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Changes in serum testosterone profile and some reproductive parameters in commercial ISA Brown cocks experimentally infected with *Salmonella Gallinarum* in Zaria, Kaduna State, Nigeria

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ABSTRACT

Aim: This study was aimed to elucidate the changes in serum testosterone profile and some reproductive parameters in commercial ISA Brown cocks experimentally infected with *Salmonella Gallinarum*.

Method and materials: A total of 40 ISA Brown cockerels, unvaccinated against fowl typhoid, but vaccinated against other infectious diseases such as Newcastle disease, Infectious bursal disease and fowl pox, were purchased from a reputable farm and brooded for four weeks and reared to 18 weeks of age and housed in deep litter system and managed intensively in the Poultry Research Pen of the Veterinary Teaching Hospital, ABU, Zaria. After attainment of reproductive age (23 weeks), the cocks were randomly allocated into two groups: infected and control of 20 birds each. Each bird in the infected group was administered orally 1ml inoculum containing 9.0 x 10^8 CFU/ml *Salmonella Gallinarum*, while birds in the control group were administered 1ml of distilled water each. Blood samples were collected on days 0, 4, 7, 14, 21, 28, 35 and 42 post infection (pi) in both groups and the harvested serum samples were used to analyse for serum concentrati on of testosterone. The cocks in both groups had their semen characteristics determined weekly for sixweeks post infection.
Cocks showing a typical clinical signs of fowl typhoid were humanly sacrified (embolism in the heart). Gross lesions, observed, were recorded, while specimens from liver, spleen and other organs were fixed in 10% neutral buffered formalin, while the testes were fixed in Bouins solution.

Results: Following infection of the infected birds, classical signs of fowl typhoid were observed. The clinical signs were moderate (24.7%) with mortality and morbidity of 37% and 50%, respectively. Serum testosterone concentration (1483±645 pg/ml) in the infected group significantly decreased ($p < 0.05$) from day 7 to lowest value of 520±394 pg.ml on day 21 pi. The control group had better semen colour, higher semen volume and better spermatozoa concentration than the infected cocks. The infected Isa brown cocks had higher percentage total spermatozoa abnormalities than the control group.

Conclusion: It was concluded that *Salmonella Gallinarum* can reduce serum testosterone concentration, semen volume, semen concentration, sperm motility and among others in ISA Brown cocks thereby resulting in reduced fertility.

Keywords: Salmonella, Cockerels, Typhoid, Isa brown.

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Introduction

Poultry production is the most efficient and cost-effective way of increasing the availability of high-protein food as eggs are known to provide the most perfectly balanced food containing all the essential amino acids, minerals and vitamins (Branckaert *et al*., 2000). In Africa, poultry farming is a major source of livelihood (Cardinale *et al*., 2004) and is the largest domestic animal stock in the world accounting for more than 30% of all animal protein produced globally (Permin and Pedersen, 2000; FAO, 2020). In Nigeria, poultry production is a

major source of income in both the semi-urban and rural settings. It provides a source of animal protein and accounts for 25% of local meat production (Agbaje *et al*., 2010). Salmonella species belong to member of the Family Enterobacteriaceae (Ferrari *et al*., 2019). They are gram negative, non-spore forming rods (Popoff *et al*., 2003). Salmonella enterica has six subspecies, namely, Salmonella enterica subsp. enterica, Salmonella enterica subsp. salamae, Salmonella enterica subsp. arizone, Salmonella enterica subsp. diarizone, Salmonella enterica subsp. houtenae, and Salmonella enterica subsp. indica (Hurley *et al*., 2014). The vast majority (about 99%) of Salmonella strains that infects humans and other warm-blooded animals belong to the species Salmonella enterica (Pławinska *et al*., 2022). The growth of poultry industry hinges on robust poultry immunity, health and production. The re-emergence of diseases will continue to be major challenges to the current situation and the strategic future of the industry (Hafez *et al*., 2020).

