

Effects of Vitamin C Supplementation on Growth Performance and Haematology of Heat-stressed Finisher Broiler Chickens

Robinson C. Unigwe^{1*}, Chigozie S. Ukwueze², and Joseph O. Uguru³

¹Department of Veterinary Biochemistry and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

²Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

³Department of Animal Science, Ebonyi State University, Abakaliki, Nigeria

*Corresponding author: robinsonunigwe@gmail.com Tel: +2348037707965

Abstract

Heat-stress is one of the major factors militating against poultry production particularly in the tropics, such as Nigeria. Supplementation of vitamin C, which is an antioxidant, has been used to reduce effects of heat-stress in poultry. Therefore, a four-week experiment was designed to assess the effects of vitamin C on heat-stressed finisher broiler chickens at Ibadan, Oyo State, Nigeria. Seventy two 4-week old unsexed Arbor Acre finisher broilers were used during January and February. They were randomly allotted to three groups replicated thrice including T₁ (control), T₂ (heat) and T₃ (heat + 100 mg/kg vitamin C) in a completely randomized design. Each replicate had eight birds. They were acclimatized for one week and given Finisher Top Feed[®] and clean water *ad-libitum* during the experiment. T₂ and T₃ groups were subjected to heat-stress by covering 3 out of 4 sides of rectangular pens and the heat sustained by two 100 watts electric bulbs per pen during the night. Daily feed intake and weekly weight gain were recorded throughout the experiment. On the 28th day, 3 ml of blood was aspirated from any 3 birds per replicate for haematological analysis. Data were analyzed and Duncan's test was used to separate significant means. Results showed no significant effect ($P>0.05$) on daily weight gain and feed conversion ratio but a decrease in feed intake by T₂. Mortality was significantly high ($P<0.05$) in T₂ compared to T₁ and zero in T₃. Haematological parameters showed no significant difference ($P>0.05$) but for white blood cells where T₃ differed ($P<0.05$), heterophils and heterophil/lymphocyte (H/L) ratio where T₂ significantly ($P<0.05$) differed, and basophils with significantly ($P<0.05$) higher T₂ and T₃. In conclusion, 100 mg/kg vitamin C had no effect on growth but improved feed intake, and reduced mortality. It enhanced leucocyte count, and reduced heterophils and H/L ratio that were elevated by heat-stress.

Keywords: Growth performance, Haematology, Heat-stress, Vitamin C

1.0 Introduction

Poultry provides organic manure for crop farming and as well a source of income and employment to millions of farmers and other people that are in its value chain industry (Jain, 2018). Unlike most monogastric animals, poultry synthesizes vitamin C for normal development and growth (Ghazi et al., 2015) but usually not sufficient for its biological demands during heat stress. Unfortunately, addition of vitamin C in the diet of poultry is often considered unnecessary because it is endogenously synthesized in the kidney and liver (Gan et al., 2018) which invariably poses a great danger to the birds in heat stress condition. In this circumstance, ascorbic acid supplementation of the diets becomes beneficial (Brake, 1987) since it enhances the antioxidant status of tissues (Gouda et al., 2020).

Poultry species are particularly sensitive to heat stress. It has been reported that modern poultry genotypes produce more endogenous body heat due to elevated metabolic activities (Sahin et al., 2003a). Excess environmental heat, coupled with increased metabolic process, is one of the major factors that negatively influence livestock production in the tropics (Sinha et al., 2017) such as Nigeria. In the event of heat stress, animals maintain homeostasis by vasodilatation and increased perspiration (Mutaf et al., 2009). Broilers as homeotherms, need the ambient temperature within the thermo-neutral zone of 18 to 24 °C (Cahaner et al., 2008), which seems difficult to attain in the tropics, such that if reared above this condition, it becomes vulnerable to heat stress (Chand et al., 2014; Yosi et al., 2016). Heat stress and high humidity adversely affect production performance as well as inhibits immune function (Chand et al., 2017). Polycarp et al. (2016) reported that oxidative stress caused by hot humid tropical condition can bring about haematological derangements in animals.

Under heat stress condition, broilers show disturbance in the acid-base balance by panting which leads to respiratory alkalosis (Syafwan et al., 2011). In broiler production, some feed additives have shown improvement in growth, feed utilization, immunity and antioxidant effect (Abudabos et al., 2016; Khan et al., 2016). Various techniques have been used to reduce heat stress in poultry such as electric fan, cooling pad system and spraying of water using fogger (Chand et al., 2016; Ullah Khan et al., 2014). High cost associated with their use has called for cheaper alternative such as nutritional therapy like vitamin C supplementation, sodium bicarbonate, and acetylsalicylic acid in drinking water. Vitamin C is a natural antioxidant that can be used to reduce the oxidative stress imposed by heat stress (Desoky & Kamel, 2018). Abidin and Khatoon (2013) reported that ascorbic acid ameliorates heat stress-associated immuno-suppression, reduced feed intake and body weight gain, oxidative stress, rise in rectal temperature and mortality in birds. In broiler, it has been shown that decreased growth and feed intake, and increased feed conversion ratio occur under heat stress (De Souza et al., 2016). Therefore, the growth performance and haematological effects of supplementing vitamin C in drinking water of finisher broilers exposed to heat-stress was conceived and experimented upon to assess if it could alleviate the negative effects of heat-stress on the parameters assessed.

