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Qualitative analysis of ejaculates and sperm production potentials of Marshall Broiler breeders fed dietary supplementation of acetylsalicylic acid

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Target Audience: *Animal Reproductive Physiologists, Poultry Farmers*

Abstract

Twenty-four broiler breeder cocks (Marshall Breed) with an average body weight of 3.5 kg were used to determine the response of seminal parameters to different levels of supplemental acetylsalicylic acid (ASA). Four treatment diets were formulated, labelled T_1 (the control), T_2, T_3 and T_4 with 0.00% (0 mg/kg), 0.05% (500 mg/kg), 0.10% (1,000 mg/kg) and 0.15% (1,500 mg/kg) ASA in the diets respectively. The cocks were randomly distributed into these diets with six replicates in each treatment and fed for sixteen weeks during which semen collection was done once weekly in the last five weeks of the trial. The ejaculates were analyzed for spermatocrit (S), sperm concentration (SC), seminal plasma (SP), live sperm, normal sperm, abnormal sperm, dead sperm and total sperm cells per ejaculate. Results showed that SP was highest ($P<0.05$) while S level was lowest ($P<0.05$) in birds fed 1,500 mg/kg ASA. Total sperm cell/ejaculate was best at 1,000 mg/kg ASA. The correlation coefficients between these seminal parameters revealed that S was 65.50% positively ($P<0.05$) correlated with SP while spermatocrit and SP were 100% negatively ($P<0.01$) correlated. Supplemental ASA at 1,000 mg/kg had the highest gonadal sperm reserves, daily sperm production and spermatogenic efficiency. In conclusion, ASA was most beneficial to the reproductive capacity of the cocks at 1,000 mg/kg of the diet.

Key words: *Acetylsalicylic acid, reproduction, semen, testicular homogenates, testicular morphometry.*

Description of the Problems

The use of acetylsalicylic acid (ASA) or aspirin as a drug of choice to combat heat stress has been recommended by many authors (1, 2). This is as a result of the physiological roles of ASA as an antipyretic, anti-inflammatory and analgesic agent. This drug has been reported to promote growth in broiler birds (3), improve egg production and livability in laying chicken (4, 5), combat wet litter in poultry houses (6) and reduce the core body temperature in livestock (2).

The broiler breeders or broiler parent stock are the male and female domestic chickens that are specially reared in breeder farms to produce hatchable eggs for the production of broiler chicks. This special line of poultry production enterprise requires that these broiler breeders are maintained on a good plane of nutrition especially that which will enhance their reproductive performance in terms of the breeding capacity of both the male and female broiler breeder stock. The problem of heat stress is ubiquitous in the tropics, broiler breeders' diets are often laced with

ASA to help reduce this environmental stressor to the barest minimum. There is however dearth of information on the effects of dietary inclusion or supplementation of ASA on the reproductive performance of broiler breeders and particularly on the males. The problems of infertility of the breeder broiler stock tilt towards the males (7, 8). This problem of infertility is further compounded especially within the tropics by the perennial heat stress known for its adverse effects on spermatogenesis (9). Since ASA is known for its anti-pyretic effects on heat-stressed animals, there is thus the need to design an experiment to test the efficacy of the dietary use of ASA on the reproductive performance of farm animals. This experiment was therefore conducted to investigate the effects of varied supplementation of ASA on the qualitative ejaculate parameters like sperm motility, spermatocrit, seminal plasma and quantitative sperm production indices like gonadal sperm reserve, daily sperm production and spermatogenic efficiency of Marshall Broiler breeder cocks.

Materials and Methods

Experimental Site

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. The farm is located in the humid rainforest zone of southwestern Nigeria, which is characterized by two rainfall peaks and high humidity during the raining season. The mean annual rainfall is about 1500mm and the rains span nine months of the year usually from March to November. The mean annual relative humidity is over 75% and that of the temperature is about 27ºC.

