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Assessment of organ weights and testicular histology of heat-stressed cockerels given lycopene and *Tetracarpidium conophorum* leaf extract

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Abstract

Heat stress has been noted to depress the functionality of poultry, resulting into lowered overall performance. Different methods of amelioration has been employed, however, there has been a developing interest in the use of phytogenic sources of antioxidant. However, documentation on the effects of administering phytogenic sources on the organ weight and histololgy of animals consuming the herbs is scanty in literature. This study was conducted to assess the relative organ weight and testes histopathology of heat-stressed cockerels given lycopene and Tetracarpidium conophorum leaf extracts (TCLE). 25-week old cockerels (n=54), were used for this experiment. They were randomly grouped into nine treatments containing: 0ml of extract / 250ml of water (control)(T1), 7.5ml of lycopene / 250ml of water (T2), 15ml of lycopene / 250ml of water (T3), 7.5ml of TCLE / 250ml of water (T4), 15ml of TCLE / 250ml of water (T5), 7.5ml of lycopene + 7.5m of TCLE / 250 ml of water (T6), 15ml of lycopene + 15ml of TCLE / 250ml of water (T7), Vitamin C at 0.1g per 250ml of water (T8), Cold temperature + Cold water (5-7^{O}C) (T9). The result showed that there was non-significant effect (P>0.05) of lycopene and TCLE on all the relative organ weight values recorded. Testes of the cockerels given the control Treatment (T1) had abnormal widening of interstitial spaces and degeneration of interstitial cells and lumen. For cockerels on T2 diets, there was an increase in both the interstitial spaces and intracellular spaces of the seminiferous tubules. Cockerels given T7 showed appreciable normal histomorphology with increased interstitial and intracellular spaces of the seminiferous tubules. Cockerels under T8, showed abnormal widening of interstitial spaces with other cells proliferating in a centripetal direction. Normal appearance without abnormal widening of interstitial spaces and degeneration of interstitial cells was observed in cockerels givenT9. In conclusion, 15ml of lycopene + 15ml of TCLE / 250ml of cool water (T7) can be administered to cockerels, to give a better testicular structure and 15 mls lycopene enhanced improved internal organ weight.

Keywords: Heat stress, Cockerels, testis, histopathology, lycopene, organ weight

Évaluation du poids des organes et de l'histologie testiculaire de coquelets soumis à un stress thermique ayant reçu du lycopène et de l'extrait de feuille de Tetracarpidium conophorum

Résumé

Il a été noté que le stress thermique déprime la fonctionnalité de la volaille, entraînant une baisse des performances globales. Différentes méthodes d'amélioration ont été employées, cependant, il y a eu un intérêt croissant pour l'utilisation de sources phytogéniques d'antioxydant. Cependant, la documentation sur les effets de l'administration de sources phytogéniques sur le poids des organes et l'histologie des animaux consommant les herbes est rare dans la littérature. Cette étude a été menée pour évaluer le poids relatif des organes et

l'histopathologie des testicules de coquelets soumis à un stress thermique ayant reçu du lycopène et des extraits de feuilles de Tetracarpidium conophorum (EFTC). Des coqs âgés de 25 semaines (n=54) ont été utilisés pour cette expérience. Ils ont été regroupés aléatoirement en neuf traitements contenant : 0 ml d'extrait / 250 ml d'eau (témoin) (T1), 7,5 ml de lycopène / 250 ml d'eau (T2), 15 ml de lycopène / 250 ml d'eau (T3), 7,5 ml de EFTC / 250ml d'eau (T4), 15ml de EFTC / 250ml d'eau (T5), 7,5ml de lycopène + 7,5m de EFTC / 250 ml d'eau (T6), 15ml de lycopène + 15ml de EFTC / 250ml d'eau (T7), Vitamine C à 0,1 g pour 250 ml d'eau (T8), Température froide + Eau froide (5-7OC) (T9). Le résultat a montré qu'il y avait un effet non significatif (P>0,05) du lycopène et du EFTC sur toutes les valeurs de poids relatif des organes enregistrées. Les testicules des coquelets ayant reçu le traitement témoin (T1) présentaient un élargissement anormal des espaces interstitiels et une dégénérescence des cellules interstitielles et de la lumière. Pour les coquelets soumis au régime T2, il y avait une augmentation à la fois des espaces interstitiels et des espaces intracellulaires des tubules séminifères. Les coquelets ayant reçu du T7 ont montré une histomorphologie normale appréciable avec une augmentation des espaces interstitiels et intracellulaires des tubules séminifères. Les coquelets sous T8 ont montré un élargissement anormal des espaces interstitiels avec d'autres cellules proliférant dans une direction centripète. Une apparence normale sans élargissement anormal des espaces interstitiels et une dégénérescence des cellules interstitielles a été observée chez les coquelets ayant reçu le T9. En conclusion, 15 ml de lycopène + 15 ml de TCLE / 250 ml d'eau fraîche (T7) peuvent être administrés aux coquelets, pour donner une meilleure structure testiculaire et 15 ml de lycopène améliorent le poids des organes internes.