2.0 Materials and Methods

2.1 Site of the study and ethical consideration

The study took place at Bora project site of the Federal College of Animal Health and Production Technology (FCAH&PT), Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is on longitude 3° 5' to 4° 36' E and latitude 7° 23' to 7° 55' N (Oladele & Oladimeji, 2011). Ibadan has mean total rainfall of 9,233.60 mm, relative humidity of 74.55% as well as mean maximum and minimum temperatures of 39.82 and 22.5 °C respectively (Egbinola &

Amobichukwu, 2013). Ethical conditions governing experimentation on life animals were strictly observed according to Ward and Elsea (1997). The ethical committee OF FCAH&PT approved the experiment.

2.2 Animals, source of heat, test ingredient, feed, design and duration

Seventy-two (72) 4-week old, unsexed finisher broilers of Arbor Acre breed were used during hot season of January and February for the experiment. The heat stress was sustained by covering (with thick black polythene nylon) three sides of a rectangular pen (2 x 1.5 m²) during the day whereas during the night hours, 100 watts electric bulbs (2 bulbs/pen) powered by standby generator were used to sustain the heat stress. Vitamin C (100 mg/tablet) was sourced from Danax Pharmacy shop, Mokola, Ibadan, Oyo State. The birds were allotted to three treatments and were replicated thrice (8 birds/replicate) in a completely randomized design as follows.

T₁= No heat from electric bulb (control)

T₂= Heat from electric bulb

T₃= Heat from electric bulb + 100 mg/kg body weight of vitamin C.

The T₃ group received 100 mg/kg of vitamin C (synthetic white VC100 by Foodchem® China) daily by dissolving a calculated quantity (100 mg/kg) in 80 ml of drinking water based on the weight of the total number of birds in a replicate and served it in a drinker 20 minutes after feed was served throughout the 28-day study. Drinking water was served *ad-libitum* after they had finished drinking the dissolved vitamin C. Throughout the study, the birds were subjected to thermal stress of over 35 °C (Chand et al., 2016). This was measured by the reading of hung digital thermometers in each of the experimental pens (Yosi et al., 2016). The duration of the experiment was four weeks (i.e. terminated at the age of 9 weeks old).

2.3 Management of the birds

The litter materials were scooped out, the cobwebs removed and the floor swept. Detergent was mixed in water and used to wash the floor and wall. Lysol® (Benzalkonium chloride) of which 4 ml was added in 2 liters of water as recommended by the manufacturer. After cleaning and disinfecting, the pens were left without stocking for 7 days to enable minimal residues of the disinfectant. On birds' arrival, they were served a mixture of water, glucose and vigorlite® (multivitamins) in order to suppress stress of transportation. The birds were put together in a pen where they received finisher Top Feed® (Crude Protein = 18% and Metabolizable energy = 2900 kcal/kg) *ad-libitum*. Clean water was served *ad-libitum* throughout the acclimatization and study period. After the acclimatization the birds were randomly allocated to treatments and replicates (n = 8). The sides of the pens that housed T₁ (control) were left opened while three sides out of four of the pens that housed T₂ and T₃ were covered with thick and black polythene to conserve excess heat in the pens. Two electric bulbs of 100 watts/pen were hung in the T₂ and T₃ pens to produce heat during the nights.

2.4 Data collection

Blood: At the end of the experiment, a bird/replicate was randomly selected, and 3 ml of blood aspirated aseptically from the wing web using needle and syringe into EDTA sample bottle for hematological analysis at the Laboratory of Veterinary Medicine, University of Ibadan, Nigeria. Heterophil: lymphocyte (H:L) ratio was determined based on standard protocol (Cengiz et al., 2015; Lentfer et al., 2015). The heterophils: lymphocytes (H/L) ratio was

determined in whole blood using Hema-3 stain (cat# 22-122911, Fisher scientific, USA), according to Zhang et al. (2009).

Body weight: Weekly weight gain was obtained by weigh-back mechanism.

Feed intake: Feed intake was calculated by subtracting the left-over feed from the feed offered.

Feed conversion ratio (FCR): FCR was got by dividing feed intake by body weight gain.

2.5 Statistical analysis

Data were statistically analysed by one-way analysis of variance using the general linear model (GLM) procedure of SAS Institute Inc (2009). Duncan's test was used to separate statistical means at $p \leq 0.05$ (Duncan, 1955).