Procurement and Management of Experimental Animals.

Thirty broiler breeder cockerels of the Marshall Breed were purchased from Obasanjo breeders' Farms, Lanlante, Oyo State, Nigeria. The birds were fourteen weeks of age at the time of their arrival and were stabilized for two weeks before the commencement of the experiment. Twenty-four birds with an average live weight of 3.5kg were randomly allotted to four dietary treatments with six replicates in each treatment. Four treatment diets were formulated in which ASA was supplemented at 0, 0.05, 0.10 and 0.15% of the diets corresponding to 0, 500, 1,000 and 1,500mg/kg of the diets respectively. 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 broiler breeders' diets.

Semen collection, through the abdominal massage method commenced on the 28th week of age till the 32nd week of age and was carried out once weekly between 16.00 hours to 18.00 hours.

Semen Collection and Evaluation

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 Rekwot (10). 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 Zemjanis (11).

Table1: Gross composition (g/100g) of the broiler breeder cocks' diets

Ingredients	T_1	T_2	T_3	T_4
Maize	55.00	55.00	55.00	55.00
Soybean	9.00	9.00	9.00	9.00
Wheat offal	21.00	21.00	21.00	21.00
Groundnut cake	9.00	9.00	9.00	9.00
Fish meal	1.00	1.00	1.00	1.00
Bone meal	2.75	2.75	2.75	2.75
Limestone	1.50	1.50	1.50	1.50
Methionine	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Breeders' Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
ASA	0.00	0.05	0.10	0.15

T_1 = Diet with 0.00% (0.00mg/kg) ASA; T_2 = Diet with 0.05% (500mg/kg) ASA; T_3 =Diet with 0.10% (1,000mg/kg) ASA;T_4 =Diet with 0.15% (1,500mg/kg)ASA; ASA = Acetylsalicylic acid.

Breeder Premix: *Supplied in a kg of the diet; Vit. A, 10,000 IU; Vit. D3, 2,800IU, Vit. E, 35,000IU, Vit. K, 1,900mg; Vit. B12, 19mg; Riboflavin, 7,000mg; Pyridoxine, 3,800mg; Thiamine, 2,200mg; Pantothenicacid, 11,000mg; Nicotinicacid, 45,000mg; Folicacid, 1,400mg; Biotin, 113mg; Cu, 8,000mg; Manganese, 64,000mg, Zinc, 40,000mg; Iron, 32,000mg; Selenium 160mg; Iodine, 800mg; Cobalt, 400mg; Choline, 475,000mg; Methionine, 50,000mg; BHT, 5,000mg; Spiramycin, 5,000mg.*

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). An improved Neubauer haemocytometer and red blood cell pipette were used to determine sperm concentration. Sperm count was done as described by Hafez, (12) 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. The seminal volume was determined by the use of a calibrated pipette, the semen was aspirated and the volume read.

Determination of gonadal sperm reserve, daily sperm production and spermatogenic efficiency.

The testicular homogenates of the individual cock was obtained by the method described by Aro (13). Briefly, the testes were dissected from each cock after slaughter and were freed from the fascia and the epididymis. Each testis was weighed using the sensitive Precisa 30000DSCS (Precisa Instrument Limited, Switzerland) weighing scale. The testis was then cut longitudinally to free the parenchyma from the tunica albuginea. Both the testicular parenchyma and the tunica albuginea were weighed and the weights recorded. The parenchyma of each testis was cut into tiny pieces with a dissecting knife and then homogenised for two minutes at 6000g broken at an interval of 1 minute. The homogenate was filtered through a double ply of clean cheese cloth and the filtrate was used for the determination of testicular sperm count. The haemocytometry method using the improved Neubauer haemocytometer was employed in the testicular sperm count. Only the elongated spermatids and fully matured sperm cells were counted in the microscopic

field using the ×40 objective lens. Counts were made in duplicates and the average value of the two was recorded. From the testicular sperm count, the total sperm cells in the two testes i.e. the gonadal sperm reserves were computed. The daily sperm production was calculated from the gonadal sperm reserves by dividing the latter by a factor of 1.93, a time divisor for domestic fowl (14). The spermatogenic efficiency or the efficiency of sperm production was determined by dividing the daily sperm production by the paired testes weight.