Among poultry diseases, fowl typhoid, caused by Salmonella Gallinarum, is one of the most important bacterial diseases that pose serious challenges to poultry production, worldwide (Saidu *et al*., 1994; Majid *et al*., 2010). This is aside the fact that it also constitutes a source of food-borne and zoonotic transmission of the disease to humans (Hafez *et al*., 2020). Salmonella infection remains a global problem and one of the most common foodborne illnesses (World Health Organization, 2021). Thus, this condition in food animals constitutes an important public health concern and, particularly, in food safety. Salmonella infection has been reported not only poultry but is also an emerging pandemic and threat to public health (Zeinab *et al*., 2020). Fowl typhoid is considered one of the most important septicaemic bacterial diseases of poultry that is associated with huge economic losses in affected farms (Ojima *et al*., 2021). The losses are incurred from the high mortality rate in chicks, retarded growth, adverse effect on egg production in infected laying birds and low fertility and hatchability of eggs laid by carriers (Saha *et al*., 2012; Haque *et al*., 2021).

Although the pathological effects of infection with Salmonella Gallinarum have been well established (Wigley *et al*. 2005; Zeinab *et al*., 2020), there are minimal reports available on changes on reproductive parameters consequent to the infections in males of the avian species. Many conditions affect the fertility of roosters and hens. In roosters, semen production and quality is affected byorchitis, epididymitis and epididymo-orchitis, which may account for impaired fertility in individual male breeders (Rafael *et al*., 2008).

Testosterone is a steroid hormone that is not only critical for male spermatogenesis, but also plays a critical role in the development of secondary sexual characteristics such as song and plumage among others (Lindsay *et al*., 2011), it also influence reproductive success due to its role in regulating aggressive behavior in both males and females during the breeding season (Hau *et al*., 2000; Sandell, 2007). Semen quality is an important indication of reproductive performance in individual male breeders. Fertilization requires good semen quality such that spermatozoa will be able to reach and penetrate the egg yolk (Mellor, 2001). The most important semen characteristics are sperm motility, concentration, per cent live spermatozoa and total abnormalities and among others. Roosters infertility is a major concern in the poultry industry because of the resulting economic losses and disruption of the production schedule (Rafael *et al*., 2008). Male fertility is based upon both the ability to perform a successful copulation and the quality of semen produced. The assessment of characteristics of semen quality of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters *et al*., 2014). Poultry industry has suffered from diseases with resultant poor fertility, poor chick quality, low performing breeds, poor weight gain and feed conversion, feeding and management problems and lack of capital (Saidu *et al*., 1994; Majid *et al*., 2010). The economic losses attributed to Salmonella Gallinarum infections in poultry industry are enormous and in most cases unquantifiable. This study evaluated changes in serum testosterone profile and some reproductive parameters in commercial ISA Brown cocks experimentally infected with Salmonella Gallinarum in Zaria, Kaduna State, Nigeria.

Materials and Methods

Ethical Statement: The handling and other experimental procedures conducted on the cocks were done based on International Guideline for the care and and use of Laboratory Animals (2011).
Ethical approval for the experimental protocol was sought from Ahmadu Bello University Committee

on animal use and care (ABUCAUC) with approval number ABUCAUC/2023/155.

Study Location: This study was carried out in the poultry research pens of Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria Kaduna State Nigeria. Zaria is situated in the Northern Guinea Savannah Zone of Nigeria, between latitude 7^0 and 11^0N, and longitude 7^0 and 44^0E; the average temperature ranges from 19^0C to 33^0C, the average rainfall of this zone ranges from 1,000 to 1,250 mm (Sawa and Buhari, 2011)

Experimental birds: A total of 40 ISA Brown cockerels vaccinated against other diseases but with the exception of fowl typhoid were purchased from a reputable farm in Jos and brooded for four weeks and reared to 18 weeks of age, They were then located to Zaria and housed in the animal research unit of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine Ahmadu Bello University Zaria Kaduna State Nigeria. The pens were thoroughly washed with detergent and sprayed with formalin at a concentration of 4 ml/1 litre of water prior to arrival of the birds. Throughout the experiment, standard commercial feed and water were provided to the birds *ad libitum*. The birds were acclimatized for a period of four weeks to get used to all the handling conditions and environment.

Bacteriological monitoring before challange: Before the establishment of infection in the experimental birds, a cloacal swabs from all the cocks using sterile swab were collected to confirm if they were free from *Salmonella* organism. This was done by immersing the cloacal swabs in a buffered peptone water which was then followed by plating the cloacal swabs in MacConkey agar (MCA) and blood agar (BA) using standard laboratory methods (Wigley *et al*., 2001; Parmer and Davies, 2007).

