Organ Weights and Testicular Histology Of Heat-stressed Cockerels Given Lycopene and *Tetracarpidium conophorum* Leaf Extract

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ABSTRACT

This study was conducted to assess the relative organ weight and testes histopathology of heat-stressed cockerels given &lycopene and African walnut (*Tetracarpidium conophorum* Mull. Arg. Hutch & Dalziel.) leaf extract (TCLE). Thirty week (30-week) old cockerels (n=54), were used for this experiment. They were randomly grouped into nine treatments containing: 0 ml of extract / 250 ml of water (control) (T1), 7.5 ml of lycopene / 250 ml of water (T2), 15 ml of lycopene / 250 ml of water (T3), 7.5 ml of TCLE / 250 ml of water (T4), 15 ml of TCLE / 250 ml of water (T5), 7.5 ml of lycopene + 7.5 ml of TCLE / 250 ml of water (T6), 15 ml of lycopene + 15 ml of TCLE / 250 ml of water (T7), Vitamin C 0.1 g per 250 ml 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 organs weight values recorded. Testes of the cockerels given the control treatment (T8) 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 spaces and intracellular spaces of the seminiferous tubules. Cockerels under T8, given Vitamin C, showed abnormal widening of interstitial spaces and degeneration of interstitial cells of the seminiferous tubules. Normal appearance without abnormal widening of interstitial spaces and degeneration of interstitial cells in cockerel given T9. In conclusion, 15 ml of lycopene + 15 ml of TCLE / 250 ml of water (T7) can be administered to cockerels in a cool environment, with an addition of cold water (T9), to give a better testicular structure, and 15 ml of lycopene / 250 ml for improved internal organ weight.

Keywords: Heat stress, Cockerel, testis, histology, lycopene, Organ weight

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INTRODUCTION

Heat stress causes depressed bodyweight gain, feed efficiency among others (Deyhim *et al.,* 1995). Oxidative stress delineates the existence of products known as free radicals and reactive oxygen species (ROS) which results into DNA damage in exposed animals. Although ROS are produced under normal physiological conditions, but become pernicious when not being eliminated (Chanda and Dave, 2009). However, some natural antioxidants have been employed to ameliorate oxidative stress to enhance overall performance of animals despite the consistent climatic change. A few of such antioxidants are lycopene and *Tetracarpidium conophorum* (Mull. Arg. Hutch & Dalziel.) leaf (TCL). A study conducted by Sahin *et al.* (2008) showed that lycopene-rich tomato powder improved feed intake, weight gain, and reduced concentration of malondialdehyde (MDA) in muscles, liver, and serum of Japanese quail raised under heat stress

Also a study conducted by Nwaoguikpe *et al. (*2012A) on *Tetracarpidium conophorum* has revealed that ingestion of its seeds improves protection against proliferous diseases, and the leave extract promotes fertility. advancing spermatozoa count in male animals. However, there is limited information on the effect of different concentrations of plant extracts on tissue structure, despite the outward improved performance shown by the animal models used for different experiments. That is why this study is designed to determine the effect of different concentrations of lycopene and TCLE on the organ weights and testis histology of experimental animals.

MATERIALS AND METHODS

Study Area

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

Experimental Animal, Management and Design

Reproductively matured cockerels, aged 30 weeks old (n=54) were used for this experiment. The birds were procured from a reputable farmer within

Kwara State. The birds 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±2^{O}C as well as cold water of about 5-7^{O}C both from 08.00 hours till 02.00 hours throughout the period of the experiment.

The treatments include the following;
T1: 0 ml of extract / 250 ml of water, T2: 7.5 ml of lycopene / 250 ml of water
T3: 15 ml of lycopene / 250 ml of water, T4: 7.5 ml of TCLE / 250 ml of water
T5: 15 ml of TCLE / 250 ml of water, T6: 7.5 ml of lycopene + 7.5 ml of TCLE / 250 ml of water, T7: 15 ml of lycopene + 15 ml of TCLE / 250 ml of water, T8: Vitamin C 0.1 g per 250 ml of water (control), T9: Cold temperature + Cold water (5-7^{o}C).