3.0 Results

3.1 Result of growth performance for heat stressed finisher broiler chickens administered with vitamin C supplement

Table 3.1 shows the results on growth performance of heat-stressed finisher broiler chickens on vitamin C supplemented water. There were no significant differences among the groups regarding average initial weight (AIW), average weight gain (AWG) and feed conversion ratio (FCR) though numerical increase occurred in T₃ with respect to AWG probably due to vitamin C supplementation since it enhances feed intake. Though there was no significant difference ($P < 0.05$), the T₃ had the best conversion of feed to meat which might be because of some anabolic factors in the vitamin C. However, there existed increased average daily feed intake (ADFI) in heat stressed and vitamin C supplemented group (T₃) which was similar ($P < 0.05$) to T₁ but differed statistically ($P < 0.05$) from T₂ that was heat-stressed. The T₂ had significantly ($P < 0.05$) higher mortality compared to T₁ whereas zero mortality was recorded against T₃ which could be associated with positive effect of vitamin C that probably suppressed the negative effect of heat stress and elevated the immunity of the birds.

Table 3.1: Growth performance (\pm SEM) of heat-stressed broiler chickens

Parameter	T ₁ (control)	T ₂ (heat only)	T ₃ (heat + vit. C)	SEM
AIW (g)	915.40 \pm 1.04	917.85 \pm 1.10	914.15 \pm 1.06	2.65
AFW (g)	2680.00 \pm 2.23	2622.20 \pm 1.76	2686.00 \pm 2.06	63.91
AWG (g)	1764.60 \pm 1.68	1704.35 \pm 1.54	1771.85 \pm 2.32	53.86
ADG (g)	63.02 \pm 1.47	60.87 \pm 1.51	63.28 \pm 1.93	3.64
ATFI (g)	6495.20 \pm 3.78 ^a	6256.40 \pm 2.36 ^b	6458.20 \pm 1.98 ^a	80.04
ADFI (g)	231.97 \pm 2.87 ^a	223.44 \pm 2.45 ^b	230.65 \pm 2.04 ^a	2.86
FCR	3.68	3.67	3.64	0.70
Mortality (%)	0.50 ^b	1.50 ^a	0.00 ^c	4.49

^{abc}: Superscripts with different letters along the same row are significantly different ($p < 0.05$). Note: AIW = Average initial weight, AFW = Average final weight, ATWG = Average total weight gain, ADG = Average daily gain, ATFI = Average total feed intake, ADFI = Average daily feed intake, FCR = Feed conversion ratio

3.2 Haematological parameters of heat stressed broiler chickens and vitamin C supplementation

Table 3.2 shows the values of haematological parameters of heat-stressed finisher broiler chickens on vitamin C supplementation. There was no significant difference ($P>0.05$) among the treatment groups (T_1 , T_2 and T_3) with respect to major haematological parameters (PCV, Hb, and RBC). Meanwhile, T_2 had numerically highest PCV, Hb and RBC possibly due to dehydration occasioned by heat stress since increased loss of water occurs due to excessive heat resulting to concentration or thickening of blood which invariably gives a false increase in blood parameters. The WBC of T_3 was significantly higher ($P<0.05$) than that of T_1 and T_2 suggesting immune-enhancing property could be inherent in vitamin C. The differentials of WBC were not statistically significant ($P>0.05$) except for the heterophil, basophil and H/L ratio. The heterophil of T_2 had the highest count and significantly differed ($P<0.05$) from T_1 and T_3 . The high heterophil could be ascribed to immunologically defensive reaction to heat stress which was mitigated by vitamin C in T_3 . The basophil of T_2 and T_3 could also have been heightened by the excessive heat. The H/L ratio of T_2 was significantly higher ($P<0.05$) than T_1 and T_3 since it has been noted that increase in heat leads to rise in the ratio due to decreased lymphocytes which are usually marginalized in heat-stress condition.

Table 3.2: Haematological parameters (\pm SEM) of heat-stressed finisher broilers

Parameter	T_1 (control)	T_2 (heat only)	T_3 (heat + vit C)	SEM
PCV (%)	29.50 \pm 2.08	35.50 \pm 1.76	33.50 \pm 2.01	2.12
Hb (g/dm)	9.75 \pm 2.75	11.60 \pm 1.45	11.00 \pm 2.67	1.74
RBC	2.70 \pm 0.56	3.51 \pm 1.05	3.42 \pm 0.48	2.92
WBC ($\times 10^3$)	13.58 \pm 4.18 ^b	12.53 \pm 1.42 ^b	16.10 \pm 2.63 ^a	1.62
PLT ($\times 10^4$)	10.25 \pm 2.09	10.05 \pm 1.87	11.15 \pm 1.53	1.14
LYM (%)	67.70 \pm 0.94	62.20 \pm 1.52	70.30 \pm 0.63	3.88
HET (%)	23.16 \pm 0.62 ^b	34.50 \pm 0.85 ^a	26.64 \pm 0.69 ^b	3.05
H/L ratio	0.34 ^b	0.55 ^a	0.38 ^b	3.06
MON (%)	3.00 \pm 2.04	3.50 \pm 1.83	2.50 \pm 2.07	1.45
EOS (%)	4.00 \pm 3.03	3.50 \pm 2.63	3.00 \pm 2.93	1.43
BA (%)	0.00 \pm 0.00 ^b	0.50 \pm 0.23 ^a	0.50 \pm 0.63 ^a	2.21