Experimental design: After attainment of reproductive age (23 weeks), the cocks were randomly allocated into two groups, that is infected and control of 20 birds each. Cocks in each group were housed at Poultry Research Pen of the Veterinary Teaching Hospital, ABU, Zaria. The cocks in the control group were inoculated each with 1 ml of sterile distilled water only per os while cocks in the infected group were inoculated orally with isolated *Salmonella* Gallinarum strain at a dose of 1.0 ml sterile saline containing $9x10^8$ cfu/ml of *Salmonella* Gallinarum. Following inoculation of the infected cocks with the *Salmonella* Gallinarum, the infected cocks were kept under strict observation for clinical signs of fowl typhoid and findings were recorded accordingly.

Blood Sampling: Blood samples were taken from wing vein from individual bird from both group using 23-gauge sterile hypodermic needles and syringes. 2 ml of blood from individual cocks were collected from both groups. Blood collection were done on days 0, 4, 7, 14 , 21, 28, 35, 42 post infection (pi). The blood was dispensed into plain sample bottles and allowed to clot at room temperature before centrifugation for 10 minutes at approximately 1000×g. The harvested serum from each cock was then emptied into micro-vials and stored at -20^oC and the harvested serum was then assayed for testosterone.

Semen collection and Evaluation: Semen samples were collected from both groups on days 0, 4, 7, 14 , 21, 28, 35, 42 post infection (pi) from each cock by abdominal massage as described by Baskt and long (2010). After stimulating the cocks, ejaculates was collected in an individual sterilized glass cup and kept in a metal chamber containing pre-warmed glass beads to avoid temperature shock. The semen samples was immediately processed for quality assessment (Coles, 1980). Semen analysis was conducted at the Artificial Insemination Unit of National Animal Production Research Institute Shika, Ahmadu Bello University Zaria. Assessment of semen quality

Semen Volume: The volume of semen was measured immediately after collection using a 1ml graduated tube according to the method of Zemjanis (1970)

Semen colour: Visually, semen colour was recorded as milky, creamy or watery and was designated 1 (milky), 2 (creamy), and 3 (watery) immediately after collection Zemjanis (1974)

pH and mass motility in raw semen: The pH of semen was measured using pH indicator strips. Evaluation of sperm motility was conducted with fresh and generally analyzed under the light microscope (10× magnifications). A drop of semen (raw or diluted) was placed onto a microscope slide and a score assigned (0-4) that estimates the percentage of motile sperm cells in the semen (Adeoye *et al*., 2017). Mass motility of sperm cells were viewed under light microscope at X40 magnifications and the percentage of motile sperm cells in the semen were estimated as described by Adeoye *et al*., (2017).

Semen concentration: The semen concentration in individual samples was evaluated using an

improved Neubauer haemocytometer as indicated by Adeoye *et al.* (2017). A drop of semen was thoroughly mixed with half-normal saline using a dilution factor of 1:20. Ten µL of the diluted semen was dropped at each side of the haemocytometer using a micropipette and allowed to settle for 5 min. and then placed under the light microscope under X40magnification. Sperm count was carried out by counting any sperm head that falls within the sub-divided smaller squares at the four edges and centre of the haemocytometer and recording the average. Sperm concentration was obtained by multiplying the number of sperm cells counted by dilution factor/volume and the multiplying factor of the chambers counted (Adeoye *et al*., 2017).

Determination of percentage of live sperm: The percentage of live sperm cells was determined by using eosin-nigrosin staining minimum of 200 live or dead sperms were counted on each slide (Shanmugam *et al*., 2014). A drop of semen sample was placed at the edge of a clean grease free glass slide and 3 drops of eosin-nigrosin stain was added to the semen. A smear was made from the mixture. Two hundred sperm cells were counted under light microscope at X40 magnification. In this principle, the live cells will not allow the dye to penetrate its cells while the dead cells will be stained by the dye appearing reddish or pinkish depending on the dye used.

Sperm morphology: This is used to check for abnormalities of the spermatozoa for both primary and secondary abnormalities which includes (head abnormalities, tail abnormalities and mid-piece abnormalities) This was done using light microscope at x400 magnification

Assay of Chicken testosterone using Enzyme-Linled Immonosorbent Assay: Chicken specific ELISA Kit (ELK Biotechnology CO., LTD) was acquired for the determination of testosterone in the ISA Brewn cocks serum and instructions in the manufacturer's manuals were strictly followed.