Mots clés : Stress thermique, Coquelets, testicules, histopathologie, lycopène, poids des organes

Introduction

Heat stress causes divers physiological and metabolic alterationin chickens, which have damaging effects on bodyweight gain, feed efficiency, serum triglyceride uric acid levels (Deyhim *et al.,* 1995), eggshell quality and livability (Mashaly *et al.,* 2004; Quinteiro-Filho *et al.,* 2010). Oxidative stress delineate the existence of products known as free radicals and reactive oxygen species (ROS), although are produced under normal physiological conditions, but become pernicious when not being eliminated (Chanda and Dave, 2009). ROS are major sources of essential catalysts that introduce oxidation *in vivo* and *in vitro* and cause oxidative stress which results into impairment of biomolecules viz. DNA/RNA, proteins and lipid peroxidation of membranes and interuption of normal cell metabolism (Spurlock and Savage, 1993).Some researchers discovered that the dietary supplementation of taurine, betaine, vitamin C, and vitamin E may enhance the growth performance and livability of birds under heat-stress conditions (Zulkifli *et al.,* 2004; Shim *et al.,* 2006; Ipek *et al.,* 2007). However, some natural antioxidants have been employed to conflict oxidative stress, proceeding as a result of heat stress. One of such antioxidants is lycopene and *Tetracarpidium conophorum* leaves (TCL)**.** Lycopene is a prevailing carotenoid pigment which is common to all fruits and vegetables, with tomatoes and their products as a fundamental source. A study conducted by Sahin *et al*. (2008) documented that lycopene-rich tomato powder is importantly better as it enhanced feed intake, weight gain, and reduced concentration of malondialdehyde (MDA) in muscles, liver, and serum of Japanese quail raised under heat stress. A study on *Tetracarpidium conophorum* has also

revealed that ingestion of its seeds improves protection against proliferous diseases, oxidative stress endothelial malfunction (Nwaoguikpe *et al.,* 2012). It also promotes fertility in both men and women and advance spermatozoa count in men. According to Ashraf and Sheik, (2015), oxidative stress and have been noted to play germane functions in testicular damage as a result of hyperthermia. Heat stress (HS) has been noted to cause testicular injury, resulting in decreased fertility. Previous research conducted by Nash and Rahman (2019) revealed that the level of testicular damage is positively proportional to the decrease in reproductive performance caused by heat stress in male animals This would in turn adversely affect the availability of animal protein sources to the ever- growing human population. There is therefore a need to find solutions to the effect of heat stress in cockerels. This study was designed to assess the organ weights and histology of heat-stressed cockerels given lycopene and *Tetracarpidium conophorum* leaf extract

Materials and methods

Study area

This study was carried out at the Broiler Unit of Teaching and Research Farm of the College of Agriculture, Kwara State University Malete, Moro Local Government Area, Kwara State, Nigeria. Kwara state, is within Guinea Savannah ecological zone of Nigeria with average day time temperature of 30-38^{o}C in the dry season and relative humidity of 55-75% with latitude 8.7082N and longitude 4.4723E.

