1-8.
2. Kikusato, M., Xue, G., Pastor, A., Niewold, T.A. and Toyomzu, M. (2012). Effects of plants-derived isoquinoline alkaloids on growth performance and intestinal function of broiler chickens under heat stress. *Poultry Science*, 100: 957-96.
3. Gupta, N. and Jani K.K. (2011). Hypertension: salt restriction, sodium homeostasis, and other ions. *Indian Journal of Medical Science*, 65(3):121-32.
4. Ravinder, D., Raj, S.B., Sajjan, S and Chanadrashekhar, S.P (2016). The effect of dietary supplementation of salts of organic acid on production performance of laying hens. *Veterinary World*, 9(12): 1478.
5. Aro, S.0. (2019a). Dietary salt potentiates folliculogenesis and modulate the functionality of the oviduct in late production layers. *Nigeria Journal of Animal Production*, 21 (3): 117-127.
6. Aro, S.0. (2019b). Qualitative analysis of ejaculates and sperm production potentials of Marshall Broiler breeders fed dietary supplementation of acetylsalicylic acid. *Nigeria Journal of Animal Production*, 21 (3): 95-106.
7. Aro, S.O., Owokotomo, E.P. and Adebayo, A.D. (2015). The effects of varying levels of dietary salt on performance and faecal parameters of late production layers. Proceedings of the 40th Annual Conference of the Nigerian Society for Animal Production, 15-19th March 2015, NAPRI/ABU, Zaria. Pp: 407-410.
8. Aro, S.O., Osho, I.B. and Awoneye,

O.O. (2017). Comparison of rectal and ambient temperatures of Isa Brown and Harco Black layers fed different levels of acetylsalicylic acid. *Animal Research International*, 14: 2691-2696.

9. Aspirin American Society of Health Sciences. (2021). Archived from the original on May 25 April 2017 via www.drugs.com.
10. Aro, S. O., Arogbodo, J. O., Faluyi, O. B. and Abere, O. (2018). Faecal bio-Physical Parameters of Two Strains of Laying Chickens Fed Dietary Supplementation of Acetylsalicylic Acid. In: Proceedings of the 43rd Annual Conference of Nigerian Society for *Animal Production (NSAP).* Owerri, Nigeria. Pp. 682-685.
11. Aro S.O. Onuoha, A.N. (2020). Performance and ambient temperature of cockerels under varied acetylsalicylic acid supplementation and different sodium chloride inclusion level. Proceedings of the 45th Annual Conference of Nigerian Society for Animal Production (NSAP*).* Bauchi, Nigeria. Pp. 72-76.
12. Silveira, M. M., Freitas, A. G. de Moraes, C. A., Gomes, F. S., Litz, F. H., Martins, J. M. S., Fagundes, N. S. and Fernandes, E. A. (2014). Feeding management strategy for male broiler breeders and its effect on body weight, hatchability and fertility. *Revista Brasileira de Ciencia Avicola*,. 16: 397-401.
13. Abioja, M.O. (2010). Tempertaure-humidity effect on eggs fertility and evalution vitamin C and cold water on broiler growth in hot season. Department of Animal Physiology, University of Agriculture Abeokuta, Nigeria, 157pp.
14. Shadmehr, S., Fatemi Tabatabaei, S. R., Hosseinifar, S., Tabandeh, M.R. and Amiri, A. (2018). Attenuation of heat stress-induced spermatogenesis complications by betaine in mice. *Theriogenology,* 106: 117-126.
15. Aro, S.O., Aletor, V.A., Tewe, 0.0., Fajemisin, A.N., Usifo, B. and Adesida, A.A., (2008). Studies on Nutritional Potentials of Cassava Tuber Wastes (CTW) Collected from a Cassava Starch Factory. Proceedings 4th Annual Conference of School of Agriculture and Agricultural Technology held at The Federal University of Technology, Akure, Nigeria, Pp 86-92.
16. Burrows, W.H. and Quinn, J.P. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*, 16, 19-24.
17. Rekwot, P.I. (2013). Artificial insemination. *Ahmad Bello University, Zaria*. pp. 1-33.
18. World Health Organiztion. (2021). WHO laboratory manual for the examination and processing of human semen (5th edition). 978924-1547789.
19. Kathleen, R.N., Deska, F.A.C., Timothy, J. Pagan, M.D. (2013). Masby's manual of diagnostic and laboratory tests (5th edition). St. L 978 Loius Missouri: ISBN 978-0-323-08949-4.
20. Gangwar, C., Atul, S., Akhil, P., Singhb, S.P., Sarvajeet, Y., Ravindra, K. nad Vijay, S. (2018). Effect of reduced glutathione supplementation on cryopreservation induced sperm cryoinjuries in Murah bull semen. *Journal of Animal Reproduction Science*, 192: 171-178.
21. Cabrera, F., Gonzalez, F., Batista, M., Calero, P., Medrano, A. and Gracia, A. (2005). The effect of removal of seminal plasma, egg yolk level and

