



Research Article

Effects of Tomato Juice Supplementation on the Reproductive Performance, Hematological Indices and Oxidative Status of Gestational Sows in Ilorin, Nigeria.

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Abstract

A ten-week trial was conducted to evaluate the efficacy of orally administered tomato juice (ToJ) on the hematological and reproductive performance of gestational sows raised at Omolara Farms, Ogbagba town, Kwara State. A Sixteen (n=16) gestational sows within 4th -6th weeks of gestation were randomly allotted into four treatment groups, having four replicates, with one sow representing a replicate Both Treatment 1 and 2 received no ToJ, but each received only 60 mls of clean water, plus 1g of Vitamin C in treatment 2., Treatment 3 and Treatment 4 received ToJ at 60mls and 120 mls respectively. At 10th week of experiment, blood samples were collected via marginal ear vein; hematological parameters evaluated include packed cell volume (PCV), red blood cell (RBC), hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), white blood cell (WBC) and platelet. Reproductive data monitored include; number of piglets per sow (NPPS), mortality rate, number of stillbirth piglets (NSBP), number of surviving piglets (NSP), number of piglets at birth (NPB). Result revealed that hemoglobin and WBC values were significantly depressed, when compared with the negative and positive controls (T1 and T2), while being significantly elevated in pregnant sows administered with 60mls and 120mls ToJ. NPPS, NPB and mortality rate were significantly elevated and an abysmal increase in NSBP at 60 mls ToJ, while the reduced values of these parameters were recorded at 120 mls ToJ, as compared to the values observed in sows given Vitamin C. Serum antioxidant enzymes were elevated at 120 ToJ. Therefore, its recommended that ToJ inclusion could enhance performance of pregnant sows up to 120 mls under tropical condition

Keywords: Gestational sows, heat stress, hematology, piglets, reproductive parameters

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Introduction

Heat stress is an eminent threat to the successful management of pigs for a profitable enterprise, especially in the tropical environment (Zhao and Kim, 2020). Heat stress strongly influences various cellular biochemical activities, particularly those that are specific to nutrient digestion and absorption.(Wei *et al.*, 2022), Incidence of climatic change has made sows particularly more susceptible to metabolic heat production (Mutua *et al.*, 2020). Elevated ambient temperature usually enhances oxidative

stress by adversely impacting the formulation of antioxidant enzymes (Huchzermeyer, 2022) as well as depressed levels of some reproductive hormones. Oxidative stress (OS) is a disproportion condition when reactive oxygen species (ROS) production surpasses cellular antioxidant capacity. OS occurs when the balance between the production of ROS and antioxidant defence is disturbed, resulting into oxidative stress (Shakeri and Le, 2022). Pregnancy has been noted to increase oxidative stress generation

by a regular systemic erythrocytic feedback, which pulsated into high amounts of promulgated reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$) and hydrogen peroxide (H_2O_2). As a result of this, productivity problems, resulting into increased rectal temperature, respiration rate and skin temperature has been on the rise, which may adversely influence parturition (Brandt *et al.*, 2021). Previous studies have shown that during pregnancy, sows suffers immensely from a variety of physiological stress conditions, resulting into depressed reproductive performance (Deng *et al.*, 2021) and under severe catabolic condition, especially during late gestation and lactation with an elevated oxidative stress (Parraguez *et al.*, 2021). This negatively impairs embryonic development; reduce feed intake and milk production in sows, in addition to delayed puberty of gilts. It usually result in summer infertility and reduced productivity (Kim *et al.*, 2022). According to Farmer and Edwards (2021) elevated metabolic heat in sows has shorter gestation period, reduced litter size and birth weight repercussions. These adverse effects of heat stress can be highly mitigated by supplementing dietary or oral antioxidants, as these substances positively influence the intestinal integrity and biochemical functionalities through the improvement of intestinal morphology and histology. Excessive reactive oxygen and radicals were actually produced from placenta and material metabolism during the early pregnancy of sows. Although oxidative stress in late gestation is more serious than in early gestation with the course of pregnancy (Prins *et al.*, 2022). Recent interest has been drawn to the use of herbal supplements in livestock production going by the reports showing their efficacy in handling some of these challenges. Of course they are natural, readily available, less toxic and acceptable as animal feed additives. It has been certified to be rich in substance containing reductive activity, such as terpenes, phenols, glycosides, saccharides, aldehydes, esters and alcohols (Reyes *et al.*, 2020). Herbal plants have also been reported to enhance fetal development by elevating fetal blood glucose and serum growth hormone levels. Phytochemical component of

