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Relative organ weights and serum biochemical indices of heat-stressed broilers given *in ovo* injection of Tomato extract at maturity

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Abstract

Two hundred and forty fertile eggs were randomly assigned to five treatment groups consisting of 3 replicates of sixteen birds each in a completely randomized design. *In ovo* injection of tomato extract (ToE) was carried out per treatment as follows: Treatment one (T1) control group (no injection), Treatment two (T2) injected with 0.5mls saline water only, Treatment 3 (T3) injected with 0.125mg lycopene/ 0.5mls of saline water, Treatment 4 (T4) injected with 0.25mg of lycopene/ 0.5mls of saline water and Treatment 5 (T5) injected with 0.25mg of Vitamin C, immediately after candling on the 18th day. After hatching, the birds in each replicate were housed in a separate pens, till the 42nd day. Selected birds were then slaughtered and organs were excised for further evaluation. Result showed that kidney and GIT weights were significantly raised in T1, except for T4 which was also elevated in GIT. The relative lung weight was significantly increased in T3 and decreased across all other treatments. The serum ALP was significantly raised in T1 and decreased in T2 and T4. AST significantly increased at T4 and T5 similar to the control and T2 value was decreased. Significantly highest relative organ weight values of the kidney and GIT were recorded in T2 with statistically similar values with control group for kidney, and T5 for GIT respectively with the significantly lowest value for GIT noted in T3. Inclusion of oral or dietary inclusion of ToE from week 3 of rearing should be recommended.

Key Words: *Broilers, in-ovo injection, Tomato, Oxidative status, Organ weight, heat-stress*

Introduction

Raising poultry birds represents an important segment of food production and enterprise in small- and large-scale animal farming to solve the challenges of food insecurity at a global level (Pius et al., 2021). One of the most important significances of broiler production is the speedy growth rate potential and quick return on investment, which makes it a very lucrative business for vast agribusiness-oriented individuals of all ages. (Kpomasse et al., 2021) Broiler chicken has been genetically selected to maximize production, in order to achieve a high growth rate and a higher percentage of carcass breast muscle to a successful elevation of 85% according to Carney et al. (2022). This has caused an elevated metabolic activity of birds beginning from the embryonic developmental stage, prompting a depletion of their glycogen reserves at the incubation stage (Givisiez et al., 2020) ushering chicken embryonic growth into an interim inhibition and limitation in growth rate. In Nigeria and some parts of the world, compensation for the glycogen losses is not always been addressed during transportation from hatchery to the farm (Akosile et al., 2023), sometimes up to about 72 hours, leading to reduced immunity status and increased mortality especially at week one of brooding. This might be due to the tendency of commercial hatcheries to maximize profit. However, to obtain a high hatchability rate and well-developed chicks. a modest enhancement in energy status at the peri-hatching phase could impose a productive impact in the quality of day-old chicks and their post-hatch performance (Geng et al., 2021; Yalcin et al., 2022). Łukasiewicz *et al*. (2024). An increasing significance has recently been appraised on the application of bioactive substances into the airspace of developing chicken embryos. This technology is known as *ovo* injection (Saeed *et al*., 2019; Abd El-Ghany 2025) This technology acts as a means of introducing nutritional supplement into the eggs before hatch to carter for the lag in time between hatching and commencement of feeding on the farm. Phyto antioxidants especially lycopene sourced from fresh tomato fruits has emerged as a functional device in revamping chicks' quality, productivity and profitability (Khan et al., 2021; Natali et al., 2025). *In ovo* technology involves the conveyance of nanoscopic amounts of bioactive substances directly into the airspace of developing embryos. Despite the immense literature available on the incorporation of lycopene from tomatoes and its products in the diet of chickens, using different methods of administration, there is little information on the use of *in ovo* method of lycopene administration. (Hidayat et al., 2023; Long et al., 2024) The effect of *in ovo* injection of lycopene on organ weight and oxidative biomarker of broiler has not been well documented. Lycopene is implicated as a protective agent against oxidative damage such as those seen in food deterioration and chronic diseases (Wang, 2023; Mousstaaid et al., 2022). However, the role of lycopene as a dietary antioxidant in broiler chickens exposed to stressors is not well known. To justify the long-term impacts of *in ovo* supplementation and its financial advantages for the poultry industry, further research is necessary.

Materials And Methods

Experimental Site

The research was conducted in a reputable hatchery at Oluyole Industrial Estate Ibadan, Oyo State and Kwara State University Teaching and Research Farm Malete, Moro Local Government Area, Kwara State, Nigeria.

Materials

Source and Processing of experimental tomato extract

Fresh tomatoes were obtained from Malete market were washed and cleaned to remove debris. After which the tomato fruits were cut into smaller sizes to ensure easy blend. After blending using a Binatone heavy duty blender, the tomato slurry was then heated at 100ºC to ensure the proper drying for the formation of tomato paste. The paste was sieved using a cheese cloth to drain out water and then left undisturbed to ensure water drain was complete. The tomato paste was stored at 4ºc till use.

