RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v23i4.6

Kolawole *et al.*/*Sokoto Journal of Veterinary Sciences*, **23**(4): 264 - 269.

Ascorbic acid effects on erythrocyte osmotic fragility in guinea fowls during seasonal variations in Zaria, Nigeria

OF Kolawole[1], TA Ige[2]*, VO Adeyemi[3], IA Elisha[4], KK Omikunle[3], MA Abdullahi[5] & MS Umar[2]

1. *Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*
2. *Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*
3. *Department of Veterinary Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*
4. *College of Agriculture, Science and Technology, Jalingo, Taraba State, Nigeria*
5. *Federal College of Animal Health and Production Technology, Vom, Plateau State, Nigeria*

*Correspondence: Tel.: +234 7032466305; E-mail: taiwoige29@gmail.com

Publication History:
Received: 20-09-2025
Revised: 28-11-2025
Accepted: 30-11-2025

Abstract

This study evaluated the effect of ascorbic acid (AA) supplementation on erythrocyte osmotic fragility (EOF) of guinea fowls across seasonal variations in the Northern Guinea Savannah zone of Nigeria. Forty healthy mixed sex guinea fowls were randomly assigned to control (n = 20) and experimental (n = 20) groups. Experimental birds received oral AA at 50 mg/kg body weight, while controls received none. Meteorological parameters, including dry- and wet-bulb temperatures, relative humidity, and temperature-humidity index (THI), were recorded during rainy, Harmattan, and hot-dry seasons. Blood samples were collected after three weeks, and EOF was determined by exposing erythrocytes to graded NaCl solutions (0.10–0.90%). Results revealed seasonal variations in THI, with the hot-dry season posing the greatest thermal load, followed by the Harmattan and rainy seasons. Haemolysis increased with decreasing NaCl concentration across all groups. Control birds exhibited significantly higher ($P < 0.05$) haemolysis than AA-supplemented birds during hot-dry and Harmattan seasons, whereas no significant differences were observed during the rainy season. The findings suggest that oxidative stress-induced erythrocyte fragility is most severe in the hot-dry season and that AA supplementation reduces haemolysis by enhancing erythrocyte membrane stability. Supplementation with AA is therefore recommended during hot-dry and Harmattan seasons to improve resilience and productivity in guinea fowls.

Keywords: Ascorbic acid, Erythrocyte osmotic fragility, Guinea fowls, Oxidative stress, Thermal stress

Introduction

Erythrocyte osmotic fragility (EOF) is a critical haematological parameter that reflects the structural and functional integrity of red blood cells (RBCs). As RBCs constitute over 99% of all cellular blood

components and play a central role in oxygen transport, their ability to withstand osmotic stress is a reliable indicator of membrane stability and overall physiological health (Muzykantov, 2010; Yu *et al.,* 2020). EOF is sensitive to both endogenous and exogenous stressors, including environmental fluctuations, such as seasonal temperature and humidity changes, which can compromise erythrocyte resilience (Igbokwe, 2018).

Seasonal heat stress disrupts oxidative balance by overwhelming the body's endogenous antioxidant defences, leading to excessive free radical production, lipid peroxidation of cellular membranes, and subsequent cellular damage (Freeman & Crapo, 1982; Altan *et al.,* 2003; Khan *et al.,* 2024). Such oxidative damage alters haematological and biochemical parameters, which are frequently used to assess stress and welfare in animals (Fazio & Ferlazzo, 2003). Notably, the erythrocytes of guinea fowls are intrinsically more fragile than those of domestic chickens, and adult guinea fowls display greater fragility than their younger counterparts (Durotoye & Oyewale, 1988).

The antioxidant defence system in animals, which comprises enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, scavenges reactive oxygen species (ROS) to preserve cellular functions (Birben *et al.,* 2012). However, during prolonged stress, this endogenous protection becomes inadequate, necessitating exogenous antioxidant support (Sies, 1997; Ahmadu *et al.,* 2016). Ascorbic acid (AA), a water-soluble vitamin, is a potent antioxidant with a well-documented role in neutralizing ROS, stabilizing cell membranes, and supporting metabolic functions such as collagen synthesis, vascular integrity, and adrenal hormone release (Padayatty *et al.,* 2003; Halliwell, 2012). Following oral administration, AA is rapidly absorbed and exerts strong free radical scavenging activity, thereby protecting cellular macromolecules and signalling pathways from ROS-induced disruption (Pardue *et al.,* 1984).