^{ab}: Superscripts with different letters along the same row are significantly different ($p<0.05$)

Note: PLT = platelet, LYM= lymphocyte, HET= heterophil, MON = monocyte, EOS= eosinophil, BA =basophil

4.0 Discussion

Broilers perform best when reared in their thermo-neutral zone above which subjects them to heat-stress and its attendant problems (Yosi et al., 2016). Ascorbic acid which is an antioxidant is synthesized in the kidney and liver of birds, therefore there is no need for its supplementation

in their feed/water (Gan et al., 2018; Khan et al., 2012). Supplementation of vitamin C in the diet of birds has been used to alleviate the negative consequences of heat stress (Amer et al., 2021; Desoky & Kamel, 2018; Gan et al., 2018; Gouda et al., 2020; Hashem et al., 2021). Vitamin C can positively influence intestinal health by enhancing tensile strength, epithelial formation, and internal protein flow (Cowieson et al., 2017; Ibrahim et al., 2020). This present study with no significant difference in weight gain contradicts the reports of some earlier authors (Amer et al., 2021; Desoky & Kamel, 2018; Gouda et al., 2020; Tavakoli et al., 2021; Zhu et al., 2019) that recorded significant difference but affirmed the reports of others (Attia et al., 2017; Nosrati et al., 2017; Yoo & Yi, 2016) that recorded only numerical improvement. These differences could be due to the variation of dosages of vitamin C (150 – 400 mg/kg body weight) used with exception of Jain (2018) that got significant difference among birds on vitamin C supplements of 0, 30, 60, 90 and 120 mg/kg feed.

Vitamin C supplementation improves feeding behaviour of birds (Amer et al., 2021). Meanwhile, Yu et al. (2021) reported no effect on growth performance of broiler chickens on vitamin C supplement. The present study's significant increase in feed intake due to supplementation of vitamin C aligned with the reports of some authors (Abudabos et al., 2018; Chand et al., 2016; Chand et al., 2017; Jain, 2018) unlike Yoo and Yi (2016) that reported no effect of vitamin C on feed intake. Nevertheless, in contrary, Sahin et al. (2003b) reported improved feed intake in quail under heat stress like the report of Livingston et al. (2022) in broilers. The no significant difference of FCR in the present study is in consonance with the reports of Yoo and Yi (2016) and Jain (2018) that reported no significant difference due to vitamin C supplementation. Al-Masad (2012), Attia et al. (2017) and Abudabos et al. (2018) reported improved FCR of heat-stressed broilers whereas Livingston et al. (2022) got higher FCR when compared with thermo-neutral birds. The significantly higher mortality recorded by heat-stressed group (T_2) was ameliorated by vitamin C supplementation. Al-Masad (2012) reported that dietary supplementation of vitamin C reduced mortality in heat-stressed birds. Other authors (Attia et al., 2017; Livingston et al., 2022; Rafiee et al., 2016) reported suppression of negative consequences usually associated with prolonged heat-stress in birds. It has been established that heat stress causes inappetence, poor weight gain, immuno-suppression and increased mortality in broilers (Chand et al., 2017; Sohail et al., 2012).

The major haematological parameters including PCV, RBC and Hb in the present study did not show significant difference among the treatments which opposes the earlier reports that vitamin C supplementation positively affected these parameters (Adenkola & Angani, 2017; Hassan & Asim, 2020; Samantaray & Parida, 2021). The difference could be due to lower vitamin C (100 mg/kg) used in the present study unlike theirs at higher doses of 150 – 300 mg/kg of diet or water. Similar to the present study, Abudabos et al. (2018) reported no significant difference in haematological parameters among broilers exposed to heat-stress and supplemented with vitamin C. Similarly, Usman et al. (2008) reported no difference in haematological parameters of quails under heat stress (38 °C) and placed on diets supplemented with vitamin C. The significantly higher leucocytes (WBC) in T_3 possibly was due to the immune enhancing property of vitamin C.

High environmental temperature has the capacity to induce heat-stress and consequently reduces circulating leucocytes in birds. Previous result observed that heat-stress prevented the releasing of leucocytes into peripheral bloodstream from the body reservoir by enhancing the secretion of corticosteroids during stress (Adenkola & Angani, 2017). Corticosteroids are known to marginalize leucocytes and prevent them from being in circulation. Under heat-stress conditions, vitamin C prevents the production of corticosteroids which is an immune suppressive hormone by inhibiting 21-hydroxylase and 11- β hydroxylase enzymes that aid its

production (Brake, 1989). By blocking this pathway, vitamin C ameliorates the negative effects that lead to immuno-suppression and poor performance in heat-stressed poultry (Pardue & Thaxton, 1986).