Sample procurement and preparation

Bright red tomatoes (*Solanum lycoperisicum*. L) were purchased from Ipata market, Ilorin, Kwara State. 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 Daugthy (1995). Tomato paste was cooked for several hours and reduced to a thick, red concentrate. Likewise, fresh sample of *Tetracarpidium conophorum* leaves 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

This involved the simple application of N-hexane and acetone to the tomato paste samples for lycopene extraction as adopted by Roldan-Gutierrez *et al.* (2007). The tomato paste was 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 *Tetracarpidium conophorum* leaves were separated, air dried, and milled into powder using a hammer mill. 542.6 g powdered plant material is added to 2713 ml 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 24 hours for continuous agitation at using rotatory mechanical shaker with 150 rev/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 using rotary evaporator at 75^{0}C and placed in the water bath of 50^{o}C. finally 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

Trt	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Testis vol. (ml)	L/T (g)	R/T (g)	Repro(g)	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	0.29±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	0.36±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	0.34±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	0.33±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	0.25±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	0.35±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

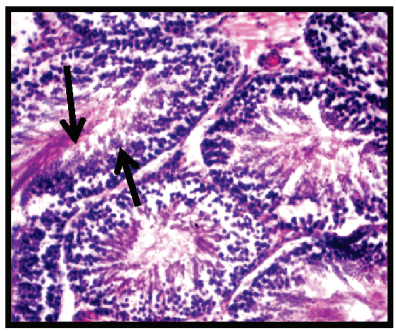
NS =not significant, Trt = Treatment, R/T = Right testis, L/T= Left testis, Repro= Reproductive system, T1=0ml of extract / 250ml of water, T2= 7.5ml ml of lycopene / 250ml of water, T3=15ml of lycopene / 250ml of water, T4= 7.5mlin of TCLE / 250ml of water, T5=15ml of TCLE / 250ml of water, T6=7.5ml of lycopene + 7.5ml of TCLE / 250ml of water, T7=15ml of lycopene + 15ml of TCLE / 250ml of water, T8=Vitamin C 0.1g per250ml of water, T9= Cold temperature + Cold water 5°C

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

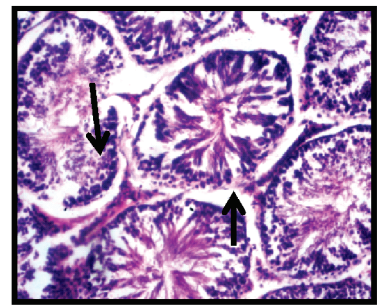
Effect of experimental inclusions on testis histology

The result of the testis histopathology showed that:

In Treatment 1, Plate A, there is normal histomorphology with typical seminiferous tubule containing different types of germ cells; spermatogonia lying on basement membrane (BM) with other cells proliferating in a centripetal direction., the transverse section of testis shows an abnormal widening of interstitial spaces (IS). (H and E x100). However, in Treatment 2, Plate B, the transverse sections testis shows a normal histomorphology with typical seminiferous tubule with an increase of interstitial spaces and increased intracellular spaces of the seminiferous tubules. The transverse section of testis shows a normal histomorphology with typical seminiferous tubule but with intercellular space. (H and E x100)



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



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

The result of the testis histopathology 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)

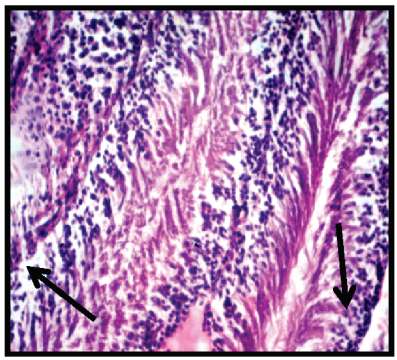


Plate C: Photomicrograph of testis of the cockerels given control diet (15 ml of lycopene / 250 ml of water)

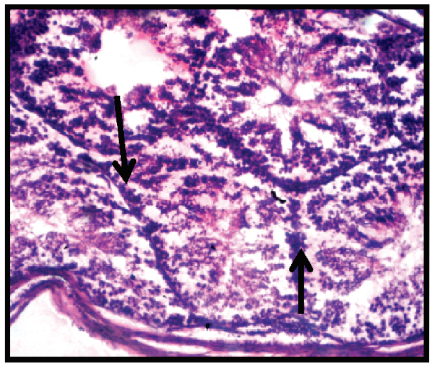


Plate D: Photomicrograph of testis of the cockerels given control diet (7.5 ml of TCLE / 250 ml of water)

The photomicrographs of chickens given 15 mls TCLE (Treatment 5) and 7.5 mls lycopene + 7.5 mls TCLE (Treatment 6), as shown in Plate E and F respectively. Observation shows that there is a normal histomorphology with 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.