Erythrocytes are particularly vulnerable to oxidative stress because of the high proportion of polyunsaturated fatty acids in their membranes (Altan *et al.,* 2003). The EOF test, which assesses haemolysis under hypotonic conditions, provides a sensitive measure of erythrocyte stability and is increasingly recognized as a biomarker of animal welfare under environmental stress (Aldrich *et al.,* 2006; Reddy *et al.,* 2019). Environmental factors, such as temperature, pH, and storage duration, significantly influence RBC osmotic behaviour by altering membrane lipids and proteins (Oyewale *et al.,* 1997; Oyewale *et al.,* 2011).

Materials and Methods

Experimental animals and management

Forty apparently healthy mixed-sex guinea fowls were randomly divided into two groups: control (n = 20) and experimental (n = 20). Birds in each group were individually tagged with numbered strips of white cloth tied to their legs for easy identification. They were housed in separate pens constructed with concrete floors, cement block walls, high-wire mesh, and asbestos roofing. The birds were managed intensively, with *ad libitum* access to water and commercial hybrid layers' mash.

Meteorological data

Meteorological parameters (wet-bulb and dry-bulb temperatures) were measured at the experimental site using wet- and dry-bulb thermometers (Brannan®, Cumbria, England). Measurements were taken three times daily (07:00 h, 13:00 h, and 18:00 h) over four days each week for three consecutive weeks. Relative humidity (RH) was calculated using the manufacturer's manual, while the temperature-humidity index (THI) was computed using the formula of Zulovich & DeShazer (1990): $THI=0.6T_{db}+0.4T_{wb}THI$. Where T_{db} is the dry-bulb temperature and T_{wb} is the wet-bulb temperature.

Experimental design

6 months old in the rainy season and reached 14 months of age by the end of the hot-dry season, with body weights ranging from 1.8 to 2.5 kg. Birds in the experimental group received oral ascorbic acid (Sigma Chemical, St. Louis, MO, USA) at a dosage of 50 mg/kg body weight (Minka & Ayo, 2010). It was prepared by dissolving 0.05 g/kg (50 mg/kg) body weight of ascorbic acid powder in 2 mL of distilled water dispensed to the birds individually using 5mL syringes. Control birds received no ascorbic acid.

Blood sample collection

At the end of the third week of each season, approximately 2 mL of blood was aseptically collected from each bird via the wing vein using 5 mL syringes and 25-gauge sterile needles. Samples were transferred into sterile screw cap test tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant and transported immediately to the Physiology Research Laboratory, Faculty of Veterinary

Medicine, Ahmadu Bello University, Zaria, for analysis of erythrocyte osmotic fragility.

Measurement of erythrocyte osmotic fragility

Stock sodium chloride (NaCl) solutions (0.10–0.90%) were prepared according to the classical method of Faulkner & King (1970), as well as procedures applied in more recent osmotic fragility studies (Alhassan *et al.,* 2010).

Percentage haemolysis was calculated according to the classical formula of Faulkner & King (1970):

% Haemolysis = Optical density of test/Optical density of standard × 100

Distilled water served as the standard. Bar charts of erythrocyte osmotic fragility were plotted by relating percentage haemolysis to saline concentrations.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using Pearson's correlation and Student's t-test in GraphPad Prism version 5.03 (GraphPad Software, San Diego, California, USA). Results with $P < 0.05$ were considered statistically significant.

Results

The study revealed marked seasonal variations in meteorological conditions in the Northern Guinea Savannah zone. The lowest dry-bulb thermometer (DBT) value was recorded during the Harmattan season (minimum 22.67 ± 2.33℃ mean 24.32 ± 1.50℃), which was lower than that recorded during the rainy season (Table 1). In contrast, the hot-dry season recorded the highest DBT values (maximum 34.5 ± 3.01℃; minimum 29.0 ± 2.65℃; mean 32.28 ± 1.67℃), which were higher than those of the Harmattan season (Table 1). Relative humidity (RH) was highest in the rainy season (75.67 ± 3.01%) and lowest in the Harmattan (32.11 ± 3.28%) (Table 1). Within each season, daily variations were observed; however, comparison of mean values across seasons showed that the hot dry season remained warmer than the Harmattan and rainy seasons. The calculated temperature-humidity index (THI) indicated that thermal load was most severe during the hot-dry season (28.50 ± 1.11), moderate during the Rainy season (26.16 ± 0.36), and lowest in the Harmattan season (20.69 ± 1.52).