Heat stress can impair the immune response by reducing the antibody production and suppressing the synthesis and function of blood lymphocytes in laying hens and broilers (Kamel et al., 2017). Vitamin C activates the phagocyte population and immune-stimulants or significantly protects WBC against hydrogen peroxide by scavenging (Carr & Maggini, 2017; Eo & Lee, 2008). Heat stress increased the heterophils count but was significantly decreased through vitamin C supplementation in T₃. Huff *et al.* (2005) reported higher heterophils count and concomitant decline in lymphocytes in chickens exposed to 2-hour heat-stress like the present study. The increased H/L ratio of heat-stressed broilers in this study could be an indication that heat impacts on physiological stress which had been validated by earlier report of Altan et al. (2003). H/L ratio measures the index of stress in birds as reported by Langsdorf and Zydney (1993). In the present study, vitamin C reduced H/L ratio significantly which agrees with the report of Altan et al. (2003) and Desoky and Kamel (2018). Heat stress elevated the basophil count in the present study which could be reactionary since heat-stress is known to be one of the most detrimental factors in overall poultry production (Lara & Rostagno, 2013).

5.0 Conclusion

There was no significant effect of heat-stress and vitamin C supplementation at 100 mg/kg on the growth performance of broilers though numerically, heat-stress negatively affected the broilers' growth performance. Numerically too, vitamin C helped to alleviate the effect of heat-stress on the birds. There was significant reduction in feed intake of heat-stressed broilers which was alleviated by vitamin C supplementation. Though FCR was not significantly affected by heat stress, however, the group supplemented with vitamin C had the best feed to meat conversion rate. Heat stress equally increased mortality whereas the vitamin C supplementation brought mortality to zero.

The PCV, RBC and Hb were not influenced by heat-stress or vitamin C supplementation. Vitamin C supplementation enhanced WBC count whereas except for heterophils, H/L ratio and basophils, the differentials of WBC were not affected by heat-stress and vitamin C supplementation. The heterophil and H/L ratio were significantly elevated by heat-stress and was assuaged by vitamin C. Vitamin C could not bring down the heat elevated basophils.

References

- Abidin, Z., & Khatoon, A. (2013). Heat stress in poultry and the beneficial effects of ascorbic acid (vitamin C) supplementation during periods of heat stress. *World's Poultry Science Journal*, 69(1), 135–152. <https://doi.org/10.1017/S0043933913000123>
- Abudabos, A. M., Al-Owaimer, A. N., Hussein, E. O. S., & Ali, M. H. (2018). Effect of Natural vitamin C on Performance and Certain Haemato-Biochemical Values in Broiler Chickens Exposed to Heat Stress. *Pakistan Journal of Zoology*, 50(3), 951–955. <https://doi.org/10.17582/journal.pjz/2018.50.3.951.955>
- Abudabos, A. M., Alyemni, A. H., Dafalla, Y. M., & Khan, R. U. (2016). The effect of phytogenic feed additives to substitute in-feed antibiotics on growth traits and blood

- biochemical parameters in broiler chicks challenged with *Salmonella typhimurium*. *Environmental Science and Pollution Research International*, 23(23), 24151–24157. <https://doi.org/10.1007/s11356-016-7665-2>
- Adenkola, A. Y., & Angani, M. T. (2017). Ascorbic acid supplementation effect on haematology and oxidative Stress Parameters of Broiler Chicken during the Hot-Dry Season in Southern Guinea Savannah. *Journal of Poultry Research*, 14(1), 28–33.
- Al-Masad, M. (2012). Effects of vitamin C and zinc on broilers performance of immunocompetence under heat stress. *Asian Journal of Animal Sciences*, 6(2), 76–84. <https://doi.org/10.3923/ajas.2012.76.84>
- Altan, O., Pabuçcuoğlu, A., Altan, A., Konyalıoğlu, S., & Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science*, 44(4), 545–550. <https://doi.org/10.1080/00071660310001618334>
- Amer, S. A., Mohamed, W. A. M., Gharib, H. S. A., Al-Gabri, N. A., Gouda, A., Elabbasy, M. T., Abd El-Rahman, G. I., & Omar, A. E. (2021) Changes in the growth, ileal digestibility, intestinal histology, behavior, fatty acid composition of the breast muscles, and blood biochemical parameters of broiler chickens by dietary inclusion of safflower oil and vitamin C. *BMC Veterinary Research*, 17(1), 68. <https://doi.org/10.1186/s12917-021-02773-5>
- Attia, Y. A., Al-Harathi, M. A., El-Shafey, A. S., Rehab, Y. A., & Kim, W. K. (2017). Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics. *Annals of Animal Science*, 17(4), 1155–1169. <https://doi.org/10.1515/aoas-2017-0012>
- Brake, J. T. (1987). Vitamin C application and recant advances. *Poultry Advisor*, 25(3), 35–40
- Brake, J. T. (1989). Role of ascorbic acid in poultry production: Ascorbic acid, stress and immunity. *Zootechnic International*, 1, 37–62
- Cahaner, A; Ajuh, J. A; Siegmund-Schultze, M; Azoulay, Y; Druyan, S; & Valle Zárate, A. (2008). Effects of the genetically reduced feather coverage in naked neck and featherless broilers on their performance under hot conditions. *Poultry Science*. 87, 2517 - 2527.
- Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. *Nutrients*, 9(11), 1211. <https://doi.org/10.3390/nu9111211>
- Cengiz, Ö; Köksa, B. H; Tatlı, O; Sevim, Ö; Ahsan, U; & Üner, A.G. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. *Poultry Science*, 94 (10), 2395 – 403. doi: 10.3382/ps/pev194
- Chand, N., Muhammad, S., Khan, R. U., Alhidary, I. A., & Rehman, Z. U. (2016). Ameliorative effect of synthetic γ -aminobutyric acid (GABA) on performance traits, antioxidant status

