# TESTICULAR MORPHOMETRY AND EJACULATE CHARACTERISTICS OF ISA WHITE COCKS FED VARIED LEVELS OF SALT (NaCl) AND SUPPLEMENTAL ACETYLSALICYLIC ACID(ASA)

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## ABSTRACT
Testicular morphometry and qualitative parameters of the semen of Isa White cocks fed varied supplemental levels of acetyl salicylic acid (ASA) and different inclusion levels of dietary salt (NaCl) were investigated in a-sixteen-week study.. A total of sixteen Isa White cocks were used for the study. The cocks were randomly distributed into four treatment diets and replicated four times where each cock served as a replicate of its treatment diet. The four experimental diets were formulated and designated as: the control (D1) with 0.00% of ASA and 0.25% NaCl while the remaining three (3) diets were labelled as D2, D3 and D4 with 0.05% ASA and 0.50% NaCl, 0.10% ASA and 0.75% NaCl and 0.15% ASA and 1.00% NaCl respectively. The cocks were subjected to semen collection once a week for 9 weeks and qualitative parameters of the ejaculates like semen volume, sperm concentration, spermatocrit, seminal plasma and viability were determined. The birds were sacrificed at the end of the experiment to determine testicular morphometry parameters. The results showed differences ($p<0.05$) in testicular weight, volume and density among the four treatment diets. Cocks fed D3 had the heaviest testicles (13.67±1.67g) and largest testicular volume (11.37±1.75ml) while cocks on D4 had the densest testicles (1.26±0.06gml$^{-1}$) in comparison with control. The total and average weekly ejaculate volume were best ($p<0.05$)) in D2 (3.35±0.85 and 0.37±0.09ml respectively). Most of the ejaculate parameters like the spermatocrit, sperm concentration, total sperm/ejaculate (TSE) and motility were adversely affected in birds fed D4 when compared with the three other diets. The best combination of dietary salt and ASA was achieved with 0.50 and 0.05% respectively. In conclusion, the use of acetylsalicylic acid (ASA) at 0.05% ASA and 0.50 % Salt (Nacl) could significantly improve semen production potential and viability thus enhancing the reproductive performance of the male domestic chicken.
**Keywords:** Acetylsalicylic acid, dietary salt, sperm motility, testicular morphometry.

## INTRODUCTION
The phenomenon of climate change has put the world on its toes since the famous Rio Earth summit of 1996. Many intervention strategies have been deployed since then to mitigate the problems of climate change on humans, livestock and plants. Livestock agriculture, because of its rate of expansion in recent times, has been fingered as a major culprit in the generation of green house gases (GHG) that are the albatrosses in the depletion of the ozone layer – a protective stratospheric screen that reduces the direct impact of the dangerous ultraviolet rays of the sun on the Earth. Animal agriculture has been predicted to double its production by 2050 as a result of greater demands engendered by increase in global population and standard of living. This increase in reality foretells more generation of these GHG at the detriments of the biosphere. For instance, the contribution of livestock to the generation of the GHG is estimated to be 9% of global $CO_2$, 35-40% of global $CH_4$ and 64% of global $N_2O$ emissions (Wikipedia: https://en.m.wikipedia.org/). To tackle this peculiar challenge, livestock practitioners have engaged in series of research channelled towards finding a lasting solution to the climate change problems. Some of such mitigation strategies are dietary manipulation and the use of feed additives, reduction of exposure of manure to water in order to reduce $CH_4$ emission and use of supplemental feed.
Dietary salt (NaCl) and acetylsalicylic acid (aspirin) have been used as feed additives to mitigate one of the ubiquitous ill effects of climate change in the tropics – heat stress. The ability of these two feed additives to reduce the core body temperature has been documented in the literature (Dai *et al*., 2009; Aro *et al*., 2017). The potentials of NaCl to perfectly play this role is hampered by its predisposition to wet litter in the poultry house thus providing a conducive environment for the proliferation of maggots that produce manure-related GHG like $CO_2$, $N_2O$ and $CH_4$. However, a recent study by Aro *et al.* (2018) reported that the use of supplemental levels of acetylsalicylic acid decreased faecal moisture and volume and improved faecal consistency in layers fed high NaCl diets. This experiment was therefore conducted to determine the optimal levels at which these two feed additives could be combined in the diet to achieve the twin effects of decreasing heat stress on livestock and controlling

wet litter in poultry houses. Also, this experiment is intended to unravel any effect such dietary combination of NaCl and ASA could have on the reproductive capacity of the birds.

