



ISSN: 2321-9122

www.biosciencejournals.com

EJBB 2015; 3 (1): 46-52

Received: 12-01-2015

Accepted: 25-01-2015

Impact Factor: 3.742

Okey, Samuel Maduabuchi

Department of Human
Physiology, Ahmadu Bello
University, Zaria, Nigeria.

Ayo, Joseph Olusegun

Department of Veterinary
Physiology, Ahmadu Bello
University, Zaria, Nigeria.

The role of co-administration of ascorbic acid and zinc gluconate on brain biochemical changes in wistar rats during the hot-humid season

Okey, Samuel Maduabuchi; Ayo, Joseph Olusegun

Abstract

The aim of the study was to investigate the effect of co-administration of ascorbic acid and zinc gluconate, which are antioxidant nutrients on brain biochemical changes in wistar rats, exposed to the hot-humid season. Fourteen adult male Wistar rats weighing 110 g - 186 g, were divided into two groups of seven rats each. The first group, which served as the control was given distilled water (1 ml/kg); rats in the second group served as the experimental animals and were first given ascorbic acid (100 mg/kg) and immediately followed by the administration of zinc (50 mg/kg). The regimens were given once daily and orally by gavage for a period of 28 days. The dry and wet-bulb temperature values were taken; and from the values, relative humidity and heat index was calculated. The rats were sacrificed on day 29 of the administration and evaluated for brain catalase, superoxide dismutase and acetylcholinesterase activities. Lipid peroxidation was determined by measuring the malondialdehyde concentration in the brain. The heat index was significantly ($P < 0.05$) different, when compared with values obtained between the hours of 7.00 h, 13.00 h and 18.00 h, indicative of the exposure of the rats to heat stress. There was no significant difference in the malondialdehyde concentration ($P > 0.05$) of both groups. There were no significant differences in activities of superoxide dismutase, catalase and acetylcholinesterase in the brain showed no significant difference ($P > 0.05$) between the groups. In conclusion, the moderate heat stress during the hot-humid season induced brain biochemical changes in Wistar rats, which may be attenuated by co-administration of ascorbic acid and zinc gluconate.

Keywords: heat stress, zinc gluconate, ascorbic acid, catalase, acetylcholinesterase, malondialdehyde.

1. Introduction

The interplay between free radicals, antioxidants and co-factors is important in maintaining health. Enzymatic and non – enzymatic antioxidants provide necessary defense against oxidative stress, induced by heat stress during the different seasons in the northern Guinea Savannah zone of Nigeria [1]. Oxidative Stress cause an increase in the body's endogenous antioxidant systems such as superoxide dismutase [2], catalase, glutathione peroxidase [3]; and also input from co-factors, including managanese, zinc, selenium and by the ingestion of exogenous antioxidants, vitamins A, C, E are involved in scavenging reactive oxygen species generated in excess during oxidative stress. [4- 6].

The hot-humid season (May-October), which has a very high rainfall (155.9 ± 26.2 mm), high temperature maxima (30.2 ± 0.6 OC), very high relative humidity ($63.2 \pm 5.6\%$) and low evaporation rate (154.2 ± 9.71 mm) [7], may induce heat stress capable of inducing oxidative stress in the body. Oxidative stress increases the generation of reactive oxygen species in the body, which may decrease the antioxidant activity in the body [6].

In the central nervous system, especially in the brain, ascorbic acid enhances neurogenesis, neural maturation and neurotransmission. It also protects the brain, since its oxidized form dehydroascorbic acid is capable of crossing the blood-brain barrier [8-9]. Zinc inhibits both excitatory and inhibitory receptors. Therefore administration of zinc chelators lowers the threshold for seizure induction, indicating that Zn^{2+} dominant effort in the normal brain is to reduce excitability. The high concentration of zinc-releasing terminals in the neocortex and limbic structure may have a special role in the synaptic plasticity that underlies learning and memory [10-13]. The aim of this study was to investigate the modulatory role of co-administration of ascorbic acid and zinc on some brain biochemical changes in Wistar rats during the hot-humid season.

Correspondence:

Okey, Samuel Maduabuchi

Department of Human
Physiology, Ahmadu Bello
University, Zaria, Nigeria.

Materials and Methods

2.1 Site of Experiment

The experiment was carried out in Physiology Research Laboratory of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria and the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital, Zaria (11° 10' N, 07° 38' E) located at the elevation of 650 m above the sea level (Zaria), in the Northern Guinea Savannah zone of Nigeria [14]. Zaria has an average annual rainfall of 1107 mm [15].