Statistical Analysis

The data obtained from the experiment were subjected to one-way analysis of variance in a Completely Randomized Design (CRD) using Statistical Package for Social Sciences (15) version 22. Significant means were separated using the Duncan Multiple range test of the same statistical package.

Results

Table 2 shows the ejaculate parameters of Marshall Broiler breeder cocks fed varied levels of dietary supplementation of acetylsalicylic acid. Percentage spermatocrit ranged from 5.00 ± 0.58 in T_4 to 6.67 ± 1.76in T_2 but significant effect of ASA supplementation on spermatocrit was only observed in T_4 while T1, T_2 and T_3 were statistically similar (P>0.05) to the control (T_1) treatment. The lowest percentage seminal plasma was recorded in T_2, T1 and T3 (93.67%) while the highest was recorded in T_4 (95.00%). An inverse relationship between the spermatocrit and seminal plasma was thus observed. This is corroborated by the correlation coefficients (Table 3) where a negative correlation coefficient of -1.00 was recorded between these two ejaculate parameters.

Table 2: Ejaculate parameters of Marshall Broiler breeder cocks fed dietary supplementation of acetylsalicylic acid (ASA)

Parameters	T_1	T_2	T_3	T_4
S (%)	6.33 ± 1.20[a]	6.67 ± 1.76 [a]	6.33 ± 0.67 [a]	5.00 ± 0.58 [b]
SP (%)	93.67 ± 1.20[b]	93.67 ± 1.76[b]	93.67 ± 0.67[b]	95.00 ± 0.58[a]
SV (ml)	0.30 ± 0.10	0.27 ± 0.07	0.27 ± 0.07	0.20 ± 0.00
SC (× 10^9/ml)	6.49 ± 0.25[a]	4.36 ± 0.82[c]	5.86 ± 0.51[b]	4.05 ± 0.88[c]
TSE (× 10^9)	1.97 ± 0.72[b]	1.19 ± 0.09[c]	4.49 ± 1.61[a]	2.21 ± 1.23[b]
AS (%)	4.70 ± 0.21[b]	6.40 ± 1.67[a]	3.82 ± 0.91[c]	6.46 ± 0.97[a]
DS (%)	8.83 ± 0.95	9.91 ± 2.28	9.27 ± 0.83	8.90 ± 0.35

[a, b, c] = means within the same row but with different superscripts are statistically (P<0.05) significant.
S = Spermatocrit; SP = Seminal Plasma; SV = Seminal Volume; SC = Sperm Concentration; TSE = Total Sperm per Ejaculate; AS = Abnormal Sperm; DS = Dead Sperm; T_1 = Treatment with 0.00% ASA; T_2 = Treatment with 0.05% ASA; T_3 = Treatment with 0.10% ASA; T_4 = Treatment with 0.15% ASA; ASA = Acetylsalicylic acid.

The seminal volume or ejaculate volume was 0.30 ± 0.10ml in T_1, 0.27 ± 0.07 ml in $T_2$and T_3 and 0.20 ± 0.00 ml in T_4. These values though numerically different, were statistically (P>0.05) insignificant. There were statistically different (P<0.05) treatment effects in sperm concentration observed in the ASA supplemented diets relative to the control. All the ASA supplemented diets showed significantly (p<0.05) lower sperm concentration values in comparison with the control diet. The highest total sperm cell per