## MATERIALS AND METHODS

**Experimental site:** The experiment was carried out at the livestock section of Teaching and Research Farms of the Federal University of Technology, Akure, Nigeria. Akure is located at an elevation of about 353m above mean sea level, on Latitude 7°18′′ north of Equator and Longitude 5°10′′ east of Greenwich meridian. The climate is of hot, wet equatorial type with a bimodal distribution pattern. The mean annual rainfall is about 1,500mm and the mean annual temperature ranges between 26-28ºC while the relative humidity averages about 77% per annum.

**Experimental animals and their management:** Twenty Isa White cockerels with an average live weight of 1.94kg at 16 weeks of age were purchased from a reliable source. Sixteen of these birds were randomly allotted to four dietary treatments comprising four birds replicated four times at one bird per replicate. Four treatment diets were formulated with four different inclusion levels of dietary salt (NaCl) and four supplemental levels of acetylsalicylic acid (ASA). Diet 1 (D1) which is the control diet had 0.25% NaCl and 0.00% ASA while D2, D3 and D4 had 0.50 and 0.05%, 0.75 and 0.10% and 1.00 and 0.15% of NaCl and ASA respectively. Table 1 below shows the gross composition of the experimental diets.

**Table 1: Gross composition (g/100g) of the cocks' diet**

| Ingredients | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| Maize | 55.10 | 55.10 | 55.10 | 55.10 |
| Fish meal | 1.00 | 1.00 | 1.00 | 1.00 |
| Groundnut cake | 9.00 | 9.00 | 9.00 | 9.00 |
| Soybean meal | 9.00 | 9.00 | 9.00 | 9.00 |
| Wheat offal | 21.00 | 20.75 | 20.50 | 20.25 |
| Bone meal | 2.75 | 2.75 | 2.75 | 2.75 |
| Limestone | 1.50 | 1.50 | 1.50 | 1.50 |
| Salt (NaCl) | 0.25 | 0.50 | 0.75 | 1.00 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine | 0.10 | 0.10 | 0.10 | 0.10 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| ASA | 0.00 | 0.05 | 0.10 | 0.15 |

These diets were fed to the birds throughout the 16-week duration of the experiment at 150g/ day/cock while water was given to the birds *ad libitum*. Routine medication and vaccination were administered as and when due. The birds were trained to respond to the abdominal massage method of semen collection for five weeks before the commencement of semen collection at the 24th week of age. Semen collection continued once weekly at 0700hours for the next 9 weeks or till the 16th week of the experiment. Data were collected on weekly weight change, daily feed intake and weekly seminal volume. The seminal qualitative parameters like the spermatocrit, seminal plasma, sperm concentration, total sperm cells/ejaculate, percentage abnormal sperm cells, sperm motility and viability were determined. All the birds were sacrificed at the end of the trial, dissected and the testes were extracted from which the testicular morphometrics like the testicular weight, volume and density were determined.

**Statistical analysis:** All data were subjected to a completely randomized design of one way analysis of variance (ANOVA) using SAS (2012 version 9.2 statistical package. Duncan's multiple range test (DMRT) of the same statistical package was used to separate differences among the means where applicable.

## RESULTS AND DISCUSSION

The testicular morphometry showing the right and left testis weight, left testicular volume and left testicular density is presented in Table 2 below. Significant ($p<0.05$) treatment effects were observed

in the weight of both the right and left testes between D1 and the other three treatment diets. The high NaCl and ASA supplemented diets had heavier testicular size in comparison with the control ranging from 8.33±1.20g in D1 to 12.33±1.34g in D3 for the right testis and from 10.33±1.20g in D1 to 13.67±1.67g in D3 for the left testis. The same trend observed with the testicular weight was also observed with the left testicular volume where the values ranged ($p<0.05$) from 8.92±1.44ml in D1 to 11.37±1.75ml in D3.

**Table 2: Testicular weight, volume and density of Isa White cock fed varied supplementation of acetylsalicylic acid (ASA) and dietary inclusion of Sodium chloride (NaCl).**

| Diets | Right testis weight (g) | Left testis weight (g) | Left testicular volume (ml) | Left testicular density ($gml^{-1}$) |
|---|---|---|---|---|
| D1 | $8.33±1.20^{c}$ | $10.33±1.20^{b}$ | $8.92±1.44^{b}$ | $1.20±0.67^{ab}$ |
| D2 | $10.33±1.67^{b}$ | $11.33±2.19^{b}$ | $9.69±1.34^{a}$ | $1.15±0.08^{b}$ |
| D3 | $12.33±1.34^{a}$ | $13.67±1.67^{a}$ | $11.37±1.75^{a}$ | $1.23±0.14^{ab}$ |
| D4 | $12.00±1.75^{a}$ | $13.33±1.20^{a}$ | $10.69±1.35^{a}$ | $1.26±0.06^{a}$ |