Erythrocyte osmotic fragility (EOF) results showed that haemolysis increased as NaCl concentration decreased across all seasons (Figure 1-3). During the hot-dry season, haemolysis was significantly higher ($P < 0.05$) in the control group compared to the experimental group at 0.5% NaCl (Figure 2). During the Harmattan season, haemolysis was significantly higher in the control group at 0.3% NaCl (Figure 3). In

Table 1: Daily fluctuations in thermal environmental conditions across seasons

Parameter	Season	Day 1	Day 2	Day 3	Mean ± SEM
Dry-bulb Temperature (℃)	Rainy	27.33 ± 0.88[a]	28.33 ± 0.88[a]	26.67 ± 0.67[b]	27.44 ± 0.48
Relative Humidity (%)	Rainy	72.33 ± 4.177[a]	73.00 ± 4.000[a]	81.67 ± 2.333[b]	75.67 ± 3.01
Temperature- humidity Index	Rainy	25.87 ± 0.636	26.87 ± 0.636	25.73 ± 0.533	26.16 ± 0.36
Dry-bulb Temperature (℃)	Harmattan	23.00 ± 2.52	22.67 ± 2.33	27.33 ± 3.18	24.32 ± 1.50
Relative Humidity (%)	Harmattan	26.67 ± 1.333	31.67 ± 6.888	38.00 ± 3.055	32.11 ± 3.28
Temperature- humidity Index	Harmattan	19.13 ± 2.174	19.20 ± 1.800	23.73 ± 2.994	20.69 ± 1.52
Dry-bulb Temperature (℃)	Hot-Dry	34.50 ± 3.01[a]	33.33 ± 2.67[a]	29.00 ± 2.65	32.28 ± 1.67
Relative Humidity (%)	Hot-Dry	38.67 ± 14.52	41.67 ± 13.30	56.33 ± 13.35	45.56 ± 5.46
Temperature- humidity Index	Hot-Dry	29.97 ± 1.732	29.20 ± 1.744	26.33 ± 1.790	28.50 ± 1.11

Values are represented as mean ± SEM. Different superscripts (a, b) within row indicates significant differences at $P < 0.05$.

Table 2: Relationships between sodium chloride concentration and percentage (%) haemolysis in experimental and control guinea fowls

Time of Blood Collection	Experimental Group (n=20)	Control Group (n=20)
Rainy season	-0.9537**	-0.9505**
Hot-dry season	-0.9303**	-0.9154*
Harmattan season	-0.9669**	-0.9290**

* = Significant ($P < 0.05$), ** = Highly significant ($P < 0.01$) correlation, *** = Very highly significant ($P < 0.001$) correlation

the rainy season, no significant differences (P > 0.05) were observed between experimental and control groups across all NaCl concentrations (Figure 1).

Correlation analysis revealed a highly significant (P < 0.01) negative association between haemolysis and NaCl concentration in all seasons (Table 2). These results demonstrate that thermal stress was most severe in the hot-dry season, moderately stressful during Harmattan, and least stressful during the rainy season.