season sperm freezabilty of camary buck (Capra hircus). *Reproduction in Domestic Animals,* 40: 191-195.
22. SPSS. Statistical Package for Social Sciences, IBM Corp. Released 2013. IBM SPSS Statistics for Windows, *Version 22.0. Armonk,* NY: IBM Corp.
23. Jimenez, T., McDermott, J.P., Sánchez, G. and Blanco, G. (2011). Na,K-ATPase alpha4 isoform is essential for sperm fertility. *National Academic Science*, 08(2):644-9.
24. Hall, J.E. and Guyton, A.C. (2006). Textbook of medical physiology. St. Louis, Mo: Elsevier Saunders (4th edition*)*. ISBN 978-0-7216-0240-0.
25. Fan, Y.P., Tang, J.J., Lu, H., Zhang, Y.C., Ruan, J.L. and Teng, X.M. (2013). Progesterone induction keeps a balanced mitochondrial activity and a low ROS productivity in human sperm. *Poultry Science,* 19: 880-5.
26. Liu, Y., Lin, J., Wu, X., Guo, X., Sun, H and Yu, B (2019). Aspirin-mediated attenuation of intervertebral disc degeneration by ameliorating reactive oxygen species in vivo and in vitro. Oxidative Medical Cell Longev: 7189854.
27. Adekunbi, D.A., Ogunsola, O.A., Oyelowo, O.T., Aluko, E.O., Popoola, A.A. and Akinboboye, O.O. (2016). Consumption of high sucrose and/or high salt diets alters sperm function in male Sprague-Dawley rats. *Egyptian Journal of Basic and Applied Science,* 3(2).
28. Al-Taei, B.S.D. (2014). Effect of Aspirin on Sperm Specification and Some Hematological Parameters in Male Albino White Rat. *Journal of Biological Research*, 8(4):0-4.
29. Fang, Y., Sun, X., Zhong, R. and Zhou, D. (2018). High salt diet decreases reproductive performance in rams and down-regulates gene expression of some components of the renin-angiotensin system in the testis. *Theriology.* 107: 127-133.
30. Vyas, A., Ram, H., Purohi, T. A. and Jatwa, R. (2016). Adverse Effects of Sub-chronic Dose of Aspirin on Reproductive Profile of Male Rats. *Journal of Pharmaceutical,* p: 1-9.
31. Adeoye, G. O., Olerofuh-Okoleh, V. U. and Chukwuemeka, U. M. (2017). Influence of breed type and age on spermatological traits in Nigerian local chickens. Journal of *Tropical Agriculture, Food, Environment and Extension,* 16: 11- 16.
32. Rajabi-Toustani, R., Akter, Q.S. and Almadaly, E.A. (2019). Methodological Improvement of fluorescein isothiocyanate peanut agglutinin (FITC-PNA) acrosomal integrity staining for frozen-thawed Janpane black bull. *Journal of Veterinary Medical. Science,* 81: 694-702.
33. Tanyildizi, S. and Turkan, B. (2003). Effects of ASA and metamizole on the hyaluronidase activity and sperm characteristics in rams. *Animal Reproduction Science,* 76(34): 195-204.
34. Zubair, M., Ahmad, M. and Jamil, H. (2014). Review on the screening of semen by hypo-osmotic swelling test. *Journal of Andrologia.* 47(7): 744-750.
35. Stutz, G., Martini, A.C., Ruiz, M., Fiol De Cuneo, J.L. and Lacuara, L.M (2000). Functional activity of mouse sperm was not affected by low doses of aspirin-like drugs. *Journal of Reproductive System,* 44(2): 117-128.