Principle of the assay method: This kit was based on sandwich enzyme-linked immune -sorbent assay technology. Capture antibody was pre -coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. Horse Radish Peroxidase -Streptavidin(HRP–Streptavidin) was added and unbound conjugates were washed away with wash buffer. Tetramethylbenzidine (TMB) substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. The optical density (O.D.) absorbancewas read at 450nm in a microplate reader, and the concentration of the analyte was extrapolated from the standard curve

Gross and Histopathological Examination: Gross lesion observed during postmortem examination were documented accordingly. Cocks showing a typical clinical signs of fowl typhoid were humanly sacrified (embolism in the heart) at Necropsy Unit of the Department of Veterinary Pathology, Faculty of Veterinary Medicine (ABU) Zaria. Gross lesions, observed, were recorded. The tissues of the liver, spleen, lung, kidney, heart and other organs were fixed in 10% neutral buffered formalin, while the testes were fixed in Bouins solution. It was then processed by paraffin embedding, sectioned at 5µm and stained with haematoxylin and eosin (Baker *et al*., 2000; Oladele *et al*., 2008). The slides were examined using light microscope at ×40, x100, x200 and x400 magnifications and the photomicrograph of lesions observed were snapped using camera and were recorded appropriately.

Statistical analysis: Data obtained were subjected to statistical analysis using Graph Pad Prism Version 8.00 for Windows, GraphPad Software, San Diego California USA. Data from the two groups was compared using the student t-test and values of $P<0.05$ were considered significant

Results and Discussion

Clinical Manifestations of Fowl Typhoid in the infected Commercial ISA Brown cocks

During the period of the study, neither morbidity nor mortalities were observed in the control group. Following challenge of the infected group, the clinical signs observed in the infected birds which Started from day 8 post-infection up to day 15 post-infection include: depression, a decrease in feed and water consumption, huddling, ruffled feathers, somnolence, greenish to yellow diarrhea, loss of body weight and among others. The morbidity rate recorded in the infected group was (50%) while, the mortality rate was 35%.

Effect of Salmonella enterica serovar Gallinarum Infection on Serum Testosterone of ISA Brown cocks

Mean testosterone concentration: The mean testosterone concentration of the *Salmonella enterica serovar* Gallinarum–infected and uninfected control

groups are presented in Figure 4.11.The mean serum testosterone concentration in the infected group (3808.00 ± 93.00 pg/ml) was not significantly different (p>0.05) from the control (3996.00 ± 98.00 pg/ml) on day 0 pi, and also on day 4 pi. Thereafter, a significant decrease (p<0.05) was observed in the serum level of this testosterone in the infected group on day 7 pi (1483.00 ± 645.00 pg/ml) than that of the control group (3779.00 ± 798.00 pg/ml). The lowest level of mean testosterone were recorded in the infected group on day 21 pi (520.00±394.00 pg/ml). Following this, a gradual rise were observed on day 21 pi until termination of the study.

Table 1: Morbidity rate in control and experimentally infected ISA Brown cocks with Salmonella Gallinarum during 42 days post infection

Days post-infection	Infected (N=20)	Control (N=20)
0	0	0
4	0	0
7	0	0
8-14	8	0
15-21	2	0
22-28	0	0
29-35	0	0
36-42	0	0
Total	10	0
MR	50%	0

- Morbidity rate= Number of sick birds/ Number of birds inoculated x 100

Table 2: Mortality rate in control and experimentally infected Isabrown cocks with *Salmonella* Gallinarum during 42 days post infection

Days post-infection	Infected (N=20)	Control (N=20)
0	0	0
4	0	0
7	0	0
8-14	5	0
15-21	2	0
22-28	0	0
29-35	0	0
36-42	0	0
Total	7	0
MR	35%	0