ejaculate ($4.49 \pm 1.61 \times 10^9$ sperm cells) was observed in the T_3 diet supplemented with 0.10% ASA while T_1, T_2 and T_4 had $1.97 \pm 0.72 \times 10^9$, $1.19 \pm 0.09 \times 10^9$ and $2.21 \pm 1.23 \times 10^9$ sperm cells per ejaculate respectively. The lowest rate of abnormal sperm cells ($3.82 \pm 0.91\%$) was observed in T_3 while the highest ($6.46 \pm 0.97\%$) was recorded in T_4. Generally, the cocks in the current study had a very low rate of abnormal sperm cells. Percentage dead sperm cells ranged from $8.83 \pm 0.95\%$ in T_1 to $9.91 \pm 2.28\%$ in T_2. These values were however statistically similar ($P>0.05$) among the treatment means.

The correlation coefficients among the ejaculate parameters of Marshall Broiler breeder cocks fed different levels of dietary acetylsalicylic acid supplementation are shown in Table 3. A strong perfect negative correlation was observed between spermatocrit and seminal plasma (-1.00). This means that an increase in the volume of spermatocrit will lead to a corresponding decrease in the volume of seminal plasma. This correlation is also statistically significant at 0.01level. Between spermatocrit and sperm concentration, a strong, positive and significant correlation coefficient of 0.655 was observed, which implies that the spermatocrit and sperm concentration are 65.50% correlated. It was also observed that a moderate, negative and non-significant correlation of -0.416 exists between seminal plasma and dead sperm cells. Seminal volume and abnormal sperm were 0.381 moderately and positively correlated while a weak, positive and insignificant correlation of 0.108 exists between seminal volume and dead sperm.

A significant strong and negative correlation of -0.661 was observed between sperm concentration and abnormal sperm while a weak, negative and insignificant correlation of 0.221 exists between sperm concentration and dead sperm. A weak, negative, insignificant correlation of -0.291 was observed between total sperm per ejaculate and spermatocrit. This means that increase in the total sperm per ejaculate will lead to an insignificant decrease in spermatocrit level. A moderate, positive but insignificant correlation of 0.381 was observed between abnormal sperm cells and seminal volume which indicates that an increase in the abnormal sperm will lead to an insignificant increase in the seminal volume. It was also observed that a moderate, positive and insignificant correlation of 0.419 exists between abnormal sperm and dead sperm. The correlation between dead sperm and total sperm per ejaculate was 0.01 and it implied a weak, positive but insignificant relationship between these two seminal parameters.

Table 3: Correlation coefficients between the ejaculate parameters of Marshall Broiler breeder cocks fed different levels of acetylsalicylic acid supplementation.

	S	SP	SV	SC	TSE	AS	DS
S	1						
SP	-1.00**	1					
SV	0.022	-0.022	1				
SC	.655*	-.655*	0.087	1			
TSE	-0.291	0.291	-0.099	-0.184	1		
AS	-0.264	0.264	0.381	-.661*	-0.325	1	
DS	0.416	-0.416	0.108	-0.211	0.01	0.419	1

*S = Spermatocrit; SP = Seminal Plasma; SV = Seminal Volume; SC = Sperm Concentration; TSE = Total Sperm per Ejaculate; AS = Abnormal Sperm; DS = Dead Sperm; ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; T_1 = Treatment with 0.00% ASA;T_2 = Treatment with 0.05% ASA;T_3 = Treatment with 0.10% ASA;T_4 = Treatment with 0.15% ASA;ASA = Acetylsalicylic acid.*

The quantitative assessment of testicular homogenates and testicular morphometry of the Marshall Breeder cocks fed varied supplementation of acetylsalicylic acid (ASA) is presented in Table 4.

Table 4: Quantitative assessment of testicular homogenates and testicular morphometry of Marshall Broiler breeder cocks fed dietary supplementation of acetylsalicylic acid.