certain herbal plants have been revealed to exert their positive antioxidant benefits towards animals, resulting in favourable performance, improvement of product quality and enhancement of internal antioxidant enzymatic activities. All these could perhaps have been possible through direct effect on specific molecular targets or indirectly by stabilizing conjugates affecting metabolic pathway. Tomatoes contain a matrix of many bioactive components including Vitamin C, Vitamin E, other carotenoids (a beta, gamma carotene, and lutein) and flavonoids. According to Bin-Jumah *et al.* (2022), the interdependent interaction of the bioactive component in tomatoes, when used in combination, might be responsible for the beneficial effects of tomato-based products.

Tomato juice has been stated to enhance antioxidant enzymes activities and upgrade the immune status (Collins *et al.*, 2022). In line with all the reported potentials of tomato and its products, little has been documented on the effect of ToJ on the gestational changes in sow. Therefore; there is the need to evaluate efficiency of tomato juice (ToJ) on the reproductive performance, oxidative status and hematological parameters of gestational sows managed under tropical environment.

Materials and Methods

Study Area

The experiment was carried out at Omolara Farms, Ogbagba town, Shao/Malete Road, Kwara State (Coordinate: 8.6003° N, 4.5521° E)

Source and Preparation of the Experimental Material

Fresh tomato fruits were obtained from Malete Market, Malete Kwara State. The ripe tomato fruits were washed to remove all adhering dirt to avoid contamination of experimental material. On-farm crushing of the fresh and cleaned tomato fruits was done daily using a rechargeable blender. This was sieved twice, using a double-layered cheese cloth to obtain a homogenous tomato juice (ToJ). The quantity of extract was determined using a measuring cylinder.

Experimental design

Sixteen (16) pregnant sows were randomly allotted into four treatments, four replicates/

treatment, with one sow allocated per replicate. The ToJ oral administration was done as follows;

- Treatment 1 - served as control received 60mls clean water only
- Treatment 2 - 1g of vitamin C /60mls of water
- Treatment 3 - 60ml of ToJ
- Treatment 4 - 120ml of ToJ

Blood Collection

At the end of the 35 days feeding trial, blood samples (5mls) was collected from the marginal ear vein at the coolest part of the day, by swapping with a cotton wool soak in xylene to make the vein prominent and 23G needle and syringe was used to collect the blood in which it was stored in plain bottles containing no anticoagulant and was taken to the laboratory. The blood was allowed to clot and was centrifuged at 3,000 rpm for 15 minutes to separate the serum from the plasma (Jimoh, 2019). The clear serum was collected and stored in another plain bottle until use for subsequent biochemical analyses.

Data collection

The reproductive performance data evaluated are as follows; No of litters/ sow, Weekly Piglet weight, Piglet mortality.

The no of litters / sow = Total litters farrowed in a time period ÷ average number of sows over the same time period

Weekly Piglet weight = Weight / piglet / week

Piglet mortality+ No of dead piglet / sow

Hematology Parameters:

Packed cell volume (PCV) = Height of red blood cell column x 100% divided by total height of blood column (%)

Red blood cell (RBC) = Number of cells per Microliter of blood (number of RBCs $\times 10^6/\mu\text{L}$)

Hemoglobin (HGB) = Hematocrit / 3 (g/dl)

Mean corpuscular volume (MCV) = MCV in fl = (Hct [in L/L]/RBC [in $\times 10^{12}/\text{L}$]) $\times 1000$ (fL)