Experimental animal and management and design

30-week old reproductively matured cockerels (n=54) were used for this experiment. The birds were procured from a reputable farm within Kwara State. The cocks were individually wing tagged for identification purpose and housed accordingly. The birds were given feed *ad - libitum* with commercial breeder mash containing 17.5% crude protein and 2700kcal metabolizable energy. Clean water was supplied *ad- libitum*. Medications and vaccinations were done as required. The cockerels were given a 2-week period to acclimatize physiologically to the environment before commencement of data collection. There were nine (9) treatments with each treatment replicated 3 times with 2 birds per replicate in a complete randomized design. Birds in treatments 1-8 (T1-T8) were subjected to an external heat source through the use of wooden charcoal after grouping them in treatments and partition the cubicle to achieve an average temperature of the pen (38±2^{O}C), while birds in T9 was given a source of temperature coolant to enhance a reduced temperature of about 25^{0}C as well as cold water of about 5-7^{O}C both from 08.00 hrs till 02.00hrs throughout the period of the experiment.

The treatments are:

T1: 0ml of extract / 250ml of water, T2: 7.5ml ml of lycopene / 250ml of water

T3: 15ml of lycopene / 250ml of water, T4: 7.5ml of *TCLE* / 250ml of water

T5: 15ml of *TCLE* / 250ml of water, T6: 7.5ml of lycopene + 7.5ml of *TCLE* / 250ml of water, T7: 15ml of lycopene + 15ml of *TCLE* / 250ml of water, T8: Vitamin C at 0.1g per 250ml of water (control), T9: Cold temperature + Cold water (5-7^{o}C)

Sample procurement and preparation

5 kg of red tomato fruit (*Solanumly coperisicum*. L) was purchased from indigenous market. The tomatoes were washed under running stream of water to remove dirt, dust and foreign materials De-heading and trimming of the tomatoes were carried out manually using a knife. These were processed into tomato paste following the protocols described by Dauthy (1995). Tomato paste was cooked for several hours

and reduced to a thick, red concentrate. Likewise, fresh sample of *Tetracarpidium conophorum* **also called** tropical African walnutIn Nigeria, among the Yoruba tribe, the walnut is known as *awusa* or *asala, ukpa*, or *oke okpokirinya* in Igbo and *gawudi bairi* in Hausa; (Kanu et al. 2015leaves were obtained from the environs of Ore in Ondo State, Nigeria. The leaves were cleaned and air dried and pulverized for subsequent usage.

Extraction of lycopene

It involves the simple application of organic solvents to the samples for lycopene extraction as adopted by Roldan-Gutierrez *et al.* (2007). The tomato paste were properly homogenized for efficient extraction of lycopene. 20g of each sample was taken in the 250mL of the conical flask. Samples were extracted overnight in the orbital shaker with the solvent mixture of 200mL of hexane and acetone in the ratio of 75:25 respectively at room temperature. The extract from each flask was filtered with Whatman No. 1 filter paper. The solvent from extract was separated at 50°C in a rotary vacuum evaporator (EYELA, N-N series, Japan) leaving behind crude extract only. The crude extract of each sample was stored at 4°C until use.

Preparation of extract from TCLE

The freshly collected leaves of *TCLE* were separated, air dried, and milled into powder using a hammer mill. 542.6g powdered plant material is added to 2713ml of ethanol and kept in conical flask, the mouth of the conical flask was covered with aluminum foil and kept in a reciprocating shaker for 24hours for continuous agitation at 150rev/min for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent. Then extract was filtered by using muslin cloth followed by whatman filter paper. The solvent from the extract was removed by using rotary evaporator at 750c and placed in the water bath at 50°C.The residue was collected and used for the experiments.

Organ weight determinations

Cockerels were selected per treatment for histopathological investigation. The dissection was carried out as described by Byanet *et al.* (2008). All animals were anaesthetized with formalin solution. An incision was made from the first cervical region up to the pelvic region. The specimens' liver, kidney, lung, heart, and spleen, both left and right testes were carefully removed, weighed of bird was obtained using a digital weighing balance various measurements of weight were taken. Sections were immediately fixed in neutral buffered formalin for further investigation.

Histopathological examination

Histopathological examination was carried out using the method of Aliyu *et al.* (2007).