- Mortality rate = Number of dead birds/ Number of birds inoculated x 100%

Effect of Salmonella Gallinarum on semen characteristics of infected and control ISA Brown cocks: The mean semen characteristics of infected and control ISA Brown cocks infected with *Salmonella* Gallinarum (Table 3). The semen volume of the control and infected Isa brown cocks was not significantly different (p>0.05) at day 0 and day 4 post-infection. However, a significant (p<0.05) decrease (p<0.05) was observed in the mean semen volume in the infected group beginning from day 7 pi to reach a minimum level on day 14 pi. Thereafter, a slight increase was observed on 21 pi until the end of the study. The mean semen color of the infected and control broiler cocks showed significantly different (p<0.05), which started on day 7 pi until the end of the study. Spermatozoa motility of the infected and control Isa brown cocks was not significantly different (p>0.05) at day 0 and 4 post infection. The sperm motility of the control Isa brown cocks was significantly higher (p<0.05) than that of the infected Isabrown cocks starting from day 7 pi until the end of the study. The infected Isa brown cocks had lower sperm motility which started dropping significantly (p<0.05) on day 7 pi until the termination of the study. The spermatozoa concentration of the infected and control group counterpart was not significantly different (p>0.05) at day 0 and day 4 post-infection. Thereafter, a sharp drop in spermatozoa concentration was observed in the infected Isa brown cocks on day 7, pi reaching its lowest level on on 14 pi and thereafter, a slight increase was recorded on day 21 pi until the end of the study. The mean PH of the semen in the infected and control Isa brown cocks followed the same pattern of changes with that of the spermatozoa concentration. The mean per cent live spermatozoa of the infected and control Isa brown cocks showed no significant different (p>0.05) on day 0 and 4 pi. Thereafter, the mean per cent live spermatozoa of the infected Isa brown cocks declined significantly (p<0.05) on day 7 pi, reaching its least level on day 14 pi and later, a slight incline in the mean per cent live spermatozoa was observed on day 21 pi in the infected group even though it was not significant. There was no significant difference (p>0.05) in the percentage total spermatozoa abnormalities in the infected and the control Isa brown cocks on days 0 and 4 pi in this study. However, a significant increase (p<0.05) in the percentage total spermatozoa abnormalities was observed in the infected Isa brown cocks on day 7 post-infection, reaching its peak on day 21 pi and thereafter, started dropping on day 28 pi until the termination of the study.

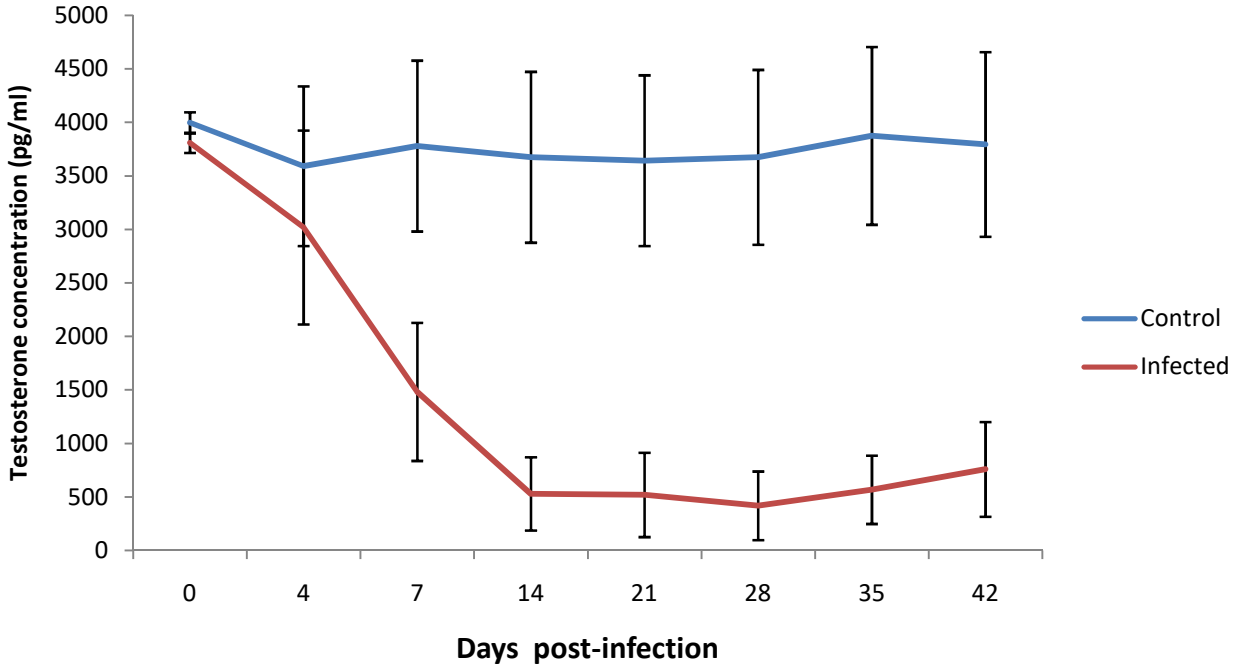

Fig 1: Mean (± SEM) of testosterone value of Isa brown cocks experimentally infected with *Salmonella enterica serovar* Gallinarum, and control cocks