2.2 Animals

A total of 14 adult male Wistar rats, weighing 110 – 186 g were used for the experiment. They were given access to growers mash (Vital Feeds, Jos, Nigeria) and water *ad libitum*. The rats were pre-conditioned to the experimental procedures at least one week before commencement of the experiment.

2.3 Drug Preparation

Ascorbic acid tablets (Mopson Pharmaceutical Limited, Lagos, Nigeria) and zinc gluconate tablets (Good 'N Natural Bohema, USA) were each dissolved in 1ml of distilled water to obtain 100 mg/ml and 50 mg/ml suspension, respectively prior to daily administration.

2.4 Treatment Protocol

The animals were grouped into two of 7 rats each:

Group I, which served as the control animals, were given distilled water at the dose rate of 1 ml/kg.

Group II, which comprised the experimental animals, were first given ascorbic acid at the dose rate of 100 mg/kg and later zinc gluconate at the dose rate of 50 mg/kg dissolved in 1 ml of distilled water.

The regimens were given once and orally by gavage for a period of 28 days.

2.5 Biochemical Test

The rats were sacrificed on day after completion of the administration, each rat was decapitated and the whole brain was collected, homogenized and evaluated for brain catalase, brain superoxide dismutase and brain acetylcholinesterase activities.

2.5.1 Effect of treatment on brain lipoperoxidation

The concentration of thiobarbituric acid-reactive substance, malondialdehyde (MDA), as an index of lipid Peroxidation, was evaluated in the brain sample using NWLSS™ NWK-MDA01 malondialdehyde Assay kit (Northwest Life Science Specialties, LLC, Vancouver, WA 98662), as stated by the manufacturer. The principle is based on the method of Janero [16].

2.5.2 Evaluation of the effect of treatments on brain catalase activity

Catalase activity was determined using NWLSS™ NWK-CAT01 catalase activity assay kit (Northwest Life Science Specialties, LLC, Vancouver, WA 98662), according to the manufacturer's instruction. The procedure used was based on the method of Beers and Sine [17] with slight modifications to increase robustness and convenience.

2.5.3 Evaluation of the effect of treatments on brain superoxide dismutase activity

Superoxide dismutase activity was analyzed using NWLSS™ NWK-SOD02 superoxide dismutase activity assay kit (Northwest Life Science Specialties, Vancouver, WA 98662) as stated by the manufacturer. The procedure was based on the method of Martin *et al.* [18] with slight modifications to increase robustness and reliability.

2.5.4 Evaluation of the effect of treatments on brain acetylcholinesterase activity

Acetylcholinesterase activity was evaluated using the method of Ellman *et al.* [19] with acetylthiocholine iodide as a substrate. Briefly, the whole brain sample of each animal was homogenized in a cold 0.1 M phosphate buffered saline (pH 7.4). Thiocholine released because of the cleavage of acetylthiocholine iodide by acetylcholinesterase was allowed to react with the -SH reagent, 5, 5'- dithiobis-(2-nitrobenzoic acid); which was reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412 nm. The extinction coefficient of the thionitrobenzoic acid was 1.36×10^4 /molar/cm. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer was then taken as a direct estimate of acetylcholinesterase activity.

2.6 Measurement of Meteorological Data

Dry-bulb temperature and wet bulb temperatures inside the laboratory at 7: 00 h , 13: 00 h and 18: 00 h were taken using the wet and dry-bulb thermometer at on days 1, 2, 3, 7, 10, 14, 17, 21, 27, 28 of the experimental period. The relative humidity was calculated using the manufacturer's manual attached and mean heat index was calculated 28.3 ± 0.57 according to Jackson [20].

2.7 Statistical Analysis

Data are expressed as mean \pm SEM. Values of the biochemical parameters were analysed using Student's t-test. The analysis was done using the Statistical Package for Social Scientist (SPSS, Chicago, IL., USA). Values of $P < 0.05$ were considered significant.