Mean corpuscular Hemoglobin (MCH) = hemoglobin ÷ hematocrit (g / dL)

White blood cell (WBC) = Number of Leucocytes per Microliter of blood (number of WBC $\times 10^6/\mu\text{L}$)

Platelet (PLT) = Average the platelet counts obtained Multiply by $15 \times 10^9/\text{L}$

Serum Biomarkers; The blood samples were

allowed to clot and centrifuged for 15 min at 3,000 rpm and sera were stored at -20°C until subsequent analysis. Malondialdehyde (MDA), Catalase, Glutathione peroxidase (GPx); Superoxide dismutase (SOD) and Glutathione reductase (GR) were analysed, using Randox kit for SOD, CAT, GST and TA, the instructions on the kits were followed to letter. The serum samples were analyzed to determine the superoxide dismutase (SOD) activity and reduced glutathione (GST) and Total antioxidant capacity (TAC) levels spectrophotometrically using commercial kits (Alagawany et al., 2018).

Statistical Analysis

The data collected were subjected to statistical analysis of variance (ANOVA) using Gen-stat software.

Significant differences between means was separated using Duncan multiple range test (DMRT)

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Results and Discussion

Table 1 shows the hematological indices/ parameters of gestational sows given ToJ at varying concentrations. Among all parameters evaluated, only the hemoglobin, platelet and white blood cells were significantly influenced by the experimental inclusions. Observation shows that the hemoglobin and white blood cell values of the pregnant sows administered with 60mls (8.72 Mmol/L and $18.0 \times 10^3/\mu\text{L}$) and 120mls (8.67 Mmol/ and $17.91 \times 10^3/\mu\text{L}$) of ToJ respectively were significantly depressed in comparison to the controls. However, the values recorded for the platelet were significantly improved at 60mls ($265.5 \times 10^9/\text{L}$) and 120mls ($357.0 \times 10^9/\text{L}$) of ToJ administration.

Table 2 shows the oxidative biomarkers of gestational sows given varying concentration of ToJ (60 and 120 mls) and Vitamin C. The catalase and malondialdehyde values did not differ significantly across the treatments, while the superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase were significantly influenced. The gestational sows administered with T3 (60 mls ToJ treatments had significantly lower SOD, GR and GPx activities, while significantly elevated highest values of the

3 enzymes were observed under T4 compared to the control.

Figure 1 shows the post-partum/piglet performance of gestational sows given ToJ. Observation shows that the number of piglets at birth was elevated in pigs under T3, administered with 120 mls ToJ, while the lowest value was recorded in pigs in T2, given 1g of vitamin C /60mls of water. However, number of still birth was elevated as the control with no administration, while pigs under T3 and T4, which received 60 and 120mls ToJ experienced no stillbirth piglets. The number of surviving piglets were similar across all treatments. The highest piglet mortality was recorded in T3 piglets (60 mls ToJ).

Reproductive efficiency is an important aspect of the swine industry (Edmunds *et al.*, 2022). Heat stress (HS) results into poor growth rate, feed intake and feed efficiency (Teixeira, 2021). Bjerg *et al.* (2020) categorized temperature exposure of sows into groups based with maximum as 27°C , greater $\geq 27^{\circ}\text{C}$ or lower than 27°C $< 27^{\circ}\text{C}$ in the week before weaning or the week around service. This recent review reported that there was no significant effect of ambient temperature on feed intake, milk yield, weight loss of sows, and litter weight gain when sows were exposed to a temperature $< 27^{\circ}\text{C}$. There is evidence that elevated environmental temperature influences an avalanche of neuro- and hormonal effect on affected animals Zahangir *et al.* (2020). Heat stress (HS) during gestation affects fetal development with innuendo for impaired muscle growth. Zhao (2022) have formerly showed that maternal HS during early to mid-gestation conciliated muscle fibre hyperplasia in developing fetal pigs. However, to our awareness, this is the first study to be documented on determining the effects of tomato juice supplementation on the post-partum performance and hematological indices of gestational sows in the tropics. Tomato is an efficient source of bioactive antioxidant components used in traditional medicine. Tomato and tomato products have been known to contain some phytochemicals that may have health benefits with epidemiological reports showing a significant association between high intakes of