The clinical signs observed in this present experiment shows that, the cocks in the infected group developed severe fowl typhoid infection which was characterized by depression, ruffled feathers, huddling, reduction in body weight, emaciation, somnolence, loss of appetite, greenish-yellow diarrhoea, paleness of the comb and wattle. This findings were were consistent with findings in previous reports (Ezema *et al*., 2009; Garcia *et al*., 2010 ; Barde *et al*., 2023). The incubation period observed in the infected Isa brown cocks in this present study was 8 days which contrast 3 days reported by Garcia *et al*. (2010), and 7 days by Chiroma *et al*. (2018).

The mean testosterone concentration of the infected Isa brown cocks shows a slight decline from day 4 post-infection in this study and reaching its lowest level on day 14 post-infection indicating that the oral challange of the infected group with Salmonella Gallinarum affected testosterone production. Testosterone plays an important role in gamete production, but also influences social and aggressive behaviour (Pikus *et al*., 2018). Testosterone is a steroid hormone that is critical for the onset of puberty in male such as development of genital tract, libido, initiation and potentiation of spermatogenesis with their interaction with androgen building proteins and follicle stimulating hormone (Hafez, 1987 and 1990). Spermatogenesis is controlled by a range of pathways, especially those including reproductive hormones. It occurs in the seminiferous epithelium and is controlled by the activity of Sertoli cells and their interaction with germ cells, testosterone, leutenizing hormone, follicle stimulating hormone and progesterone. The control group maintained there mean testosterone concentrations from the day of the study until the day of the termination of the study. In this study, the decline in the serum levels of testosterone observed in the infected Isa brown cocks is an indicative disruptive effect of Salmonella Gallinarum lipopolysaccharide on spermatogenesis. The maintenance of the male reproductive function depends readily on effective spermatogenesis and the synthesis of testosterone. Tissue damage and inflammation caused by bacterial infection can lead to male infertility by negatively interfering with spermatogenesis and testosterone production (Schuppe *et al*., 2008). The reduction in serum level of testosterone observed in the infected Isa brown cocks may also probably be due to necrosis of leydig cells and desquamation of seminiferous epithelium and these cells are responsible for the production of testosterone. The pathological lesions mentioned may have disturbed the serum hormone levels, as the affected cells were

Table 3. The mean semen characteristics of infected and control ISA Brown cocks infected with Salmonella Gallinarum