3.0 Results

3.1 Thermal Environment Data

Tables 1 and 2 show the thermal environment data and heat index, recorded during the study period. The overall heat index recorded during the experimental period was $28.3^{\circ}\text{C} \pm 0.57$. The mean heat index for 7.00 h, 13.00 h and 18.00 h were $25.6^{\circ}\text{C} \pm 0.48$, $28.3^{\circ}\text{C} \pm 0.79$ and $31.0^{\circ}\text{C} \pm 0.84$ respectively. Minimum and maximum values of recorded heat index recorded at 7.00 h and 18.00 h were 24°C and 34°C , respectively. The heat index obtained at 7.00 h was lower than the value, recorded either at 13.00 h or 18.00 h and was significantly different ($P < 0.05$). Also the heat index recorded at 13.00 h when compared with that of 7.00 h and 18.00 h was significantly different ($P < 0.05$). Furthermore, the highest heat index was recorded at 18.00 h.

Table 1: Mean values of thermal environmental parameters recorded during the study period

Thermal environment data	Mean \pm SEM	Maximum	Minimum
Ambient temperature	26.2 \pm 0.35	30	23
Relative humidity	81.2 \pm 1.52	92	67
Heat index	28.3 \pm 0.57	34	24

Table 2: Diurnal variations in heat index during the study period.

Heat Index	Mean \pm SEM	Maximum	Minimum
7.00 h	25.6 \pm 0.48 ^a	29	24
13.00 h	28.3 \pm 0.79 ^b	33	25
18.00 h	31.0 \pm 0.84 ^c	34	26

Values with different superscript letters are significantly difference ($P < 0.05$)

3.2 Brain Lipid Peroxidation Assessment

Figure 1 represents brain malondialdehyde concentration in μmolml^{-1} of control and ascorbic acid + zinc groups. Malondialdehyde concentration was higher in the ascorbic acid and zinc group (2.13 ± 0.15) than in the control group (1.74 ± 0.12), but the difference in the concentrations was not statistically significant ($P > 0.05$).

3.3 Brain Catalase Activity

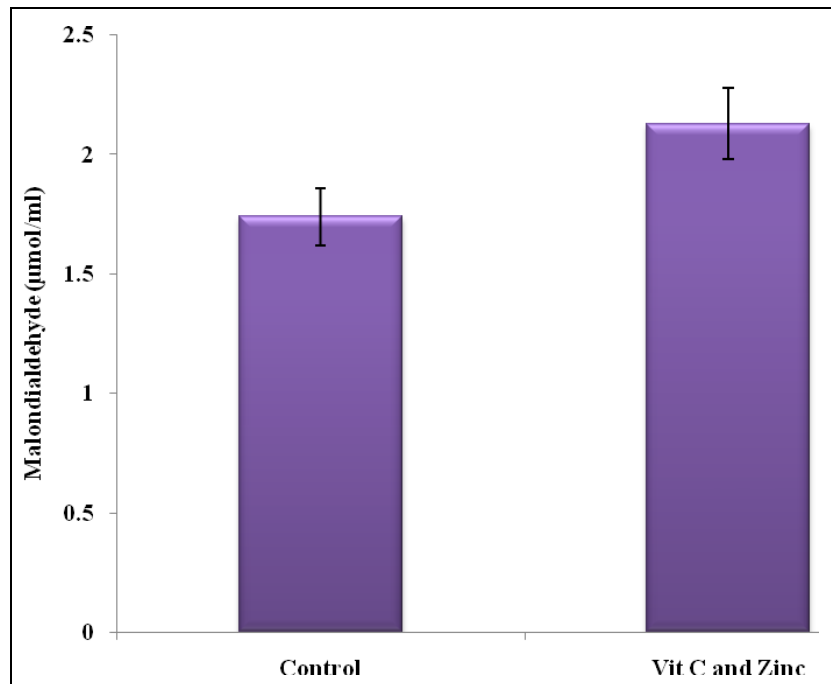
Figure 2 shows brain catalase activity in IU/ml of control and ascorbic acid + zinc groups. There was no significant difference ($P > 0.05$) in the activities of the control group with respect to the ascorbic acid + zinc group, and the values obtained were 44.7 ± 1.66 and 44 ± 1.88 , respectively.

3.4 Brain Superoxide Dismutase Activity

Figure 3 shows brain superoxide dismutase activity in IU/ml of control and ascorbic acid + zinc groups. There was no significant difference ($P > 0.05$) between the activities of the control group with respect to that of the experimental group, and values were 1.83 ± 0.1 and 2.03 ± 0.15 , respectively.