tomatoes or tomato-based products (Kucuk, 2002). Recent studies have suggested a protective role for lycopene, an antioxidant carotenoid, in the prevention of stress including environmental stress, with tomatoes and tomato products acting as its major dietary source. Sahin *et al.* (2008). As a result of this, there is an elaborated interest in the antioxidant components in tomatoes, which contains lycopene, ascorbic acid, phenolics and flavonoids (Lima *et al.*, 2022). Other carotenoids, include phytoene, phytofluene, and provitamin A carotenoid β carotene (Antonio *et al.*, 2022), which may have a synergistic effect with lycopene. In the present study, elevated number of still-births, in the control with no supplemental administration showed an obvious influence of environmental heats stress which adversely negates the survivability of the new born as oxidative stress, peculiar to pregnancy cannot be ruled out. The findings recorded in this study aligns with the report of Liu *et al.* (2022), who documented that serious impacts of gestational heat stress adversely affected foetal development, resulting into reduced farrowing rate of sows, inferior growth rate and reduced survival rate. The zero stillbirth experienced in experimental pigs under T3 and T4, which received 60 and 120 mls ToJ is a reflection of the ability of lycopene to extinguish singlet oxygen and other oxygenated species, to achieve cellular protection against oxidative damage. Perhaps, lycopene intercepted the oxidative species before damage occurred, thereby preventing still birth (Edge and Truscott 2018). After the inclusion of tomato extract at 4g per kg body weight, Tesby (2020) observed that lycopene component in tomatoes alleviated oxidative stress in pregnant animals, which corroborates the positive influence of the number of piglets at birth in this experiment, as elevated in pigs administered with 120 mls ToJ under T3. The lowest number of piglets at birth recorded in pigs given 1g of vitamin C could have meant that there is an existing synergy between the various components, which enhanced the mechanism of action of phytogetic antioxidants above the synthetic antioxidants sources commonly used during stress.

The depressed value of platelet, packed cell volume (PCV), hemoglobin, and white blood cell in pregnant sows observed in those administered

with 60mls and 120mls of tomato extracts could be as a result of the low bio-availability of lycopene present in fresh tomato juice as compared to other processed tomato products such as pomace, ketchup and tomato powder. The depressed hemoglobin and WBC values of the pregnant sows, administered with 60mls of ToJ observed in this study is in tandem with the report of Tesby (2020), who observed a significantly lower mean values for HB, RBC and MCHC respectively, when he administered tomato juice to rats. However, in contrast to this, he noted a significantly elevated white blood cell value compared to the control. Lycopene, found in tomato and tomato powder is known to have an effective free radical scavenging activity and this action could be beneficial to livestock. (Fachinello *et al.*, 2020). The significantly improved platelet at 60mls and 120mls of ToJ administration is an indication of quick blood clotting, which will act to prevent loss of blood in case of injuries. Also in line with the submission of Videla *et al.*(2020), tomato juice supplementation had no significant effects on the biological response values evaluated under thermo-neutral conditions in growing Japanese quails. Parallel to the results of the present study, Jain *et al.* (1999) reported that live weight and feed intake were not affected by lycopene supplementation in rats under thermo-neutral conditions. Dietary supplementation with tomato products increased serum lycopene levels and reduced endogenous levels of oxidation of lipids, proteins, lipoproteins and DNA (Antonio *et al.*, 2022). This is in line with the report of Tesby (2020), who stated that lycopene bioavailability in tomato paste is higher in fresh juice. Inclusion of ToJ positively improved some post-partum performance of heat-stressed gestational sows in comparison to synthetic antioxidants, influenced the physiological characteristics of animals, and in turn improved reproductive performance of sows in the tropics (Mizael *et al.*, 2020).