PARAMETERS	Groups	SAMPLING INTERVAL IN DAYS							
		0	4	7	14	21	28	35	42
Sperm volume (ml)	Control	0.64 ±0.027	0.67±0.031	0.71 ±0.018[a]	0.70 ±0.021[b]	0.70±0.026[c]	0.07±0.026[d]	0.70±0.025 [e]	0.72±0.013[f]
	Infected	0.65±0.027	0.65±0.027	0.34 ±0.043[a]	0.2 ±0.021[b]	0.22±0.02[c]	0.22±0.02[d]	0.22±0.02[e]	0.2±0.026[f]
Color (1-3)	Control	2.50±0.22	2.20±0.25	2.10±0.19 [a]	2.40±0.26[b]	2.30±0.21[c]	2.50±0.27[d]	2.70±0.32[e]	2.30±0.24[f]
	Infected	2.30±0.21	2.00±0.21	1.20±0.13[a]	1.00±0.00[b]	1.00±0.00 [c]	1.00±0.00[d]	1.00±0.00 [e]	1.00±0.00[f]
Sperm mortility %	Control	82.00±3.50	81.00±4.40	84.00±3.50 [a]	82.00±3.50[b]	87.00±2.00[c]	85.00±2.10	83.00±1.40	86.00±1.50 [f]
	Infected	82.00±2.30	83.00±2.30	51.00±3.00 [a]	40.00±1.80 [b]	40.00±1.90 [c]	41.00±1.70 [d]	42.00±1.20 [e]	43.00±2.00 [f]
Sperm PH	Control	6.8.00±0.25	6.60±2.70	7.20±0.097[a]	7.30±0.087 [b]	7.30±0.89 [c]	7.50±0.94[d]	7.20±0.34[e]	7.30±0.25 [f]
	Infected	6.70±0.21	6.80±0.20	4.10±0.31[a]	4.10±0.43[b]	3.80±0.29[c]	3.80±0.31[d]	4.50±0.34[e]	4.70±0.21[f]
Sperm concentration	Control	193.0±8.30	197.0±8.60	205.0±13.00 [a]	208.0±15.00 [b]	226.0±8.40 [c]	232.0±4.00[d]	241.0±3.00[e]	232.0±4.40[f]
(x10⁶)	Infected	205.0±3.5	208.0±3.70	91.00±15.00 [a]	33.00±1.80[b]	35.00±1.90 [c]	38.00±1.30[d]	39.00±1.70[e]	45.00±1.80[f]
Per cent live	Control	77.3±12.2	74.3±0.7	78±0.7 [a]	69.0±o.11 [b]	68.1±0.9 [c]	74.7±11.1[d]	75.09±0.9 [e]	76.32±0.7 [f]
spermatozoa (%)	Infected	75.0±0.9	77.9±11.1	42.2±0.8 [a]	41.8±12.0 [b]	41.9±0.9 [c]	43.8±0.6[d]	43.9±0.9 [e]	45.7±0.9 [f]
Total sperm	Control	7.4±2.6	6.2±1.7	8.3±1.6 [a]	6.1±1.9 [b]	7.1±2.2 [c]	5.9±1.5	4.8±2.3	5.4±1.6
abnormalities (%)	Infected	7.4±2.3	6.9±2.0	4.2±1.5 [a]	3.2±1.3 [b]	3.1±1.1[c]	4.1±1.5	4.1±1.3	3.9±1.3

Values with the same superscript alphabets along the same column are significantly different with $p<0.05$

associated with the production of testosterone. Tissue damage and inflammation caused by bacterial infection can result to male infertility by negatively interfering with spermatogenesis and testosterone production (Schuppe *et al*., 2008). The reduction in the serum concentration of the testosterone level in the infected Isa brown cocks in this study may also be due to the lesions caused by the Salmonella to the spermatozoa which includes, chromosome breakage, alteration in cell membrane structure, injury to the acrosome, and mitochondrial dysfunction (Li *et al*., 2018; Zeyad; *et al*., 2018a)

The cocks in the control group had higher semen volume starting from day 4 post-infection and throughout the period of the study than the infected Isabrown cocks. Similarly, Isa brown cocks in the control group had higher semen and better semen colour starting from day 7 post-infection up to the end of the study than the infected cocks. The decreae in mean semen volume observed in the infected Isa brown cocks in this study may be attributed to the Salmonella Gallinarum lipopolysaccharide on semen of the infected cocks. The control Isabrown cocks had better spermatozoa concentration when compared with the infected Isa brown cocks. The significantly decrease semen motility observed in the infected Isa brown cocks in this study were in part similar to the findings of Vizzier-Thaxton *et al*. (2006) who reported that Salmonella can attach to the head, midpiece, and tail of broiler rooster sperm, which could have a negative effect on sperm motility there by resulting in sperm abnormalities and subsequent infertility. A similar suggestion was made in colibacillosis in human semen which causes a decrease in motility with subsequent, infertility (Auroux *et al*., 1991; Diemer *et al*., 1996; Haines *et al*., 2013).

The PH in the infected Isa brown cocks started reducing on day 7 post-infection and reaching its lowest levels on 21 and 28 post-infection respectively. The PH of the control group was higher than the infected Isa brown cocks, which begin on day 7 post-infection till the end of the study. Bussalleu *et al*. (2005) reported that semen PH may not be a contributing factor to sperm immotility but bacteria infection can affect sperm's ability to swim, supporting previous research in boar semen.

Conclusion

It was concluded that Salmonella Gallinarum, can reduce serum testosterone concentration, semen volume, semen concentration, sperm motility and among others in ISA Brown cocks thereby resulting in reduced fertility.

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