HPLC quantification /Analysis of lycopene

Lycopene was analysed using reversed-phase high performance liquid chromatography using isocratic elution and UV detection at 472 nm (Waters, Zellik, Belgium).

Total lycopene was quantified by summing the peak area of all lycopene and the Z isomers and based on the standard curve of all lycopene (Lee and Chen 2001).

Statistical analysis

All data from this study were subjected to descriptive statistics and one-way analysis of variance (ANOVA) in a completely randomized design using statistical for social science (SPSS) vision 23(IBM 2013), Means were separated using Duncan multiple range test DMRT .

Results and discussion

Table1 shows the effect of lycopene and *TCLE on relative organ weight of heat stressed cockerels*. There was nosignificant effect (P>0.05) of lycopene and *TCLE* on all the organs monitored across the treatments.

However, cocks givenT3 had heavier liver, kidney, heart, left testes and right testes than the control group (T8) and the rest of the bird, while birds given T7 had more of testes volume (1.42), lung (0.64), and reproductive system (1.39) than the rest of the birds including control group. It has been asserted that cockerel with high bodyweights usually have well develop internal organs and bigger testes (Ubah *et al.,* 2017). The results obtained for this study are comparable to those values observed by Akomolafe *et al.* (2017) on the research conducted on rat given TCLE through feed.

Table: Effect of lycopene and *Tetracarpidium conophorum* leaf extract (TCLE) on the relative organ weights of heat-stressed cockerels

Trt	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Testesvol. (ml)	L/testes (g)	R/testes (g)	Repro. ogr	LUNG (g)
1	1.07±0.24	0.30±0.06	0.10±0.00	0.43±0.06	1.01±0.11	0.50±0.03	0.46±0.08	1.00±0.09	0.44±0.13
2	1.41±0.32	0.37±0.02	0.12±0.03	0.30±0.17	1.10±0.77	0.49±0.38	0.47±0.36	1.05±0.80	0.54±0.05
3	1.27±0.20	0.58±0.38	0.11±0.02	0.55±0.04	1.37±0.13	0.64±0.06	0.67±0.14	1.31±0.22	0.49±0.19
4	1.12±0.19	00.29±0.04	0.10±0.02	0.41±0.09	1.02±0.07	0.51±0.03	0.490.05	1.05±0.10	0.42±0.08
5	1.29±0.07	00.36±0.05	0.10±0.03	0.46±0.09	1.29±0.20	0.56±0.06	0.54±0.07	1.18±0.15	0.50±0.10
6	1.34±0.34	00.34±0.08	0.09±0.01	0.43±0.11	1.26±0.44	0.54±0.37	0.54±0.30	1.20±0.58	0.55±0.17
7	1.08±0.07	00.33±0.07	0.10±0.01	0.51±0.17	1.42±0.34	0.61±0.16	0.67±0.26	1.39±0.41	0.64±0.15
8	1.17±0.18	00.25±0.01	0.10±0.03	0.38±0.08	0.96±0.18	0.45±0.07	0.43±0.09	1.03±0.18	0.39±0.09
9	1.04±0.24	00.35±0.11	0.08±0.03	0.43±0.08	0.97±0.07	0.49±0.03	0.44±0.04	1.04±0.11	0.49±0.07
	NS	NS	NS	NS	NS	NS	NS	NS	NS

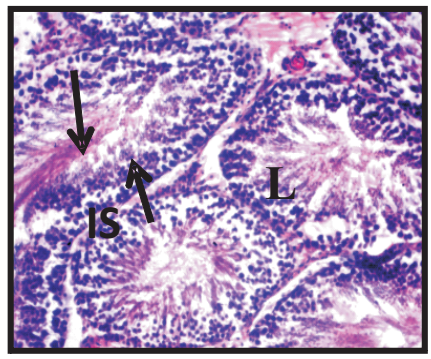
NS: Not significant T1: 0ml of extract / 250ml of water, T2: 7.5ml ml of lycopene / 250ml of waterT3: 15ml of lycopene / 250ml of water, T4: 7.5ml of TCLE / 250ml of water
T5: 15ml of TCLE / 250ml of water, T6: 7.5ml of lycopene + 7.5ml of TCLE / 250ml of water, T7: 15ml of lycopene + 15ml of TCLE / 250ml of water, T8: Vitamin C at 0.1g per 250ml of water (control), T9: Cold temperature + Cold water (5 -7^{o}C) R Testes -Right testes, L -Testes-Left testes, Repro Org - Reproductive organ. Trt-Treatment