Parameters	T_1	T_2	T_3	T_4
GSR (× 10^9)	5.15 ± 2.43^b	6.24 ± 1.55^b	7.27 ± 0.76^a	5.58 ± 1.03^b
DSP (× 10^9)	2.67 ± 1.26^b	3.23 ± 0.81^b	3.76 ± 0.40^a	2.89 ± 0.54^b
SE (× 10^6)	117.06 ± 68.77^b	166.19 ± 70.69^a	179.14 ± 34.52^a	137.88 ± 22.65^b
LTW (g)	25.25 ± 4.55	23.47 ± 4.00	22.73 ± 1.66	21.13 ± 0.90
RTW (g)	25.10 ± 3.50^a	21.03 ± 4.62^a	20.97 ± 2.86^b	20.43 ± 1.96^b
PTW (g)	50.35 ± 8.05^a	44.50 ± 8.52^a	43.70 ± 4.39^a	41.57 ± 1.19^b
WTP (g)	24.05 ± 4.02^a	21.12 ± 4.25^a	20.73 ± 2.18^a	19.67 ± 0.59^b
WTA (g)	1.13 ± 0.01	1.13 ± 0.01	1.12 ± 0.01	1.12 ± 0.00
ATW (g)	25.18 ± 4.03^a	22.25 ± 4.25^a	21.85 ± 2.19^a	20.78 ± 0.59^b

^{a,b} = Means in the same row but with different superscripts are statistically (P0.05) significant.
GSR = Gonadal Sperm Reserve; DSP = Daily Sperm Production; SE =Spermatogenic Efficiency; LTW = Left Testis Weight; RTW = Right Testis Weight; PTW = Paired Testis Weight; WTP = Weight of Testis Parenchyma; WTA = Weight of Tunica Albuginea; ATW = Average Testis Weight; T_1 = Treatment with 0.00% ASA;T_2 = Treatment with 0.05% ASA;T_3 = Treatment with 0.10% ASA;T_4 = Treatment with 0.15% ASA;ASA = Acetylsalicylic acid.

The quantitative indicators of testicular homogenates like the gonadal sperm reserve (GSR), daily sperm production (DSP) and spermatogenic efficiency (SE) followed similar trend in their response to dietary supplementation of ASA. These quantitative parameters had their least values in T1 (5.15 ± 2.43× 10^9, 2.67 ± 1.26 × 10^9 and 117.06 ± 68.77 × 10^6 sperm cells) for GSR, DSP and SE respectively and highest values in T3 (7.27 ± 0.76 × 10^9, 3.76 ± 0.40 × 10^9 and 179.14 ± 34.52 × 10^6) for GSR, DSP and SE respectively. The left testis weight ranged from 21.13 ± 0.90g in T4 to 25.25 ± 4.55g in T_1 with no statistical significant difference among the treatment means but were comparatively heavier than the left testis. Statistical significant differences exist among the right testis weight. Cocks fed the T_1 diet had the heaviest testicular weight (25.10 ± 3.50g) while those fed the T_4diethad the lowest testicular weight (20.43±1.96g). The paired testis weight (PTW), weight of testicular parenchyma (WTP) and average testis weight (ATW) followed similar trend. They were statistically similar in T_1 (50.35 ± 8.05g, 24.05 ± 4.02g and 25.18 ± 4.03g), T_2 (44.50 ± 8.52g, 21.12 ± 4.25g and 22.25 ± 4.25g) and T_3 (43.70 ± 4.39g, 20.73 ± 2.18g and 21.85 ± 2.19g respectively) but significantly heavier than the respective values recorded in T_4 (41.57 ± 1.19g, 19.67 ± 0.59g and 20.78 ± 0.59g). The weights of tunica albuginea for T_1, T_2, T_3 and T_4 were 1.13 ± 0.01 g, 1.13 ± 0.01 g, 1.12 ± 0.01 g and 1.12 ± 0.00 g respectively and showed no statistical significant differences among the treatment means.

The correlation coefficients between the testicular homogenate parameters and testicular morphometry of Marshall Broiler breeder cocks fed varied dietary supplementation of acetylsalicylic acid are as shown in Table 5.