Oxidants have been noted to carry out a bi-role as either a toxic substance, which are harmful or helpful to the body in form of a beneficial compound. In case an over production of oxidants occurs, free radicals or reactive oxygen species results, cumulative production of this causes oxidative stress. In the present study, the

exposure of the gestational sows to temperatures had significant effects on the activities of enzymatic antioxidants. The significantly ($P<0.05$) depressed level of SOD, GR and GSH-Px noticed in T3 gestational sows, could have resulted from the increased concentrations of ROS/RNS, that surpassed the scavenging abilities of the enzymatic and non-enzymatic antioxidants in the sows, which could not be enhanced at 60 mls ToJ supplementation. However the elevated serum enzymatic antioxidants supplementation of 120 mls ToJ (T4) that showed the elevated serum antioxidant level is a reflection of the antioxidant-enhancing capabilities, which was also revealed in the post-partum performance of the gestational sows in Figure 1. Li *et al.* (2021) reported a similar result of improved antioxidant capacity and reproductive performance after supplementing the feed of pregnant sows with catechin (200 mg/kg) under normal physiological states. The findings in this study corroborated the outcome of investigation by Choe *et al.* (2020), who reported an improvement in the level of antioxidant enzymes (GST, GSH, and SOD) of alcohol-induced liver damage in rats, after administering herbal extracts. Redoy *et al.* (2020) also recorded a positive result, similar to the findings of this research, after using plantain (*Plantago lanceolata* L.) and/or garlic leaf (*Allium sativum*) as dietary supplements to enhance growth and serum antioxidants in sheep. In line with the findings of Li *et al.* (2022), enzymatic enzymes such as SOD, CAT, GSH-Px, interacts among themselves to effectively neutralize the detrimental effect of ROS, where SOD catalyzes the removal of O_2 , via the mechanism of disintegration into O_2 and H_2O_2 being reduced by CAT. A higher serum concentration of the serum enzymes, the better the ROS scavenging ability, which prevents damage to the tissues to achieve post-partum reproductive performance of animals and a concomitant improvement in the piglet growth and litter size .

Therefore, it can be concluded that oral inclusion of ToJ for gestational sows up to 120 mls can enhance reproductive. However, further studies can be conducted using higher concentration of tomato juice, to evaluate its efficacy on

reproductive performance and hematology of gestational sows as well as in other animal species.

Ethical Statement

Consent for the present study was obtained with due institutional guidelines for humane animal treatment and complied with relevant legislation from the ethical Review Board of the Centre for Research and Innovation, Kwara State University, Malete, Nigeria

Conclusion and Recommendations

Therefore, it can be concluded that oral inclusion of ToJ as phytogetic supplements in gestational sows up to 120 mls can enhance reproductive. However, further studies can be conducted using higher concentration of tomato juice, to evaluate its efficacy on reproductive performance and hematology of gestational sows as well as other animal species.

Declaration of competing interest.

The authors have no relevant financial or non-financial interests to disclose

Author's Contributions.

- i. Ojo O.A, designed, monitored the data collection and wrote the manuscript
 - ii. Awoniyi D. Collected the data and managed the experimental animals throughout the experimental period. .
- This work was carried out in collaboration with the authors.

All authors have approved the final article is true and included in the disclosure

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Table 1: Hematological Parameters of Gestational Sows given ToJ at varying concentrations

Parameters	T1 Control)	(T2 C)	(Vit. ToJ)	T3(60 mls ToJ)	T4(120 mls ToJ)	LSD (P<0.05)	CV (%)	SEM
HGB(Mmol/L))	10.55 ^{ab}	14.55 ^a	8.72 ^b	8.67 ^b	5.01	17.00	1.80	
PLT (X10^9/L)	210.0 ^{cd}	166.0 ^d	265.5 ^b	357.0 ^a	46.72	6.70	16.83	
PCV(%)	43.5	35.8	37.7	36.4	8.76	8.20	3.15	
RBC(10 ¹² /μL)	6.95	9.11	9.57	10.62	4.01	15.90	1.45	
WBC (10^3/μL)	25.66 ^a	22.32 ^a	18.0 ^b	17.91 ^b	3.89	6.70	1.40	
MCH(pg)	18.77	17.32	18.58	17.32	5.96	11.90	2.15	
MCV(fl)	72.8	70.8	73.4	75.1	7.85	3.90	2.83	