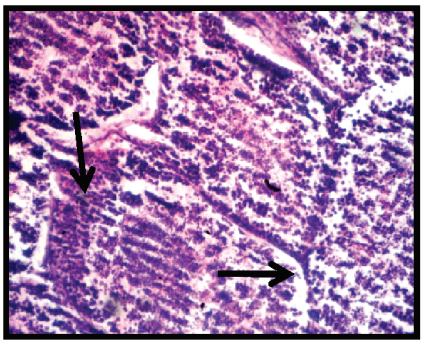


Plate E: Photomicrograph of testis of the cockerels given 15 ml of TCLE / 250 ml of water

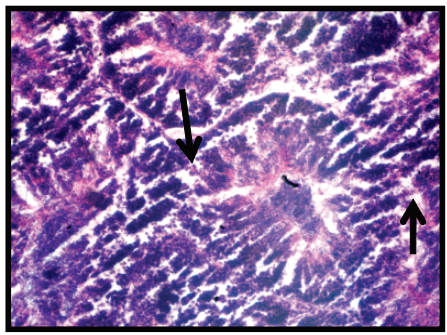


Plate F: Photomicrograph of testis of the cockerels given 7.5 ml of lycopene + 7.5 mls TCLE / 250 ml of water

In Treatment 7, Plate 7G, there is 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.

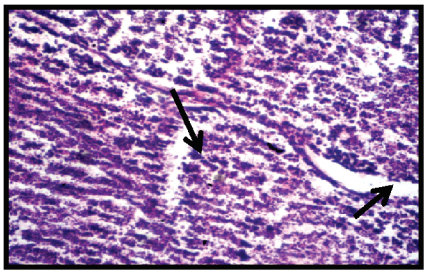


Plate G: Photomicrograph of testis of the cockerels given 15 ml of lycopene + 15 mls TCLE / 250 ml of water

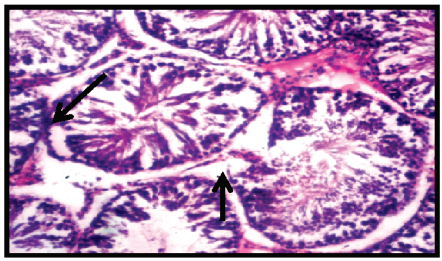


Plate H: Photomicrograph of testis of the cockerels given 0.1 g vitamin C/250 ml of water)

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

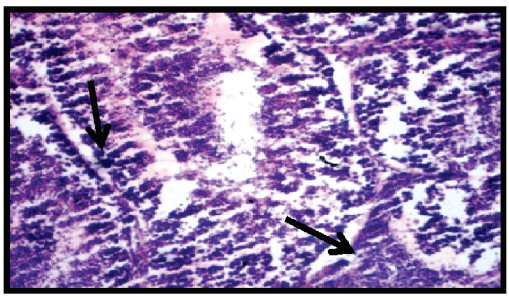


Plate I: Photomicrograph of testis of the cockerels given Cold water +reduced micro environmental temperature

DISCUSSION

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 reduced secretion of testosterone by these cockerels and negatively affect their reproductive performance. Testosterone is a reproductive hormone necessary for the development of primary sexual development, testicular descent, spermatogenesis, enlargement of the penis and testes, and increasing libido (Nasar, 2021). A deviation from the normal concentration of this hormone could adversely affect reproduction. The result of this study is in tandem with the findings of Shaugnessy et al. (2008) who treated adult rats with busulfan to remove the germ cell population and ablate leydig cells in the seminiferous tubule, after which he observed a significant depression in the testosterone levels within 24 h of treatment. 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). The result in this study also correspond with 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 7.5 ml/250 ml of water led to increase in both the interstitial spaces and intracellular spaces of the seminiferous tubules compared with birds on T8, who recorded 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 in T2 (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 space in the cockerel's testis indicated an accelerated blood flow

and the level of testosterone hormone, which resulted in improved spermatogenesis in the testicle and concomitant increase in 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), 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 with increased intracellular spaces of the seminiferous tubules. This is similar to what was obtained in control group (T8 (Plate H). This might be due to increased lycopene content beyond the requirement of the cockerel, thereby affecting the cockerel negatively as it was recorded to lead to degeneration of interstitial cells and widening of interstitial spaces. These changes are consistent with testicular degeneration observed 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 15 ml 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 of testis 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 spermatogonia cells and spermatocytes in broiler subjected to cool and controlled environment with fresh cool water.

CONCLUSION AND RECOMMENDATION

Inclusion of lycopene and TCLE at the experimental dosage levels adopted in this study did not induce any significant cellular damage of the testis in the experimental birds. Hence, the dietary treatments especially at 15 ml of lycopene + 15 ml of / 250 ml of water could be regarded as safe for medicinal use. Also, 15 ml of lycopene / 250 ml of water (T3) can be given to the birds as they gave better testicular structure.

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