Table 5: Correlation coefficients between the testicular homogenate parameters and testicular morphometry of Marshall Broiler breeder cocks fed varied dietary supplementation of acetylsalicylic acid.

	GSR	DSP	SE	LTW	RTW	PTW	WTP	WTA	ATW
GSR	1								
DSP	1.00**	1							
SE	.912**	.912**	1						
LTW	-.649*	-.648*	-.831**	1					
RTW	-0.36	-0.359	-.640*	.812**	1				
PTW	-0.517	-0.516	-.764**	.943**	.960**	1			
WTP	-0.516	-0.515	-.764**	.943**	.960**	1.00**	1		
WTA	-0.53	-0.528	-.610*	.678*	.720*	.736**	.735**	1	
ATW	-0.517	-0.516	-.764**	.943**	.960**	1.00**	1.00**	.736**	1

*GSR = Gonadal Sperm Reserve; DSP = Daily Sperm Production; SE =Spermatogenic Efficiency; LTW = Left Testis Weight; RTW = Right Testis Weight; PTW = Paired Testis Weight; WTP = Weight of Testis Parenchyma; WTA = Weight of Tunica Albuginea; ATW = Average Testis Weight; ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.*

The quantitative study of the testicular homogenates revealed highly significant and strongly positive correlations among the three homogenate parameters. Both the gonadal sperm reserves (GSR) and daily sperm production (DSP) are 1.00 positively correlated with each other and both are 0.912 positively correlated with spermatogenic efficiency (SE). A quantitative increase in one of the three parameters would therefore lead to a quantitative increase in the other two.

The testicular morphometry parameters like the weight of the right testis (RTW), weight of the left testis (LTW), paired testicular weight, (PTW), weight of testicular parenchyma (WTP), weight of tunica albuginea (WTA) and average testis weight (ATW) were either moderately or strongly negatively correlated with the testicular homogenate parameters. The LTW for instance was -0.649, -0.648 and -0.831 correlated with GSR, DSP and SE respectively while at the same time being 0.812, 0.943, 0.943, 0.678 and 0.943 strongly and positively correlated with RTW, PTW, WTP, WTA and ATW respectively. The general observation was that all the testicular morphometry parameters were negatively correlated with the testicular homogenate parameters but were at the same time strongly and positively correlated with each other.

Discussion

The spermatocrit fraction of the ejaculates of the Marshall Breeder cocks (Table 2) was lowest in those fed the 0.15% ASA supplemented diet. This spermatocrit fraction is the cellular portion of the ejaculate made up entirely of the sperm cells or spermatozoa. Lower spermatocrit fraction of the ejaculate

thus implies reduced capacity of the cocks fed the T_4 diet to produce spermatozoa. There is therefore the tendency of the cocks to produce oligospermic ejaculates if the cocks' diets are supplemented with 0.15% ASA as observed in the present study. Oligospermia (16) is a pointer to reduced reproductive performance of the male breeder stock. The significantly lower spermatocrit recorded in cocks fed the T_4 diet implied that the reproductive capacity of breeder cocks could be compromised at 0.15% ASA supplementation.

The seminal plasma is the liquid component of the ejaculate. The higher seminal plasma in the ejaculates of cocks fed the T_4 diet is reflective of the higher fluidity of the ejaculates when compared to those obtained from cocks fed the T_1, T_2 and T_3 diets. In other words the ejaculates from cocks that fed theT_4 diet were the most dilute or least concentrated and hence those with the least spermatocrit fraction. This shows the inverse relationship that exists between seminal plasma and spermatocrit values. It is implied therefore that increase in seminal plasma volume vis-à-vis spermatocrit volume would negatively affect male fertility hence; dietary supplementation of ASA at 0.15% as observed in the current study could lower spermatocrit and spermatozoa concentration of the broiler breeder cock especially of the Marshall breeders. The semen or ejaculate volume in the cocks, though not statistically significant was generally low across all treatments but still compared with 0.28ml (280 ± 11µl) reported by Riaz *et al.* (17) on Hubbard broiler breeders but fell below 0.55ml reported by Omoruyi *et al*. (18). This could be attributed to the tender age of the birds (28-32 weeks) at the time of collection. Adeoye *et al*. (19) reported on the influence of age at the time of collection on the volume of the ejaculate of the avian species of livestock.