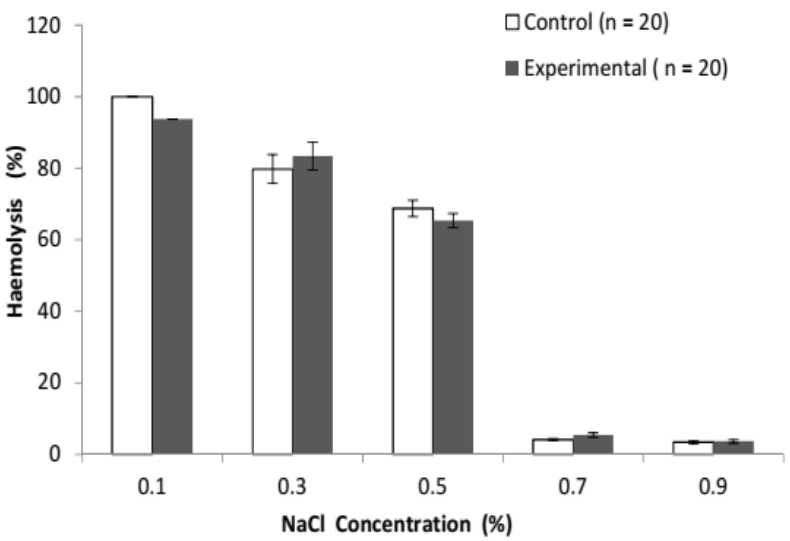

Figure 1: Percentage haemolysis in experimental (supplemented with ascorbic acid, n = 20) and control (non-supplemented with ascorbic acid, n = 20) guinea fowls during the rainy season. NaCl = Sodium chloride

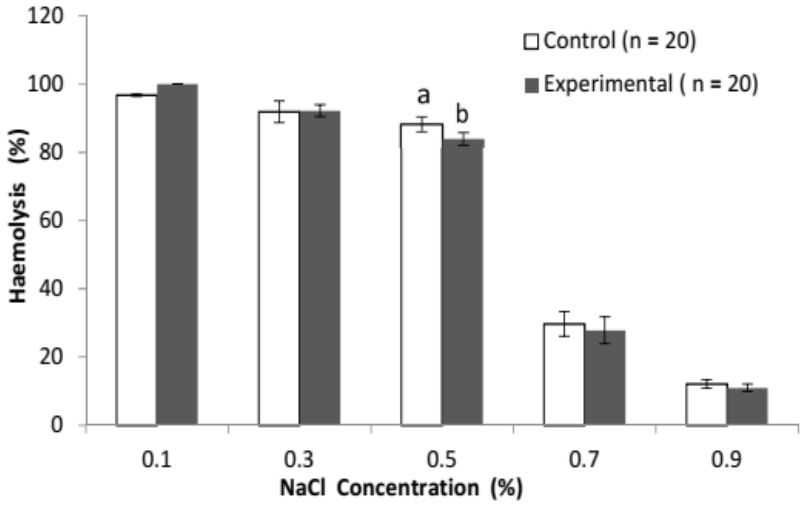

Figure 2: Percentage haemolysis in experimental (supplemented with ascorbic acid, n = 20) and control (non-supplemented with ascorbic acid, n = 20) guinea fowls during the hot-dry season.
NaCl = Sodium chloride.
a,b = Values with error bars having different superscript letters are significantly (P < 0.05) different

Discussion

The present study demonstrated marked seasonal variations in meteorological conditions within the Northern Guinea Savannah. The lowest DBT and RH were recorded during the Harmattan season, whereas the hot-dry season produced the highest DBT and THI, indicating the greatest overall thermal load. These observations are consistent with earlier reports by Ayo *et al.* (1998), who described Harmattan as a cold-dry, dusty season with low RH, and the hot-dry season as characterized by high ambient temperatures and moderate humidity.

The increased haemolysis observed during the hot-dry season may be attributed to oxidative stress resulting from heat load, a finding consistent with Altan *et al.* (2003), who reported that heat stress induces free radical generation that overwhelms the antioxidant defence system, leading to cell membrane damage and haemolysis. Stress-induced free radicals trigger lipid peroxidation, causing erythrocyte membrane deterioration (Minka & Ayo, 2013a).

Similarly, the increased haemolysis observed during the Harmattan season may be associated with the cold-dry and dusty environmental conditions typical of this period, which promote erythrocyte dehydration and heighten oxidative stress on cell membranes (Ayo *et al*., 1998; Oyewale *et al.,* 2011; Elbaz *et al.,* 2023). These seasonal patterns support the conclusion that both extreme heat and harsh cold-dry conditions adversely affect erythrocyte stability.