- and immune response in broiler exposed to cyclic heat stress. U; Alhidary, I. A; & *Environmental Science and Pollution Research International* Z. Rahman (Ed.), 23(23), 23930–23935. <https://doi.org/10.1007/s11356-016-7604-2>
- Chand, N., Naz, S., Khan, A., Khan, S., & Khan, R. U. (2014). Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. *International Journal of Biometeorology*, 58(10), 2153–2157. <https://doi.org/10.1007/s00484-014-0815-7>
- Chand, N., Naz, S., Maris, H., & Khan, R. (2017). Effect of betaine supplementation on the performance and immune response of heat stressed broilers. U; Khan, S; and Qureshi, M.S. *Pakistan Journal of Zoology*, 49, 1857–1862.
- Cowieson, A. J., Zaefarian, F., Knap, I., & Ravindran, V. (2017). Interactive effects of dietary protein concentration, a mono-component exogenous protease and ascorbic acid on broiler performance, nutritional status and gut health. *Animal Production Science*, 57(6), 1058–1068. <https://doi.org/10.1071/AN15740>
- De Souza, L. F. A., Espinha, L. P., De Alneida, E. A., Lunedo, R., & Furlan, R. L ; & Macari, M. (2016). How heat stress continuous or cyclical interferes with nutrient digestibility energy and nitrogen balances and performances in broiler. *Livestock Science*, 192, 39–43.
- Desoky, A. A., & Kamel, N. N. (2018). Effect of feed restriction and vitamin C supplementation on growth performance, carcass characteristics and some blood parameters of broiler reared under heat stress. *Egyptian Journal of Nutrition and Feeds*, 21(3), 823–832. <https://doi.org/10.21608/ejnf.2018.75800>
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1–42. <https://doi.org/10.2307/3001478>
- Egbinola, C. N., & Amobichukwu, A. C. (2013). Climate variation assessment based on rainfall and temperature in Ibadan, South-Western, Nigeria. *Environmental Earth Sciences*, 3(11), 32–45.
- Eo, J., & Lee, K. J. (2008). Effect of dietary ascorbic acid on growth and non-specific immune responses of tiger puffer, *Takifugu rubripes*. *Fish and Shellfish Immunology*, 25(5), 611–616. <https://doi.org/10.1016/j.fsi.2008.08.009>
- Gan, L., Fan, H., Nie, W., & Guo, Y. (2018). Ascorbic acid synthesis and transportation capacity in old laying hens and the effects of dietary supplementation with ascorbic acid. *Journal of Animal Science and Biotechnology*, 9, 71. <https://doi.org/10.1186/s40104-018-0284-7>
- Ghazi, S., Amjadian, T., & Norouzi, S. (2015). Single and combined effects of vitamin C and oregano essential oil in diet, on growth performance, and blood parameters of broiler chicks reared under heat stress condition. *International Journal of Biometeorology*, 59(8), 1019–1024. <https://doi.org/10.1007/s00484-014-0915-4>