The dietary supplementation of ASA in the diets of the breeder cocks had a positive influence on the total number of sperm cells/ejaculate (TSE) and on the percentage of abnormal sperm (AS) cells at 0.10% supplemental level. This is because the highest TSE and lowest AS were recorded at this supplemental level but at 0.15% ASA supplementation, the values observed for these two parameters became inferior relative to the control. It could therefore be suggested that the 0.10% ASA supplementation is the optimum level to enhance TSE and reduce sperm morphological abnormalities in Marshall Broiler breeder cocks. All cocks used in the present study had very low rate of sperm abnormality (3.82 ± 0.91-6.46 ± 0.97%) when compared with 11.6 ± 8.5% reported by Bah *et al*. (20). Increased TSE and lowered AS are indices of good semen quality in the male stock. Percentage dead sperm cell (DS) was equally low among treatments in the current study in contrast with what is reported in some literature (20, 21). This difference could be age-related or due to livability (22, 23) conferred on the birds by dietary ASA supplementation.

The strong, highly significant and perfect negative correlation (-1.00) observed between spermatocrit and seminal plasma (Table 3) underscores the inverse relationship between these two seminal parameters. The implication of this is that an increase in the spermatocrit value will lead to a corresponding decrease in seminal plasma value and vice versa. Spermatocrit measures the proportion of cellular elements or sperm cells in any given volume of the ejaculate. Therefore, the higher the spermatocrit is, the more spermatozoa concentrated is the ejaculate. This is corroborated in the current study by the strong, positive and significant correlation observed between spermatocrit and sperm concentration. The reproductive significance of these relationships is that given volume for volume, more semen doses would be processed from an ejaculate with higher spermatocrit value than

the one with a lower spermatocrit value. It was also observed that sperm concentration is significantly, strongly and negatively correlated with the proportion of abnormal sperm cells in the ejaculate. This is not unexpected because the higher the ratio of abnormal sperm cells in the total ejaculate, the weaker such ejaculates parameters like livability, motility, sperm swim-up and even sperm concentration. Determination of the proportion of abnormal sperm cells in the ejaculate is important for assessing the sperm cells for their ability to fertilize the oocyte (21, 24).

The gonadal sperm reserve (GSR) was best improved with dietary ASA supplementation at 0.10% (Table 4) beyond which a decrease in performance was recorded at 0.15% supplementation. The 0.10% ASA level could therefore probably be the optimum level of utilization of ASA in the feed of Marshall Broiler breeders as far as this trial is concerned. The GSR measures the sperm producing ability of the testicles *in situ* and is therefore one of the yardsticks for measuring the reproductive health in any population of male livestock species. All GSR values (5.58 ± 1.03-7.27 ± 0.76 × 10^9) obtained under the current study are higher than values (2.92 ± 0.29 - 4.83 ± 1.22 × 10^9) reported by Orlu and Egbunike (25) probably as a result of breed differences (26). These authors experimented with Barred Plymouth Rock cocks which are of a lighter breed than the Marshall Breed used in the present study.

Daily sperm production (DSP) and spermatogenic efficiency (SE) had their best response in terms of the enhancement of the reproductive performance of the Marshall Breeder cocks at 0.10% ASA supplementation. While DSP measures the quantity of sperm cells produced per day by the testicles, SE accounts for the number of sperm cells produced per gram testis. In essence, both like the GSR are quantitative measurement of the sperm producing ability of the testis *in situ.* It could be observed that while ASA had numerically higher sperm producing effects on the breeder cocks at all the three ASA supplementation levels relative to the control, it was at the 0.10% supplementation that the effect was statistically significant relative to the control and to the other two ASA levels. It is therefore safe to suggest 0.10% ASA supplementation as used under this present study in Marshall Breeder cocks' diets as the optimum supplemental level.