The present study further supports earlier findings that ascorbic acid (AA) supplementation stabilizes membrane integrity and reduces susceptibility to lipid peroxidation, as demonstrated in humans (Tauler *et al.,* 2003), goats (Minka & Ayo, 2007; Minka & Ayo, 2013b), and

poultry (Minka & Ayo, 2013a), particularly during the hot-dry and Harmattan seasons. In the current study, AA-supplemented guinea fowls consistently showed reduced haemolysis compared to controls, particularly during the hot-dry and Harmattan seasons. This suggests that AA ameliorates oxidative damage by scavenging free radicals, delaying haemoglobin denaturation, and reducing cell destruction (Minka & Ayo, 2013a). Overall, these findings demonstrate that EOF is a reliable biomarker for assessing thermal stress in guinea fowls, and that AA supplementation effectively reduces haemolysis, especially under hot-dry and Harmattan conditions. The rainy season was the least adverse to guinea fowl health and productivity. This study concluded that seasonal variations in thermal environment significantly influenced oxidative stress and erythrocyte osmotic fragility in guinea fowls, with the hot-dry season posing the greatest challenge, followed by the Harmattan. Supplementation with ascorbic acid markedly reduced haemolysis, highlighting its protective role against oxidative stress. Therefore, guinea fowls should be supplemented with ascorbic acid during the hot-dry and Harmattan seasons to enhance resilience, maintain productivity, and safeguard health under adverse environmental conditions.

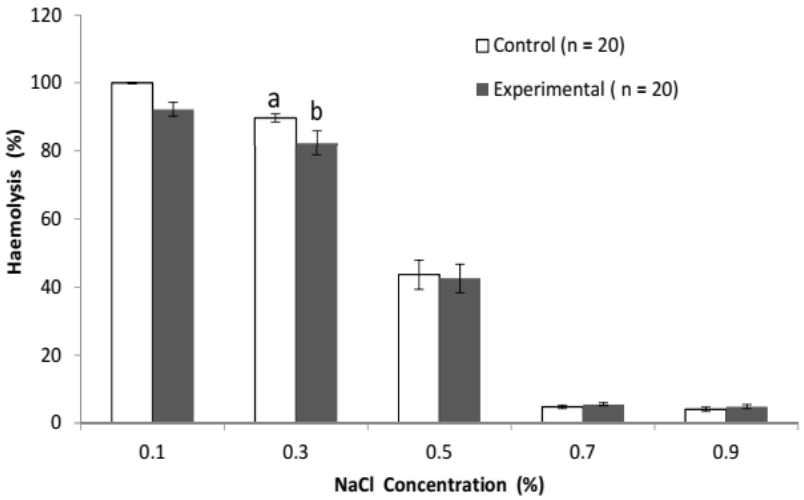

Figure 3: Percentage haemolysis in experimental (supplemented with ascorbic acid, n = 20) and control (non-supplemented with ascorbic acid, n = 20) guinea fowls during the harmattan season. NaCl = Sodium chloride.
a,b = Values with error bars having different superscript letters are significantly ($P < 0.05$) different

Acknowledgements
The authors acknowledge their respective universities and institutes for their cooperation.

Funding
No funding was received.

Conflict of Interest
The authors declare that there is no conflict of interest.

References

Ahmadu S, Mohammed AA, Buhari H & Auwal A (2016). An overview of vitamin C as an anti-stress agent in poultry. *Malaysian Journal of Veterinary Research,* **7**(1): 9–22.

Aldrich KJ, Saunders DK, Sievert LM & Sievert G (2006). Comparison of erythrocyte osmotic fragility among amphibians, reptiles, birds, and mammals. *Transactions of the Kansas Academy of Science,* **109**(3–4): 149–158.

Alhassan AW, Adenkola AY, Yusuf A, Bauchi ZM, Saleh MI & Ochigbo VI (2010). Erythrocyte osmotic fragility of Wistar rats administered ascorbic acid during the hot-dry season. Journal of Cell and Animal Biology, **4**(2): 29–33.

Altan Ö, Pabuçcuoğlu A, Altan A, Konyalioğlu 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.

Ayo JO, Oladele SB, Fayomi A, Jumbo SD & Hambolu JO (1998). Body temperature, respiratory and heart rate in the Red Sokoto goat during the harmattan season. *Bulletin of Animal Health and Production for Africa,* **46**(3): 161–166.

Birben E, Sahiner UM, Sackesen C, Erzurum S & Kalayci O (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal,* **5**(1): 9–19.

Durotoye LA & Oyewale JO (1988). A comparative study of erythrocyte osmotic fragility between the guineafowl (Numida meleagris galeata) and the Nigerian domestic fowl (Gallus domesticus). *Bulletin of Animal*

Health and Production in Africa, **36**(1): 73–76.