Methods

Extraction of lycopene from tomato paste

The method involved the simple application of organic solvent to the samples as adopted by Roldan-Gutierrez *et al.* (2007). 50g of tomato paste was extracted using 500 ml of solvent hexane and acetone in the ratio 75:25 respectively using a DLAB SK-0330-Pro orbital shaker at 250 rpm (revolution per minute) for 12 hours. Extract was then filtered with Whatman No 5 filter paper. Solvent from the extract was separated at 50ºC using a STUART RE300DB water bath and rotary evaporator, leaving behind the tomato extract which was then stored at 4ºC until use.

In ovo injection procedure

Out of the set fertile eggs, 240 were selected and hatched at Oluyole Industrial Hatchery, Ibadan, Oyo State. The *in ovo* injection took place at a reputable hatchery at Broncho, Oluyole Ibadan Oyo state. The 240-fertile eggs used for this research were selected after the 18th day candling and the eggs were grouped into 5 treatments (48 per treatment).

- Treatment 1 control (T1) - 0mls of saline water- (SW) (no injection)
- Treatment 2 (T2) - 30mls of saline water in 0.5mls/egg
- Treatment 3 (T3) - 0.125mg of ToE in 0.5mls of SW/egg
- Treatment 4 (T4) - 0.25mg of ToE in 0.5mls of SW/egg
- Treatment 5 (T5) - 0.25mg of Vitamin C in 0.5mls of SW/egg

The eggs were disinfected with alcohol and cotton wool before the injection commenced to remove any possible contaminant. A tiny puncture was made at the apex of each egg air cell using a 2mls syringe and needle. Immediately after the injection the punctured area was sealed using a sterile sealant and the eggs were returned into the incubator. The chicks were collected on day 21 after hatching and synthetic 5-color identifiers were manually applied on the down feathers of the chick on a one-color per treatment basis, to differentiate each treatment. The chicks were then transported to the Poultry Unit of the Kwara State University Teaching and Research Farm, Malete under a cool temperature to avoid external stress.

Management of Experimental Birds

The brooding pen was cleaned, disinfected and prepared upon arrival of the chicks. Heat was supplied adequately, and vaccination was done weekly. A digital hygrometer was placed in the brooding pen to take record of the daily maximum and minimum relative humidity and temperature.

Feeding of Experimental Animal

The experimental diet given to the bird are as below;

Table 1: Formulated feed ingredients fed to the experimental birds during the experiment.

Ingredients	*Percentage %*
Maize	58.50
Soybean meal	34.50
Lysine	0.20
DL-Methionine	0.24
Toxin binder	0.10
Avatec	0.07
Enzymes	0.05
Salt	0.34
Limestone	0.80
DCP	2.60
Palm oil	2.20
Broiler premix	0.40
Total	100

Data Collection

Blood collection

On day fifty-six of the experiment, 5 ml of blood was collected from the jugular vein of 3 randomly selected birds per replicate in plain bottles. The samples were kept in an air-tight container with crushed ice till they were transported to the laboratory for serum biochemical evaluation. The blood samples were then centrifuged, and serum was separated and stored at -20ºC until assayed for measuring albumin, globulin, total bilirubin, total protein and other relevant parameters.

Organ Weights

On day fifty-six, two chicks whose body weights were similar to the group average were selected from each pen and slaughtered by severing the carotid artery and jugular veins. Carcass weight measurements were done after defeathering. heads, necks, and feet were removed from the birds. The weights of internal organs were expressed as a percentage of live body weight.

Results And Discussion

The phytochemical analysis of the tomato sample used in this experiment is as revealed in Table 1. Observation shows that the tomato powder contains flavonoid, carotenoid, lycopene and Beta carotene. The flavonoid value ranged from 33.33-33.59 mg/100g, total carotenoid is 8.70- 8.77mg/g, lycopene; 555.36-561.60mg/g and beta carotene ; 23.74-23.70 mg/100 g respectively. Table 2 shows the relative organ weights of broilers given *in ovo* injection of lycopene at varying concentrations. The organs evaluated were spleen, liver, kidney, gizzard, gastro Intestinal Tract (GIT), heart, Lungs, and adrenal gland. It was observed that significantly ($p>0.05$) highest relative organ weight values of the kidney and GIT recorded in T2 had statistical ($p>0.05$) similarity with the control group values for kidney, and with T5 for GIT respectively and the significantly ($p>0.05$) lowest value for GIT was recorded in T3. All other organs evaluated were not significantly ($p>0.05$) influenced by the experimental injection.