3.4 Assessment of Brain Acetylcholinesterase Activity

Figure 4 shows brain acetylcholinesterase activity in control and experimental groups, showed no significant difference ($P > 0.05$). Value in $\text{nMolmin}^{-1}\text{ml}^{-1}$ of acetylcholinesterase activity in control group was 23 ± 1.21 , while in rats treated with ascorbic acid and zinc gluconate group, the activity was 19.57 ± 1.41 .

**Fig 1:** Effect of ascorbic acid + zinc gluconate on brain malondialdehyde concentration in wistar rats during the hot-humid season. ($n = 7$).

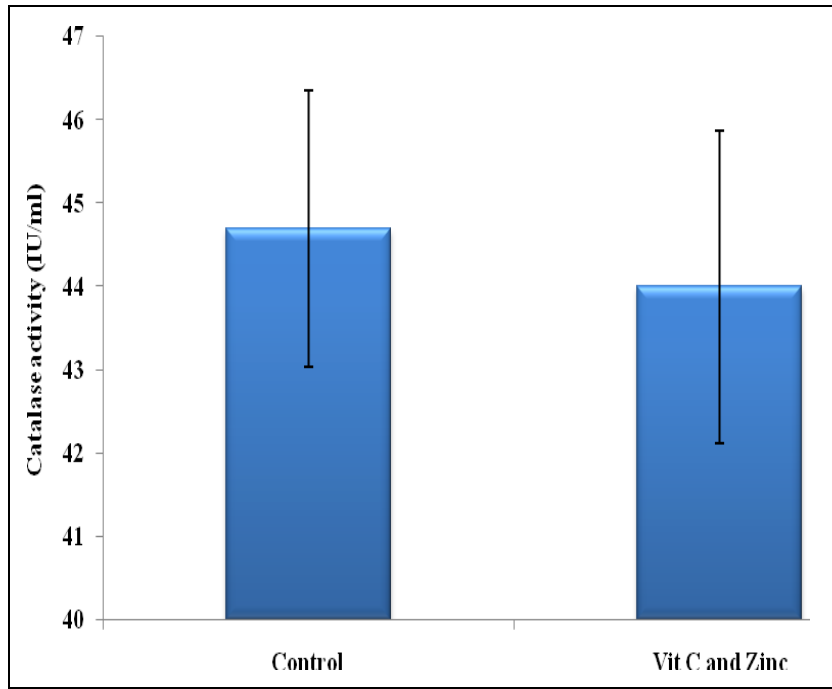


Fig 2: Effect of ascorbic acid + zinc gluconate on brain catalase activity of wistar rats during the hot-humid season. (n = 7)

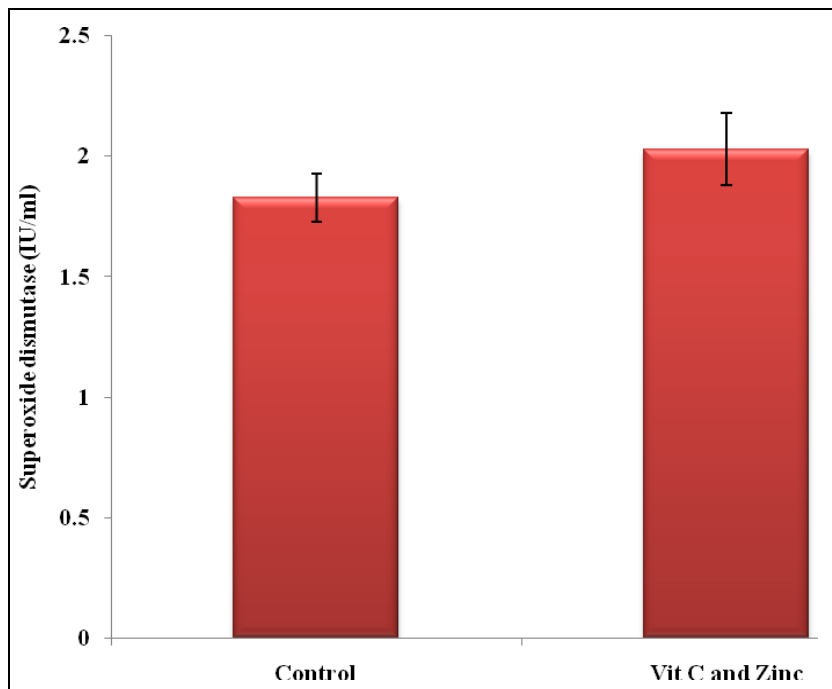


Fig 3: Effect of ascorbic acid + zinc gluconate on brain superoxide activity of wistar rats during the hot-humid season. (n = 7)