Elbaz AM, Zaki EF, Salama AA, Badri FB & Thabet HA (2023). Assessing different oil sources efficacy in reducing environmental heat-stress effects via improving performance, digestive enzymes, antioxidant status, and meat quality. *Scientific Reports,* doi.10.1038/s41598-023-47356-6.

Faulkner WR & King JW (1970). Manual of Clinical Laboratory Procedures (second edition). Cleveland, Ohio: Chemical Rubber Company. Pp 354.

Fazio E & Ferlazzo A (2003). Evaluation of stress during transport. *Veterinary Research Communications,* **27**(1): 519–524.

Freeman BA & Crapo JD (1982). Biology of disease: Free radicals and tissue injury. *Laboratory Investigation,* **47**(5): 412–426.

Halliwell B (2012). Free radicals and antioxidants: Updating a personal view. *Nutrition Reviews,* **70**(5): 257–265.

Igbokwe NA (2018). A review of the factors that influence erythrocyte osmotic fragility. *Sokoto Journal of Veterinary Sciences,* **16**(4): 1–23.

Khan MZ, Khan A, Chen W, Chai W & Wang C (2024). Advancements in genetic biomarkers and exogenous antioxidant supplementation for safeguarding mammalian cells against heat-induced oxidative stress and apoptosis. *Antioxidants,* **13**(3): 258.

Minka NS & Ayo JO (2007). Physiological responses of transported goats treated with ascorbic acid during the hot-dry season. *Animal Science Journal,* **78**(2): 164–172.

Minka NS & Ayo JO (2010). Physiological responses of erythrocytes of goats to transportation and the modulatory role of ascorbic acid. *Journal of Veterinary Medical Science,* **72**(7): 875–881.

Minka NS & Ayo JO (2013a). Ameliorating effect of melatonin on colonic temperature and erythrocyte fragility of Japanese quails (*Coturnix coturnix japonica*) transported by road. *European Poultry Science*, **77**(2): 137–143.

Minka NS & Ayo JO (2013b). Physiological and behavioral responses of goats to 12-hour road transportation, lairage and grazing periods, and the modulatory role of ascorbic acid. *Journal of Veterinary Behaviour,* **8**(5): 349–355.

Muzykantov VR (2010). Drug delivery by red blood cells: Vascular carriers designed by mother nature. *Expert Opinion on Drug Delivery,* **7**(4): 403–427.

Oyewale J, Dzenda T, Yaqub L, Akanbi D, Ayo J, Owoyele O, Minka N & Dare T (2011). Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and blood storage. *Veterinarski Arhiv,* **81**(4): 459–470.

Oyewale JO, Okowumi FO & Olayemi FO (1997). Haematological changes in West African Dwarf goats following haemorrhage. *Journal of Veterinary Medicine Series A,* **44**(10): 619–624.

Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O & Lee J (2003). Vitamin C as an antioxidant: Evaluation of its role in disease prevention. A review. *Journal of the American College of Nutrition,* **22**(1): 18–25.

Pardue SL, Thaxton JP & Brake J (1984). Plasma ascorbic acid concentration following ascorbic acid loading in chicks. *Poultry Science,* **63**(12): 2492–2496.

Reddy PR, Kumar BR, Prasad CS, Venkataseshiah C & Hyder I (2019). Erythrocyte fragility-based assessment of true thermal resilience in tropical small ruminants. *Biological Rhythm Research,* **51**(7): 1063–1074.

Sies H (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology,* **82**(2): 291–295.

Tauler P, Aguiló A, Gimeno I & Others (2003). Influence of vitamin C diet supplementation on endogenous antioxidant defences during exhaustive exercise. *Pflugers Archiv – European Journal of Physiology,* doi.10.1007/s00424-003-1112-1.

Yu YB, Park HJ & Kang JC (2020). Effects of dietary ascorbic acid on growth performance, haematological parameters, antioxidant and non-specific immune responses in starry flounder, Platichthys stellatus. *Aquaculture Reports,* doi.10.1016/j.aqrep.2020.100419.

Zulovich JM & DeShazer JA (1990). Estimating egg production declines at high environmental temperatures and humidity. American Society of Agricultural Engineers, Paper 904021, St. Joseph, Michigan, ASAE Paper No: 90-4021.