Orlu and Egbunike (14) reported on DSP determined both histometrically and through the testicular homogenate method as used in the present study. Their study gave 1.85 ± 0.22 × 10^9 for Barred Plymouth Rock cocks determined through the testicular homogenate method, a value quite low when compared with 2.67 ±1.26 - 3.76 ± 0.40 × 10^9 obtained in the present study. This difference could be attributed partly to the breed effect between these two breeds and partly to the probable ameliorative effect of ASA on sperm producing capacity of these broiler breeders.
Observation of the left and right testicular weight revealed their often highlighted anatomical weight differences and the values obtained support the popular report that the left testis is always heavier than the right testis in the avian species of livestock (27, 28, 29, 30). The right testicular weights were affected by the dietary supplementation of ASA in the diets of the experimental cocks at levels beyond 0.05%. Decrease in testicular weight of the breeder cocks may hamper their capacity to maximally produce sperm cells, thus depressing their reproductive efficiency and breeding soundness (29, 13). The paired testis weight (PTW), weight of testicular parenchyma (WTP) and average testis weight (ATW) followed the same trend in their response to dietary ASA supplementation. They all differed significantly relative to the control in all these testicular morphometry

parameters at 0.15% ASA supplementation. The PTW, WTP and ATW are all parameters factored from the weight of the testis and hence any agent that depresses the testicular weight will invariably affect the morphometric expression of these three parameters. These results on testicular weight once more revealed that the 0.10% ASA supplementation is the optimum if the breeding efficiency of cocks is not to be compromised.

The testicular homogenate parameters (GSR, DSP and SE) were all significantly and positively correlated with each other and negatively correlated with all the testicular morphometry parameters (Table 5). The three homogenate parameters were only significantly correlated with the left testis weight while the spermatogenic efficiency (SE) is the only testicular homogenate parameter that was significantly correlated with all the testicular morphometry parameters. This shows that the SE is a better sensitive statistic for determining the correlatedness of testicular homogenate parameters with the testicular morphometry parameters. It is equally noteworthy to stress that all the testicular morphometry parameters are positively and significantly correlated with each other.

Determination of coefficients of correlation among the semen and testicular parameters as carried out in the present study is a useful tool for studying the relationships among such parameters. For instance, Mphaphathi *et al.* (31) correlated body weight with semen characteristics in Venda chickens and observed weak but positive relationships between body weight, semen volume (0.38) and sperm concentration (0.23) and a negative relationship between body weight and semen p^H(-0.11). Similarly a weak, positive correlation (0.09) was observed between semen volume and sperm concentration in the present study. However a significant ($P<0.001$), positive and strong (0.92) relationship was observed between semen volume and sperm concentration by Omoruyi *et al.* (18). Fluctuation in the coefficient of variation of the ejaculate parameters of these avian species of livestock as observed in these cited literature could have been caused by breed differences.

Conclusions and Application

1. The spermatocrit fraction of the ejaculates was lowest in cocks fed the 0.15% ASA supplemented diet.
2. The 0.10% ASA supplementation seemed the optimum level to enhance total sperm/ejaculate (TSE) and reduce sperm morphological abnormalities in Marshall Broiler breeder cocks.
3. The gonadal sperm reserves (GSR), daily sperm production (DSP) and spermatogenic efficiency (SE) are best improved at 0.10% ASA supplementation.
4. The use of ASA in broiler breeder cocks' diets is therefore most beneficial to their reproductive capacity at 0.10% supplementation level.
5. Beyond this level of supplementation, the reproductive performance of broiler breeder cocks could be compromised.

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