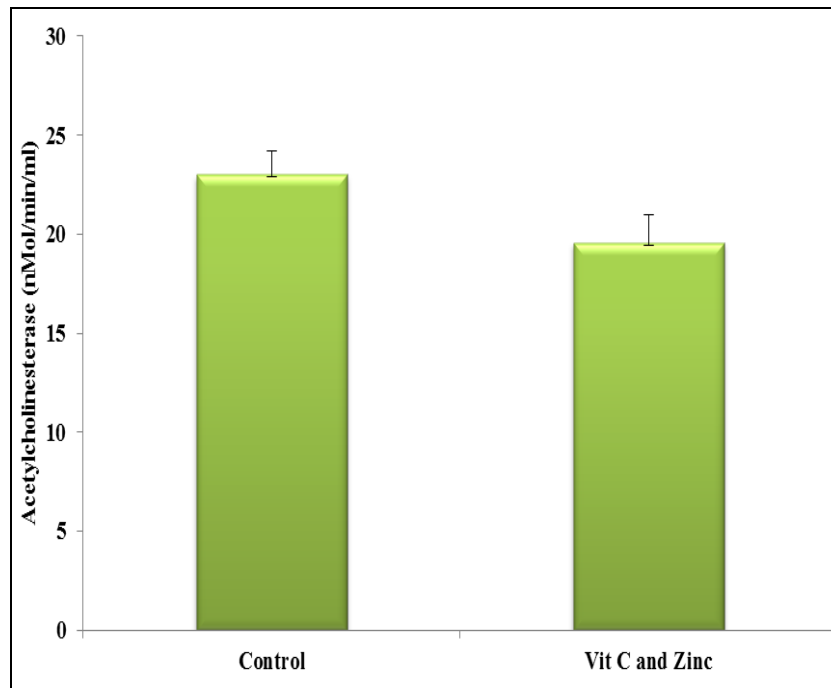


Fig 4: Effect of brain acetylcholinesterase activity of rats, during the hot-humid season. (n = 7).

4. Discussion

The thermal environmental conditions such as relative humidity, ambient temperature, thermal radiation and air speed form a complex system that acts upon human body [21-22]. The conditions are outside the thermal comfort zone. A relevant indicator of the thermal comfort is the temperature–humidity index, also known as heat index, it is the apparent temperature felt by the individual, resulting in behavioural responses and biochemical changes [23-24]. The present results show that mean overall heat index, recorded during the experimental period, corresponding to the peak of rainfall in the study area (Zaria), was $28.3^{\circ}\text{C} \pm 0.57$. The heat index obtained during the study period showed that the experimental animals were exposed to moderate heat stress, characteristic of the hot-humid season (August – September), which was the peak of rainfall, characterized by increased ambient temperature and high relative humidity. The high heat index obtained in the present study is consistent with the findings of Igono and Aliu [7] and Ayo *et al.* [21] that the hot-humid (rainy) season in the Northern Guinea Savannah zone of Nigeria is thermally stressful. Heat stress occurs when there is an imbalance between heat production within the body and its dissipation, resulting in several physiological and behavioural responses in an attempt to adjust to the thermally stressful environment [25].

Heat stress is well known to cause increased lipid peroxidation and reduction in the concentrations of vitamin A, C, E and some minerals in the serum and tissue [26-27]. The malondialdehyde concentrations of $1.74 \pm 0.12 \mu\text{mol/ml}$ and $2.13 \pm 0.15 \mu\text{mol/ml}$ obtained in control and treated rats respectively showed that rats in the ascorbic acid + zinc group had higher malondialdehyde concentration than the control, but the values did not differ significantly. This finding disagrees with the report of Sahin *et al.* [28-30], that serum and tissue malondialdehyde concentrations were lowered in

the group supplemented with dietary ascorbic acid and zinc in heat-stressed poultry.