$^{a,ab,\ b,\ c}$ *= Means in the same column but with different superscripts are statistically ($p<0.05$) significant; D1= Diet with 0.00% ASA and 0.25% NaCl; D2= Diet with 0.05% ASA and 0.50 % NaCl; D3 = Diet with 0.10% ASA ;and 0.75% NaCl; D4= Diet with 0.15% ASA ;and 1.00% NaCl; ASA = Acetylsalicylic acid; NaCl = Sodium chloride.*

The lightest testicles ($1.15±0.08 gml^{-1}$) were observed in cocks fed D2 while the densest testicles ($1.26±0.06\ gml^{-1}$) were from cocks fed D4. The reproductive capacity of the male animals is closely associated with their testicular size. Males with bigger testes have bigger testicular parenchyma and hence more sperm producing architecture than individuals with smaller testes. The testicular weight in the current study compared with 10-12g obtained by Obidi *et al*. (2008) for Shika Brown breeder cocks. There is also a direct relationship between testicular function and volume. The higher the testicular volume, the more the capacity of the testes to produce sperm cells through increased production of interstitial cell stimulating hormone and testosterone. Feeding ASA in combination with NaCl at 0.10 to 0.75% respectively as used in the current study could improve the reproductive performance of Isa White cocks.

Table 3 shows the ejaculate volumes collected weekly from Isa White cocksfed supplemental levels of acetylsalicylic acid (ASA) and dietary inclusion of sodium chloride (NaCl). The weekly ejaculate volume in D1 ranged from 0.10ml in week 1 to 0.43ml in week 4, 7 and 8. In D2, it ranged from 0.15ml in week 2 to 0.58ml in week 9; it was between 0.18ml in week 2, 4 and 6 and 0.28ml in week 7, 8 and 9 in D3 while it ranged from 0.13ml in week2 to 0.40ml (week 4) in D4. The total ejaculate volume for the entire period of collection ranged ($p<0.05$) from 2.02ml in D4 3.35ml in D2 while the weekly average of the ejaculate were 0.28ml, 0.37ml, 0.25ml and 0.22ml for D1, D2, D3 and D4 respectively.

**Table 3: Weekly ejaculate volume (ml) of Isa White cocks fed supplemental levels of acetylsalicylic acid (ASA) and dietary inclusion of Sodium chloride (NaCl).**

| Weeks | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| 1 | $0.10±0.00^{c}$ | $0.18±0.03^{b}$ | $0.20±0.00^{a}$ | $0.15±0.05^{bc}$ |
| 2 | $0.15±0.03^{ab}$ | $0.15±0.03^{ab}$ | $0.18±0.03^{a}$ | $0.13±0.03^{b}$ |
| 3 | $0.18±0.08^{b}$ | $0.30±0.09^{a}$ | $0.28±0.09^{a}$ | $0.25±0.05^{a}$ |
| 4 | 0.43±0.06 | 0.50±0.15 | 0.35±0.06 | 0.40±0.06 |
| 5 | $0.23±0.05^{a}$ | $0.28±0.06^{a}$ | $0.18±0.05^{b}$ | $0.18±0.05^{b}$ |
| 6 | $0.23±0.05^{a}$ | $0.28±0.06^{a}$ | $0.18±0.05^{b}$ | $0.18±0.05^{b}$ |
| 7 | $0.43±0.20^{a}$ | $0.53±0.17^{a}$ | $0.28±0.12^{b}$ | $0.15±0.05^{c}$ |
| 8 | $0.43±0.09^{a}$ | $0.55±0.18^{a}$ | $0.28±0.10^{b}$ | $0.28±0.09^{b}$ |
| 9 | $0.38±0.09^{b}$ | $0.58±0.08^{a}$ | $0.28±0.09^{c}$ | $0.30±0.07^{bc}$ |
| Total ejaculate volume/diet (ml) | $2.56±0.65^{ab}$ | $3.35±0.85^{a}$ | $2.21±0.59^{b}$ | $2.02±0.50^{b}$ |
| Weekly average volume/diet (ml) | $0.28±0.07^{ab}$ | $0.37±0.09^{a}$ | $0.25±0.07^{b}$ | $0.22±0.06^{b}$ |

$^{a,ab,b,bc,c}$ *= Means in the same row but with different superscripts are statistically ($p<0.05$) significant; D1=Diet with 0.00% ASA and 0.25% NaCl; D2= Diet with 0.05% ASA and 0.50 % NaCl; D3 = Diet with 0.10% ASA ;and 0.75% NaCl; D4= Diet with 0.15% ASA ;and1.00% NaCl; ASA = Acetylsalicylic acid; NaCl = Sodium chloride.*