- Gouda, A., & Amer, S. A., Gabr, S., & Tolba, S. A. (2020). Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. A; Gabr, S; & Tolba, S. A. *Tropical Animal Health and Production*, 52(6), 2987–2996. <https://doi.org/10.1007/s11250-020-02316-4>.
- Hashem, M. A., Abd El Hamied, S. S., Ahmed, E. M. A., Amer, S. A., & Hassan, A. M. (2021). Alleviating effects of vitamins C and E supplementation on oxidative stress, hematobiochemical, and histopathological alterations caused by copper toxicity in broiler chickens. *Animals (Basel)*, 11(6), 1739. <https://doi.org/10.3390/ani11061739>
- Hassan, A. A., & Asim, R. A. (2020). Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress. *Iraqi Journal of Veterinary Sciences*, 34(2), 357–363. <https://doi.org/10.33899/ijvs.2019.125950.1195>
- Huff, G. R., Huff, W. E., Balog, J. M., Rath, N. C., Anthony, N. B., & Nestor, K. E. (2005). Stress response differences and disease susceptibility reflected by heterophil to lymphocyte ratio in Turkeys selected for increased body weight. *Poultry Science*, 84(5), 709–717. <https://doi.org/10.1093/ps/84.5.709>
- Ibrahim, R. E., Ahmed, S. A. A., Amer, S. A., Al-Gabri, N. A., Ahmed, A. I., Abdel-Warith, A. A., Younis, E. M. I., & Metwally, A. E. (2020). Influence of vitamin C feed supplementation on the growth, antioxidant activity, immune status, tissue histomorphology, and disease resistance in Nile tilapia, *Oreochromis niloticus*. Aquatic Report. *Aquaculture Reports*, 18, 100545. <https://doi.org/10.1016/j.aqrep.2020.100545>
- Jain, G. (2018). Neeraj and R. Pandey. Effect of vitamin C on growth performance of caged broilers. *Advances in Biological Research*, 9(2), 178–181
- Kamel, N. N., Ahmed, A. M. H., Mehaisen, G. M. K., Mashaly, M. M., & Abass, A. O. (2017). Depression of leukocyte protein synthesis, immune function and growth performance induced by high environmental temperature in broiler chickens. *International Journal of Biometeorology*, 61(9), 1637–1645. <https://doi.org/10.1007/s00484-017-1342-0>
- Khan, R. U., Naz, S., Nikousefat, Z., Selvaggi, M., Laudadio, V., & Tufarelli, V. (2012). Effect of ascorbic acid in heat-stressed poultry. *World's Poultry Science Journal*, 68(3), 477–490. <https://doi.org/10.1017/S004393391200058X>.
- Khan, R. U., Chabdt, N., & Ali, A. (2016). Effect of organic acid on performance of Japanese quail. *Pakistan Journal*, 48, 1799–1830
- Ullah Khan, R. U., Naz, S., & Dhama, K. (2014). Chromium: Pharmacological applications in heat-stressed poultry. *International Journal of Pharmacology*, 10(4), 213–217. <https://doi.org/10.3923/ijp.2014.213.217>
- Langsdorf, L. J., & Zydney, A. L. (1993). Effect of uremia on the membrane transport characteristics of red blood cells. *Blood*, 81(3), 820–827

- Lara, L. J., & Rostagno, M. H. (2013). Impact of heat stress on poultry production. *Animals: An Open Access Journal from MDPI*, 3(2), 356–369. <https://doi.org/10.3390/ani3020356>
- Lentfer, T. L., Pendl, H., Gebhardt-Henrich, S. G., Fröhlich, E. K., & Von Borell, E. (2015). H/L ratio as a measurement of stress in laying hens – methodology and reliability. G; Fröhlich, E. K. F; & Von Borell, E. *British Poultry Science*, 56(2), 157–163. <https://doi.org/10.1080/00071668.2015.1008993>
- Livingston, M. L., Pokoo-Aikins, A., Frost, T., Laprade, L., Hoang, V., Nogal, B., Phillips, C., & Cowieson, A. J. (2022). Effect of Heat Stress, Dietary Electrolytes, and vitamins E and C on Growth Performance and Blood Biochemistry of the Broiler Chicken. *Frontiers in Animal Science*, 3, 807267. <https://doi.org/10.3389/fanim.2022.807267>
- Mutaf, S., Kahraman, N. S., & Firat, M. Z. (2009). Intermittent partial surface wetting and its effect on body-surface temperatures and egg production of white and brown domestic laying hens in Antalya (Turkey). S; & Firat, M. Z. *British Poultry Science*, 50(1), 33–38. <https://doi.org/10.1080/00071660802592399>
- Nosrati, M., Javandel, F., Camacho, L. M., Khusro, A., Cipriano, M., Seidavi, A., & Salem, A. Z. M. (2017). The effects of antibiotic, probiotic, organic acid, vitamin C, and Echinacea purpurea extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *Journal of Applied Poultry Research*, 26(2), 295–306. <https://doi.org/10.3382/japr/pfw073>
- Oladele, B. M., & Oladimeji, B. H. (2011). Dynamics of urban land use changes with remote sensing: Case study of Ibadan, Nigeria. *Journal of Geography and Regional Planning*, 4(11), 632–643
- Pardue, S. L., & Thaxton, J. P. (1986). Ascorbic acid in poultry: A review. *World's Poultry Science Journal*, 42(2), 107–123. <https://doi.org/10.1079/WPS19860009>
- Polycarp, T. N., Obukowho, E. B., & Yusoff, S. M. (2016). Changes in haematological parameters and oxidative stress response of goats subjected to road transport stress in a hot humid tropical environment. *Comparative Clinical Pathology*, 25(2), 285–293. <https://doi.org/10.1007/s00580-015-2179-8>
- Rafiee, F., Mazhari, M., Ghoreishi, M., & Esmaeilipour, O. (2016). Effect of lemon verbena powder and vitamin C on performance and immunity of heat-stressed broilers. *Journal of Animal Physiology and Animal Nutrition*, 100(5), 807–812. <https://doi.org/10.1111/jpn.12457>
- Sahin, K., Onderci, M., Sahin, N., Gursu, M. F., & Kucuk, O. (2003b). Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. *Journal of Nutrition*, 133(6), 1882–1886. <https://doi.org/10.1093/jn/133.6.1882>
- Sahin, K., Sahin, N., & Kucuk, O. (2003a). Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of