Sahin *et al.* [28] and Sahin and Kucuk [31] showed that the release of corticosterone and catecholamines initiates lipid peroxidation in membranes of several cells during heat stress, resulting in impaired antioxidant status, decrease serum and tissue concentration of exogenous antioxidants. Catalase and superoxide activities obtained in the present study with values of $44.7 \pm 1.66 \text{ IU/ml}$ and $44 \pm 1.88 \text{ IU/ml}$, $1.83 \pm 0.1 \text{ IU/ml}$ and $2.03 \pm 0.15 \text{ IU/ml}$ respectively for control and ascorbic acid + zinc treated groups showed no significant difference ($P > 0.05$). This finding demonstrated that the thermal environment prevalent during the study period were moderately stressful to the rats, although the increase in enzymatic antioxidant system may be due to the body's response to high reactive oxygen species generated during the hot-humid season [32]. Acetylcholinesterase activity in the brain of control and ascorbic acid + zinc treated group with values of $23 \pm 1.21 \text{ nMolmin}^{-1}\text{ml}^{-1}$, $19.57 \pm 1.41 \text{ nMolmin}^{-1}\text{ml}^{-1}$, respectively showed no significant difference ($P > 0.05$). The lack of increase in acetylcholinesterase activity recorded in the present study disagrees with the findings that increased acetylcholinesterase activity is one of the body's mechanisms of acclimatization to stress, which may have been reduced by ascorbic acid and zinc gluconate [33-35]. The hot –humid season was moderately thermally stressful, although the high compensatory endogenous antioxidant system in the Control rats was witnessed in the study, resulting from narrow diurnal variations in heat index during the hot-humid season.

Conclusion

The hot-humid season is moderately thermally stressful and its negative effects may be attenuated by combined administration of ascorbic acid and zinc gluconate.

Acknowledgement

The Authors do appreciate Mr. Eze E. Daniel, Mr. Isa Ahmed-Shiref, Dr. O. Tunde, Mr. A. Jimoh and Mr. P. Mshelia for all their support during this research work.

Conflicts Of Interest

The Authors declare that, there are no conflicts of interest in this research work.