Table 1: Phytochemical analysis of the tomato sample used in this experiment

Tomato Samples	*Flavonoid (mg/100g)*	*T. Carotenoid mg/g*	*Lycopene mg/g*	*Beta Carotene mg/100g*
1	33.58974	8.768	555.36	23.7432
2	33.33333	8.704	561.6	23.7008
Average	33.46	8.74	548.98	23.72

Table 2 shows an array of relative organs weights evaluated in the experimental animals including the spleen, liver, kidney, gizzard, gastro intestinal tract (GIT), heart, lungs, Adrenal gland. The kidney and GIT weights were significantly ($p>0.05$) raised in T1 (control) with a resultant lowered value across other treatments, except for T4 which had elevated values in GIT. The relative lung weight was significantly ($p>0.05$) increased in T3 and decreased across all other treatments. All other evaluated organs were not significantly ($p>0.05$) influenced by experimental inclusion.

Table 2: Relative Organ Weights of heat-stressed birds given *in ovo* injection of lycopene and Vitamin C

Relative Organ Weight	*Control (T1)*	*0.5mls of Saline Water/egg (T2)*	*0.125mg Lycopene in 0.5mls in saline water per/egg (T3)*	*0.25mg Lycopene in 0.5mls in saline water per/egg (T4)*	*0.25mg Vit C in 0.5mls in saline water per/egg (T5)*	*SEM*
Spleen	0.086	0.108	0.111	0.110	0.116	0.018
Liver	2.361	2.368	2.435	2.219	2.049	0.117
Kidney	0.487^{a}	0.353^{b}	0.249^{c}	0.256^{c}	0.238^{c}	0.0186
Gizzard	4.846	4.389	4.584	5.243	5.425	0.325
Git	14.058^{a}	12.090^{bc}	11.418^{c}	14.012^{a}	13.426^{b}	0.360
Heart	0.452	0.534	0.514	0.451	0.492	0.022
Lungs	0.002^{c}	0.359^{ab}	0.430^{a}	0.118^{bc}	0.0717^{c}	0.170
Ad Gl	0.001	0.001	0.001	0.001	0.001	0

SW; Saline Water, Vit C: Vitamin C: GIT: Gastrointestinal Tract ; T – Treatment ; Ad Gl- Arenal Gland.

Table 3 shows the Serum biochemical analysis response of broilers given *in ovo* injection of lycopene at varying concentrations. Observation shows that there was no significant effect of ToE injection across the treatments for the Albumin (g/dl), alanine phosphatase, ALP Alanine amino transferase (AST) and total protein.

In contrast to this, serum blood urea nitrogen (BUN), ALP, AST, and Potassium were significantly influenced ($p>0.05$). Results showed that BUN was significantly elevated in T3 and T5 with a concomitant depression in T4. The serum ALP was significantly ($p>0.05$) raised in T1 and decreased in T2 and T4 in the present research. AST values were significantly ($p>0.05$) increased at T4 and T5 similar to the control and T2 value also decreased. The potassium value showed significantly ($p>0.05$) low at T1 and T4 but high across other treatments.

Table 3 -Effect of *in ovo* injection of ToE on serum biochemical indices of broiler chickens

Parameters	*Control (no injection) (T1)*	*Sterile water (0.5ml (T2)*	*Lyc (0.125mg/ 0.5ml Sw (T3)*	*Lyc. (0.25mg/ 0.5ml SW) (T4)*	*Vit.C 5mg/0.5mlSw) (T5)*	*SEM*	*S/L*
BUN(mM/l)	15.2127^{ab}	15.354^{ab}	19.270^{a}	14.770^{b}	18.686^{a}	1.73	
Albumin (g/dl)	0.8325	1.1362	0.8836	1.0896	1.1804	0.11	NS
ALP (U/I)	183.54^{a}	136.99^{b}	159.47^{ab}	132.79^{b}	140.91^{ab}	40.58	
AST(U/I)	240.61^{a}	216.11^{b}	231.75^{ab}	241.58^{a}	248.07^{a}	16.74	
Total Protein (mg/dl)	3.2078	3.2977	3.3472	3.5567	3.4960	0.17	NS
ALT(U/I)	119.641	120.859	120.583	119.641	119.865	0.93	NS
TBIL (mg/dl)	2.2392	2.2626	2.2572	2.3455	2.4053	0.03	NS
Potassium(pm)	8.944^{b}	11.361^{a}	11.667^{a}	11.944^{a}	10.278^{ab}	1.61	