- broiler chickens reared at a high ambient temperature (32°C). *Nutrition Research*, 23(2), 225–238. [https://doi.org/10.1016/S0271-5317\(02\)00513-4](https://doi.org/10.1016/S0271-5317(02)00513-4)
- Samantaray, B. P., & Parida, S. P. (2021). Vitamin-C treated haematology assessment of Swiss albino mice (*Mus musculus*). *Biointerface Research in Applied Chemistry*, 11(2), 9044–9050. <https://doi.org/10.33263/BRIAC112.90449050>
- SAS Institute, Incorp. (2009). SAS/STAT R_9.2. *User's guide* (2nd ed). SAS Institute, Inc.
- Sinha, R; Lone, S. A; Ranjan, A; Rahim, A; Devi, I; & Tiwari, S. (2017). The impact of climate change on livestock production and reproduction: ameliorative management. *International Journal of Livestock Research*, 7 (6), 1 - 8
- Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., Shabbir, M. Z., & Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Z; & Rehman, H. *Poultry Science*, 91(9), 2235–2240. <https://doi.org/10.3382/ps.2012-02182>
- Syafwan, S., Kwakkel, R. P., & Verstegen, M. W. A. (2011). Heat stress and feeding strategies in meat-type chickens. P; & Verstegen, M. W. A. *World's Poultry Science Journal*, 67(4), 653–674. <https://doi.org/10.1017/S0043933911000742>
- Tavakoli, M., Bouyeh, M., & Seidavi, A. (2021). Effects of dietary vitamin C supplementation on growth performance, carcass characteristics, gastrointestinal organs, liver enzymes, abdominal fats, immune response, and cecum microflora of broiler chickens. *Journal of Animal Science*, 31(1), 67–78. <https://doi.org/10.22034/as.2021.42573.1589>
- Usman, B. A., Mani, A. U., & Muiyiwa, O. B. (2008). Effect of dietary treatments of ascorbic acid on the blood parameters, egg production and quality in quail (*Coturnix coturnix japonica* M) subjected to heat stress. *International Journal of Poultry Science*, 7(4), 344–349. <https://doi.org/10.3923/ijps.2008.344.349>
- Ward, J. W., & Elsea, J. R. (1997). *Animal case and use in drug fate and metabolism, methods and techniques*, 2 p. 431. Marcel Dekker.
- Yoo, J., & Yi, Y., Koo, J (2016). Growth performance, intestinal morphology, and meat quality in relation to alpha-lipoic acid associated with vitamin C and E in broiler chickens under tropical conditions. B; Jung, S; Yoon. *Revista Brasileira de Zootecnia*, 45 (3) 113–120. JU. HB. Department of Health; Heo & Heo, J. M.
- Yosi, F., Widjastuti, T., & Setiyatwan, H. (2016). Performance and physiological responses of broiler chickens supplemented with potassium chloride in drinking water under environmental heat stress. *Asian Journal of Poultry Science*, 11(1), 31–37. <https://doi.org/10.3923/ajpsaj.2017.31.37>
- Yu, D. G., Namgung, N., Kim, J. H., Won, S. Y., Choi, W. J., & Kil, D. Y. (2021). Effects of stocking density and dietary vitamin C on performance, meat quality, intestinal

- permeability, and stress indicators in broiler chickens. *Journal of Animal Science and Technology*. SY, 63(4), 815–826. <https://doi.org/10.5187/jast.2021.e77>
- Zhang, L; Yue, H. Y; Zhang, H. J ; Xu, L; Wu, S. G; Yan, H. J; Gong, Y.S; & Qi, G. H. (2009). Transport stress in broilers: I. blood metabolism, glycolytic potential, and meat quality. *Poultry Science*, 88, 2033 – 3041
- Zhu, Y. F., Li, S. Z., Sun, Q. Z., & Yang, X. J. (2019). Effect of in ovo feeding of vitamin C on antioxidation and immune function of broiler chickens. *Animal*, 13(9), 1927–1933. <https://doi.org/10.1017/S1751731118003531>