The average weekly ejaculate volume, though similar ($p>0.05$) when compared between the control and the ASA/NaCl treated groups, D2 gave the best statistic as far as this parameter is concerned. It showed that ASA when combined with NaCl at 0.05 and 0.50% of the diet would encourage maximum semen production. Though collected when these roosters were at a relatively young age (24-32 weeks), the figures still fell within the range of 0.2-0.5ml reported by Hafez and Hafez (2000)
The qualitative characteristics of the ejaculates of Isa White cocks fed dietary supplementation of varied levels of acetylsalicylic acid (ASA) and dietary inclusion of sodium chloride (NaCl) are as presented in Table 4.

**Table 4: Qualitative characteristics of the ejaculates of Isa White cocks fed dietary supplementation of varied levels of acetylsalicylic acid (ASA) and dietary inclusion of Sodium chloride (NaCl).**

| Parameters | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| Seminal volume (ml) | 0.30±0.10 | 0.27±0.07 | 0.27±0.07 | 0.20±0.07 |
| Spermatocrit (%) | 6.00±0.82$^{a}$ | 5.50±0.64$^{a}$ | 6.25±1.93$^{a}$ | 4.00±2.83$^{b}$ |
| Seminal Plasma (%) | 94.00±0.82 | 94.50±0.65 | 93.75±1.93 | 96.00±2.83 |
| Sperm concentration ($\times10^{9}$) | 6.83±0.41$^{b}$ | 6.64±0.34$^{b}$ | 8.55±0.88$^{a}$ | 4.35±2.57$^{c}$ |
| TSE ($\times10^{9}$) | 2.05±0.72$^{a}$ | 1.79±0.09$^{a}$ | 2.31±0.61$^{a}$ | 0.87±0.07$^{b}$ |
| Sperm motility (%) | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 62.50±23.93$^{b}$ | 50.00±28.89$^{b}$ |
| Live sperm concentration ($\times10^{9}$) | 5.24±0.45$^{a}$ | 5.12±0.32$^{a}$ | 5.40±1.20$^{a}$ | 3.84±2.37$^{b}$ |
| Dead sperm concentration ($\times10^{9}$) | 1.59±0.19$^{b}$ | 1.45±0.15$^{b}$ | 3.16±1.50$^{a}$ | 1.07±0.64$^{c}$ |
| % live sperm | 76.50±3.43$^{a}$ | 76.98±1.70$^{a}$ | 65.22±14.04$^{b}$ | 43.32±25.40$^{c}$ |
| % dead sperm | 23.50±3.43$^{b}$ | 23.02±1.70$^{b}$ | 34.75±14.00$^{b}$ | 56.68±5.87$^{a}$ |
| % abnormal sperm | 18.50±2.40$^{a}$ | 17.75±3.84$^{a}$ | 20.23±4.18$^{a}$ | 13.00±7.90$^{b}$ |

$^{a,b,c}$ *= Means in the same row but with different superscripts are statistically ($p<0.05$) significant; D1=Diet with 0.00% ASA and 0.25% NaCl; D2= Diet with 0.05% ASA and 0.50 % NaCl; D3= Diet with 0.10% ASA ;and 0.75% NaCl; D4= Diet with 0.15% ASA ;and1.00% NaCl; ASA = Acetylsalicylic acid; NaCl = Sodium chloride; TSE = Total sperm/ejaculate.*

All the ejaculate parameters varied significantly among the treatment means with the exception of seminal volume and seminal plasma. The spermatocrit, total sperm cells/ejaculate, live sperm concentration, percentage dead sperm and percentage abnormal sperm cells in D2 and D3 compared statistically ($p>0.05$) with values obtained in D1 while these values were significantly ($p<0.05$) depressed in D4. However, D1 and D2 had similar values in terms of sperm concentration, sperm motility, dead sperm concentration and percentage live sperm. The decrease in sperm motility was very obvious in D3 (62.50±23.93%) and D4 (50.00±28.89%) when compared with D1 (100.00±0.00%) and D2 (100.00±0.00%). Sperm motility and more importantly progressive motility is very crucial to the capacity of the sperm to fertilize the ovum. Therefore, using ASA and NaCl in combination above 0.05 and 0.50% of the diet respectively could compromise the reproductive capacity of Isa White roosters.

## CONCLUSION AND RECOMMENDATION

The significant depression of most of the reproductive indices in D4 showed that as far as reproduction in male poultry species is concerned, caution should applied not to use ASA and NaCl in combination above 0.10 and 0.75% respectively in the diet.

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