Reference

- Sinkalu, V.O., Ayo, J.O., Adelaiye, A.B., Hambolu, J.O., 2009. Influence of co-administration of vitamins A and E on diurnal variation in rectal temperature of Black Harco pullets during the hot-dry season. *Biological Rhythm Research*, 40(3): 257-264.
- Heyland, D.K., Dhaliwal, R., Suchner, U., Berger, M.M., 2005. Antioxidant nutrients: systematic review of trace element and vitamins in critically ill patient. *Intensive Care and Medicine*, 31: 327-337.
- Landis, G.N., Tower, J., 2005. Superoxide dismutase evolution and life span regulation. *Mechanism of Ageing and Development*, 126: 128-132.
- Rahman, K., 2007. Studies on free radical, antioxidants and co-factor. *Clinical Interventions in Aging*, 2(2): 219-236.
- Edem, D.O., 2009. Vitamin A: A review. *Asian Journal of Clinical Nutrition*, 1: 65 – 82.
- Sunil Kumar, B.V., Kumar, A., Kateena, M., 2011. Effect of heat stress in tropical livestock and different strategies for its amelioration. *Journal of Stress Physiology and Biochemistry*, 7(1): 45-54.
- Igono, M.O., Aliu, Y.O., 1982. Environmental profile and milk production of Fresian-Zebu cross in Nigerian guinea savanna. *International Journal of Biometerology*. 26 (2): 115-120.
- Naidu, K.A., 2003. Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal*, 2-7.
- Harrison, F.E. and May, J.M., 2009. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radical Biology and Medicine*, 46: 719-730.
- Takeda, A., 2001. Zinc homeostasis and functions of zinc in the brain. *Biomaterials*, 14(3-4): 343-351.
- Frederickson, C.J., Won Suh, S., Siva, D., Frederickson, C.J., Thompson, R.B., 2000. Importance of zinc in the central nervous system: the zinc-containing neuron. *The Journal of Nutrition*, 130(5): 1471-1483.
- Sensi, S.L., Paoletti, P., Bush, A.I., Sekler, I., 2009. Zinc in the physiology and pathology of the central nervous system. *Nature Review Neuroscience*, 10: 780-791.
- Tian, Y., Yang, Z., Zhang, T., 2010. Zinc ions modulator effects on excitability and synaptic transmission in hippocampal CA1 neurones in Wistar rats. *Neuroscience Research*. 68(3): 167-175.
- Akpa, G.N., Asiribo, O.E., Alawa, J. P., Dim, N.L., Osinowo, O.A., Abubakar, B.Y., 2002. Milk production by agropastoral Red Sokoto goats in Nigeria. *Tropical Animal Production*, 34: 526-533.
- Abdulwahab, A., Mabrouk, M.A., Ayo, J.A., Ambali, F.A., Muftau, S., Adenkola, A.Y., Salawu, E.A., 2010. Effects of co-administration of antioxidants on erythrocyte osmotic fragility of wistar rats during the hot-dry season. *European Journal of Scientific Research*, 46: 73-79.
- Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid Peroxidation and peroxidative tissue injury, *Free Radical, Biology Medicine*, 9: 515-540.
- Beer Jr, R.F., Sizer, I.W., 1952. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195: 133-140.
- Martin Jr, J.P., Dailey, M., Sugarman, E., 1987. Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation, *Archives of Biochemistry and Physics*, 255: 329-336.
- Ellman, G.C., Cortney, K.O., Andres, V., Fetherstone, R.M., 1961. A new and rapid colometric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7: 88-95.
- Jackson, K.Y., 2009. Heat index calculator. National weather service forecast office. Available at [http://www.crh.noaa.gov/jkl/?n=heat index calculator] accessed (05/06/2013).
- Ayo, J.O., Dzenda, T., Zakari, F.O., 2008. Individual and diurnal variations in rectal temperature, respiration and heart rate of pack donkeys during the early rainy season. *Journal of Equine Veterinary Science*, 28(5): 281- 288.
- Ayo, J.O., Obidi, J.A., Rekwot, P.I., 2011. Effect of heat stress on the well-being, fertility, and hatchability of chickens in the North Guinea Savannah zone of Nigeria: a review. *International Scholarly Research Network Veterinary Science*, doi:10.5402/2011/838606.
- Kendall, P.E., Webster, J.R., 2009. Season and Physiological effects the circadian body temperature rhythm of dairy cows. *Livestock Science*, 125(2): 155-160.
- Dzenda, T., Ayo, J. O., Lakpini, C. A.M., Adelaiye, A. B., 2011. Diurnal, seasonal and sex variations in rectal temperature of African Giant rats. *Journal of Thermal Biology*, 36: 255-263.
- Enander, A.E., 1990. Thermal stress and human performance. *Scandinavian Journal of Work and Environmental Health*, 16(1): 44-50.
- Sahin, K., Sahin, N., Onderci, M., Yaralioglu, S., Kucuk, O., 2001. Protective role of supplemental Vitamin E on lipid peroxidation, vitamin E, A and some mineral concentrations of broilers rear under heat stress. *Vet. Med-Czech*, 46(5):140-144.
- Altan, O., Pabuccuoglu, A., Altan, A., Konyalioglu, S., Bayraktar, H., 2003. Effect of thermal stress on oxidative stress, lipid peroxidation and some stress parameter in broilers. *British Poultry Science*, 44(2): 545-550.
- Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Kuck, O., 2003a. Dietary Vitamin C and Folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. *The Journal of Nutrition*. 133(6): 1882-1886.
- Sahin, K., Sahin, N., Onderci, M., Gursu, M.F., Issi, M., 2003b. Vitamin C and E can alleviates negative effects of heat stress in Japanese quails. *Food Agriculture and Environment*, 1(2): 244-249.
- Sahin, K., Sahin, N., Kucuk, O., Hayirli, A., Prasad, A.S.

2009. Role of dietary zinc in heat stress poultry. A review. *Poultry Science*, 88(10): 2176-2183.
31. Sahin, K., Kucuk, O., 2003. Zinc supplementation alleviates heat stress in laying Japanese quail. *The Journal of Nutrition*, 133(9): 2808 – 2811.
32. Park, H., Ahn, I., Park, K., Hyun, K., 2008. Response of antioxidant defense systems to thermal stress in the Antarctic Clam, *Laternula elliptica*. *Antarctic Science*, 20(6): 521-526.
33. Hussein, M.F., Mohammed, M. I., Selim, M.F., El Din, M.A., 1983. Ambient temperature effects on brain and spinal cord acetylcholinesterase activity of two poikilotherms. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 75(2): 357-359.
34. Mohammed, I.M., Mora, S.A., Mervat, F.S., 1983. Effect of ambient temperature on brain acetylcholinesterase activity and protein content in three Egyptian vertebrates. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 76(1): 127-130.
35. Adejumo, D.O., Egbunike, G. N., 1988. Effect of thermal stress and water deprivation on the acetylcholinesterase activity of pig brain and hypophyses. *International Journal of Biometeorology*, 32(2): 108-111.