Effect of lycopene and TLCE on the histology of heat-stressed cockerels

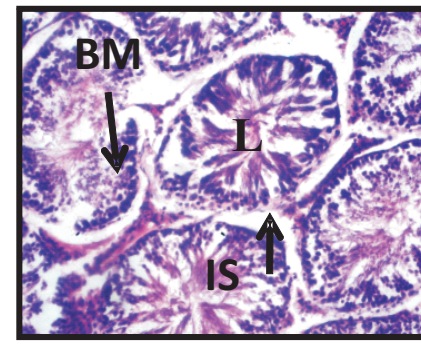
Effect of experimental inclusions on testis histology

The result of the testis histology showed that in treatment 1 (Plate A), observation showed that there was normal histomorphology of the cocks testes, with typical seminiferous tubule, containing different types of germ cells. spermatogonia was noted to be lying on basement membrane (BM) with other cells proliferating in a centripetal direction.The transverse section of the testis showed an abnormal widening of interstitial spaces (IS) (H and E x100). However, in treatment 2 (Plate B), the transverse sections of the testis showed a normal histomorphology with typical seminiferous tubule, increased interstitial spaces and intracellular spaces. The transverse section of testis shows a normal histomorphology with typical seminiferous tubule, with intercellular space. (H and E x100).

Testicular histopathology of cockerels in Treatment 3 and 4, represented as Plate C and D respectively, showed that the transverse section of testis had abnormal widening of interstitial spaces (IS) with degeneration of interstitial cells and increased intracellular spaces of the seminiferous tubules (H and E x100)



A Plate A: Photomicrograph of testis of the cockerels given control diet(0ml of extract / 250ml of water).



B Plate A: Photomicrograph of testis of the cockerels given control diet(7.5ml of lycopene / 250ml of water).

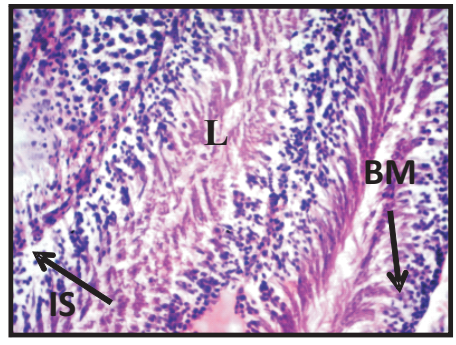


Plate C: Photomicrograph of testis of the cockerels given control diet(15ml of lycopene / 250ml of water)

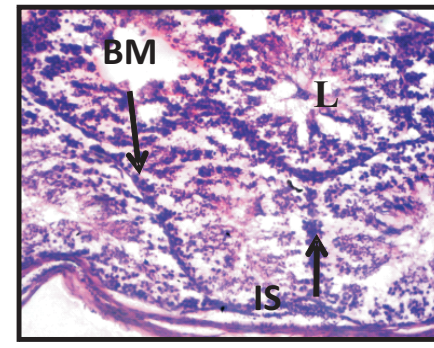


Plate D: Photomicrograph of testis of the cockerels given control diet(7.5ml of TCLE / 250ml of water)

The photomicrograhs of chickens given 15ml TCLE (Treatment 5) and 7.5ml lycopene + 7.5 ml TCLE (Treatment 6) are shown in Plates E and F, respectively. Observation showed that there was a normal histomorphology having typical seminiferous tubule with maintained integrity, but with increase of interstitial spaces. Containing different types of germ cells; spermatogonia lying on basement membrane with other cells proliferating in a centripetal direction.