HGB-,Hemoglobin PLT- Platelets, PCV Packed cell volume, RBC-Red blood cell, WBC-White blood cell, MCH- Mean corpuscular Hemoglobin, MCV- Mean Corpuscular Volume

Table 2: Oxidative biomarkers of gestational sows given ToJ and vitamin c at varying concentrations

PARAMETERS	T1 Control)	(T2 (Vitamin C)	T3 (60 mls ToE)	T4 (120 mls ToE)	LSD (P<0.05)	CV (%)	SEM
Catalase (nmol/ min/mL)	4.02	4.82	7.42	8.51	63.00	134.30	22.69
Superoxide dismutase (U/mL)	63.40 ^b	78.02 ^{ab}	68.92 ^b	97.02 ^a	163.2	5.88	7.70
Glutathione reductase (mU/mL)	9.82 ^{ab}	12.22 ^{ab}	11.60 ^{ab}	16.09 ^a	4.21 ^b	12.20	1.52
Glutathione peroxidase (GSH-Px) (U/ mL)	366.00 ^{ab}	393.2 ^{ab}	381.2 ^{ab}	463.2 ^a	117.2	10.50	42.2
Malondialdehyde (nmol/mL)	11.82	13.66	12.98	13.75	4.27	11.80	1.54

Means within the column with different superscript ^{abcd} are significantly different (p < 0.05)

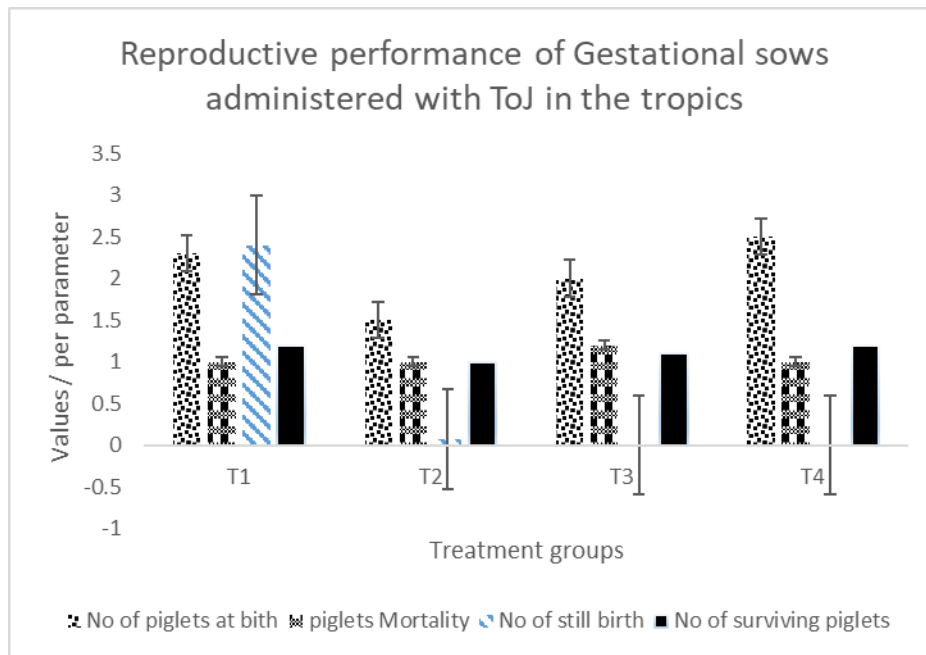


Figure 1: Effect of ToJ on the Number of piglets, piglets mortality, Number of still birth, and Number of surviving piglets. T1= Control, T2 = Vitamin C, T3 = 60ml ToJ, T4 = 120ml ToJ