Discussion

Various compounds naturally present in dietary plants known as phytonutrients are significant secondary plant metabolites that are beneficial for animal health and production. They range from been antimicrobial, modulate immunity, improved animal overall health, reduce stress and boosted antioxidant enzymatic activities. Tomatoes have been documented to contain different types of carotenoids. Recent studies have suggested that carotenoids such as lycopene averts oxidative damage by 'mopping up' free radicals in livestock as antioxidants thereby protecting cellular components (Black et al., 2020; Collins et al., 2022). Flavonoids contains outstanding antimicrobial and antioxidant properties which secures livestock health and immunity. Bahramibanan et al., 2025) stated that the serum blood urea nitrogen (BUN) concentration in animals is used as a marker to determine kidney integrity, protein metabolism and overall health of animal. Herbal inclusions or injections has been detected to impact livestock's BUN serum levels, often determined by a host of other factors such as herbal specie, dosage and specific innate characteristics of the animal. Supplemental inclusion of herbs can either depress or elevate BUN. Elevated BUN in T3 could result from the potential of ToE which favourably contribute to protein metabolism or protein turnover. This might have resulted into increased nutrient useage and easy digestibility in the treatment group. This finding agrees with the report of Redoy et al. (2020) who observed an increased BUN value after a dietary supplementation of sheep with plantain and/or garlic leaf. Furthermore, the comparable values of BUN noticed in T5 diet groups could be due to the Vitamin C supplementation. Although chickens can produce vitamin C, however, research has shown that during stress, innate vitamin C production may not meet up with the daily demand of the chickens. Therefore, an additional supplementation of vitamin C could enhance protein metabolism as a cofactor in collagen. This is in tandem with the documentation of Semsirmboon et al. (2023) who reiterated this as stated above. The increased ALP observable in T1 could have resulted from the exposure of the chickens to heat stress without any remediation. This agrees with the observation of Tang et al. (2022), who recorded an increase in the serum concentration of liver enzymes injury biomarkers in broilers after their exposure to heat stress for 7 and 14 days respectively. As supported through the study conducted by Hu et al. (2020), heat stress causes programmed cell death or apoptosis, in any organ present in livestock, including the liver. Studies have shown that acute heat stress can increase the number of liver cells undergoing apoptosis. The decreased ALP value in T2 and T4, is also similarly observed in the decreased value of AST in the present research. This could have been an expression of the efficacy of in ovo injected tomato extract, which averted apoptosis and liver injury therefore the ALP was reduced. However, the reason for the elevated AST values is not very clear as at the time of writing this manuscript. Although in certain studies, conducted by Yang et al., (2023), tomato extract showed hepatoprotective ability. Although certain studies have reflected the AST-lowering capacity of tomato extract, while a few has shown its AST-increasing tendencies, when combined with other substances like isoniazid (INH). The potassium value was significantly low at T1 and T4, but high across other treatments. The elevated potassium levels observed in all treatment except T1 and T4 could have been because of tomato fruit's notable viable source of potassium, which has been further concentrated through the processing of tomato fruit into paste in this study, which must have naturally boosted the serum potassium levels. Lu et al. (2022) authenticated the result stated in this study after reviewing the potassium concentration in processed tomato fruits with a significant amount of potassium up to 1220 mg per cup measurement. Organ weight is rated important in broiler chickens because it mirrors their immune, growth rate, and nutritional status. It is germane in determining the effect of breed, diet and environment on the animal's overall performance and productivity. Elevated GIT in T4 could be due to the presence of the various phytonutrients introduced into the eggs during the in ovo injection of ToE. This could have improved the weight of the organs that made up GIT organs like thymus, duodenum, jejunum, liver, leading to a concomitant rise in GIT weight. This also include the elevated lungs. In relation to the report of Wu et al. (2024), inclusion of tomatoes has been shown to increase the weight of different organs of the GIT in livestock. The lowered values of the GIT noted in T2, T3 and T5 could have been due to non- injection of ToE into T2 only 0.5ml saline water, the low quantity of ToE T3 (Lyn 0.125 mg/0.5 ml of saline water (SW) and T5 0.125 mg Vitamin C/0.5 ml SW. After in ovo injection of ToE, Inclusion of oral or dietary ToE from week 3 of rearing would be recommended.

Declaration

The authors of this manuscript hereby declare that this manuscript have not be published elsewhere

Ethical Approval Certificate

The experimental procedures of this study were approved by the Institutional Research Ethics Committee in charge of management and use of research animals in Kwara State University, Malete, Kwara State, in accordance with the NIN guide for the management of laboratory animals with ethical approval number KWASU/CR&D/REA/2024/00101.

Author Contribution Statement

Olayinka Ojo -Investigation, Conceptualization, Project administration, supervision, conceptualization, methodology, and writing the original draft

Adenike Omolara Awoyinka- Data collection,Methodology

Moyinoluwa Orewale Onikeku- methodology, review and editing

Ademola Johnson Ajiboye Project administration, conceptualization, methodology, review

1Oluwatoyin Christianah Ogunmokun--Project administration, methodology, review and editing

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Conflict of Interest

The authors declare no conflict of interest

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