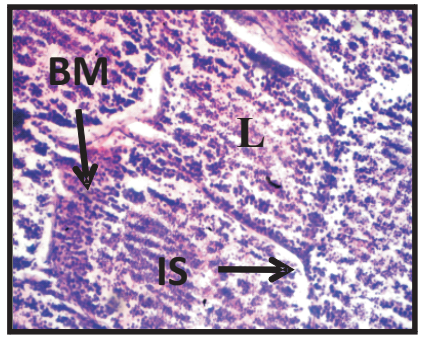


Plate E: Photomicrograph of testis of the cockerels given 15ml of TCLE / 250ml of water

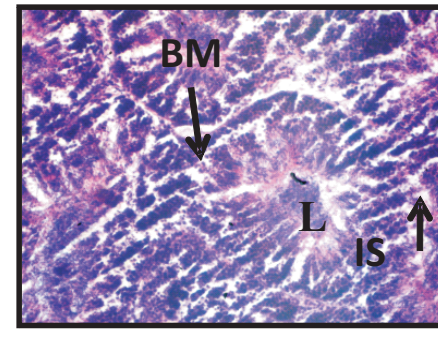


Plate F: Photomicrograph of testis of the cockerels given 7.5ml of lycopene + 7.5mls TCLE / 250ml of water

In treatment 7, Plate 7G, there was an increased interstitial spaces and intracellular spaces of the seminiferous tubules. However, in treatment 8, Plate 8H, an abnormal widening of interstitial spaces (IS) was noticed, with degeneration of interstitial cells with increased intracellular spaces of the seminiferous tubules.

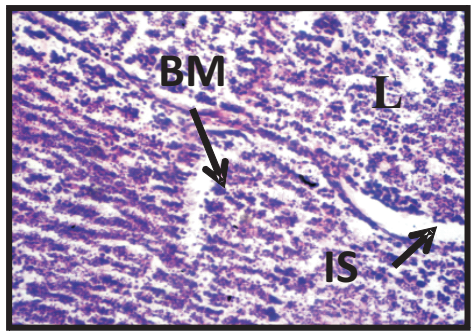


Plate G: Photomicrograph of testis of the cockerels given 15ml of lycopene + 15mls TCLE / 250ml of water

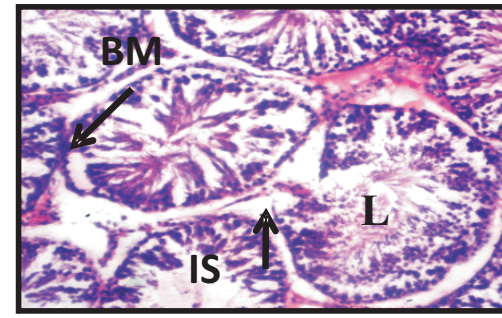


Plate H: Photomicrograph of testis of the cockerels given 0.1g vItaimin C/250ml of water)

It was recorded in Treatment 9 (Plate I) that there was seminiferous tubule with maintained integrity but with increase of interstitial spaces. Normal histomorphology with typical seminiferous tubule containing different types of germ cells and spermatogonia lying on basement membrane with other cells proliferating in a centripetal direction were also noted (H and E x100).

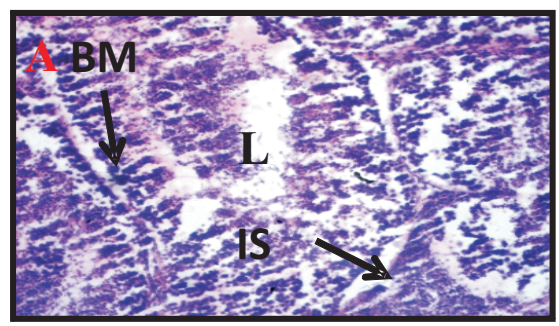


Plate I: Photomicrograph of testis of the cockerels given Cold water +reduced micro environmental temperature

The abnormal widening of interstitial spaces and degeneration of interstitial cells and lumen observed in plate A, similar to plate H (Control-Vitamin C treatment) could be an indication of depletion in the spermatogenic cell layers of the seminiferous tubules in the testis which led to larger lumens of the seminiferous tubules and fewer germ cells. The interstitial cells consist of leydig cells, fibroblast, collagenous fibres and reticular fibres, lymphatic vessels and blood vessels. Interstitial cells are responsible for the secretion of testosterone, hence their degeneration could lead to abnormal secretion of testosterone by these cockerels and negatively affect their reproductive performance. Also, it can be observed that some spermatozoids were present in the luminal space of seminal tubes. Hence, a

degenerated lumen might have caused the death of matured and immature spermatozoa resulting in infertility and low reproductive performance (Ojobor *et al.,* 2017). This study also corroborated the findings of Orji *et al.* (2018) who reported an attenuated testicular damage in rat given Vitamin C.

Observation shows that cockerels on T2 (plate B) which received the administration of lycopene at 7.5ml/250ml of water had increase in both the interstitial spaces and intracellular spaces of the seminiferous tubules compared with birds on T8 with abnormal widening of interstitial spaces and degeneration of interstitial cells of the seminiferous tubules. The number and density of spermatozoids cells in the lumen area of the seminal tubes are more (Plate B). This indicated an increased production of sperm cell and the associated hormone (testosterone). The number of spermatozoids is related to the spermatogenesis process, which is also influenced by the testosterone hormone. Increased testicular interstitial and intracellular spaces in the cockerel's testis indicated an accelerated blood flow and the level of testosterone hormone, which will result in improved spermatogenesis in the testicle and finally increased fertility. It has been reported that lycopene protect cells and tissue damage caused by ROS (Palozza *et al.,* 2011) and has been ranked as more potent in this activity compared to other carotenoids. This finding is in line with the report of Hamzehnezhad *et al.* (2019) on testis of broiler chickens given ginger. The results also agreed with findings of Morakinyo *et al.* (2008) and Bordbar *et al.* (2013).

Cockerels given T3, (Plate C), T4 (Plate D), T5 (Plate E) and T6 (Plate F) respectively showed a similar form of abnormal widening of interstitial spaces with degeneration of interstitial cells and increased intracellular spaces of the seminiferous tubules. This is similar to what was obtained in control group.Thisobservation might be due to increased lycopene content beyond the requirement of the cockerel thereby affecting the cockerel negatively as it led to degeneration of interstitial cells and widening of interstitial spaces. These changes are consistent with testicular degeneration as reported by Foster and Ladds (2007). Cockerels in T7 (plate G) showed appreciable normal histomorphology with increased interstitial spaces and intracellular spaces of the seminiferous tubules, contrary to what was obtained in T8, indicating that the combination of lycopene and TCLE at 15mLs each can be used to improve the reproductive capability of the cockerels as there was no record of abnormality in the testis. This is consistent with the report of Ojobor et al. (2017) who observed normal histomorphology with increased interstitial connective tissue and robust seminiferous tubular lumen containing sperm cells in rat after been administered orally with *T. conophorum* leaf extract. It can also be observed that feeding cockerels with Vitamin C (T8) might not improve the sperm cell production capability of the cockerel as it failed to cushion the occurrence of abnormal widening of interstitial spaces and degeneration of interstitial cells of the seminiferous tubules. This suggest that Vitamin C might not be a suitable additive to ensure improved production of quality spermatozoa by the seminiferous tubules of the cockerel's testis. Orji *et al.* (2018) also reported a scanty germinal cells in the epithelium of seminiferous tubules and empty spermatids in some part of seminiferous tubules in rat given Vitamin C. Occurrence of normal appearance without abnormal widening of interstitial spaces and degeneration of interstitial cells in cockerel given T9 (plate I) showed that birds in cool environment

and with cold water normally experience less heat stress and are able to perform better than those that are heat stressed. This is similar to the report of **Hussain *et al.* (2011) who observed normal diameter of seminiferous tubules with no degeneration of** interstitial cells but with **presence of** spermatogonial cells and spermatocytes in broiler chicken subjected to cool and controlled environment with fresh cool water.

Conclusion

Inclusion of lycopene and *TCLE at the dosage levels adopted in this study* did not induce hepatotoxicity and stress related problem that could induce cellular damage of the liver in the experimental birds. Hence, the dietary treatments especially at 15ml of lycopene + 15ml of / 250ml of water could be regarded as safe for medicinal use at the test doses.Also, 15ml of lycopene / 250ml of water can be given to the birds as they gave better internal organ weight and better testicular structure.

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