Oxidative stress biomarkers in West African Dwarf goats reared under intensive and semi-intensive production systems

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Abstract

This study explored the variation in physiological oxidative status of West African Dwarf (WAD) goats as the rainy season progressed in a humid climate in south-west Nigeria. A total of 24 growing WAD bucks, averaging 10.5 kg $\pm$ 0.78 liveweight, were reared under intensive and semi-intensive management systems during the rainy season. Twelve (12) bucks were used for each management system. Animals raised intensively were fed *Megathyrsus maximus* hay *ad libitum*, while those reared semi-intensively were allowed to graze freely in a fenced paddock. Their diets were supplemented with a maize grain-based concentrate. Blood samples were collected at the onset of the experiment and fortnightly throughout the 16-week experimental period. Oxidative stress biomarkers in blood were measured and analysed separately for each rearing system using repeated measures analysis. Means of oxidative stress biomarkers measured at the beginning and end of the rainy season were compared using a T-test. Results showed that intensively managed goats had significantly higher levels of bilirubin and uric acid in the early season than in the late rainy season. In semi-intensively managed goats, bilirubin, uric acid, and glutathione (GSH) levels were significantly higher at the start compared with the end of the rainy season. It could be concluded that the antioxidant capacity of WAD goats was greater early in the rainy season compared with the late rainy season under both management systems. Thus, during the early raining season WAD goats tend to have higher antioxidant capacity and, consequently, better immune responses, while the opposite is true during the late rainy season.

Keywords: bucks, immune response, season

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Introduction

West African dwarf (WAD) goats are an extremely important livestock species in developing countries such as Nigeria. They produce milk, meat, skins, manure and are commonly used for sacrificial purposes and ceremonies (Aziz, 2010). Despite their unique and desirable characteristics, which include their hardy nature, adaptability to harsh climates, excellent grazing habits and physiological characteristics; very little research effort has been directed towards their physiological responses to challenges posed by the environments in which they are reared.

Different management systems and the stage of the rainy season can induce various levels of discomfort in WAD goats and thus affect their physiological functions and performance. West African dwarf goats have been reported to be well adapted to the agro-ecological conditions, but not under all production systems. These goats are reared mainly through extensive and semi-intensive production. There is little or no documentation of their performance under a full intensive production system except for research and on a few commercial farms. Ajala *et al*. (2008) confirmed this in their report, which indicated a low percentage of WAD goats under the intensive system of management. Furthermore, in the face of recent climatic change, research has not been carried out to re-check and affirm their productivity. In semi-intensive systems, animals are not completely confined, but are allowed to move around in search of feed or simply to exhibit normal behaviours. This is the most common system in most humid tropics because it is characterized by high production potential and is less labour intensive than the intensive and extensive systems (Ajala *et al*.,

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2008). Under this system, animals are allowed to graze between 5 and 6 hours a day before supplementing with forage or crop residues. In some instances, they may be provided with shelter against adverse weather conditions (Ajala *et al*., 2008). To meet the demand for animal protein, farmers are shifting to intensive management systems, which involve the total confinement of animals, resulting in restriction of movement and of other natural behaviours of animals (Thornton, 2010). In this type of system, animals are provided with feed and water (Weaver, 2005). These production systems are likely to impose various levels of stress on animals, resulting in variations in their physiological responses, and consequently productivity levels. Intense and prolonged exercise and physical activity can result in oxidative damage to proteins and lipids in the contracting myocytes (Radovanović *et al*., 2004; Powers & Jackson, 2008), which may be a possibility in animals reared semi-intensively. In addition, the various production systems are intrinsically linked with animal health, production and welfare problems (Celi & Chauhan, 2013). Oxidative stress occurs when there is an imbalance between the production of reactive oxygen molecules and the ability of a biological system to detoxify these reactive oxygen intermediates or to repair the resulting damage (Celi, 2011). Diseases and metabolic disorders such as acidosis, ketosis, sepsis, mastitis, enteritis, pneumonia and respiratory disorders are a result of oxidative stress (Celi, 2011).

The ambient environment affects the performance, adaptability, and profitability of animals by changing the physico-biochemical (respiratory rate, heart rate, pulse rate, elevated blood levels, etc.) and hormonal profile of livestock. The change in ambient temperature results in increase in body surface temperature, respiration rate (RR), heart rate and rectal temperature (RT), which affects feed intake, production and reproductive efficiency of animals. The progression of a season is accompanied by changes in climatic conditions and in quantity and quality of available feed for animals. Change in environmental temperature is also known to have a significant effect on oxidative stress biomarkers. Therefore, temperature is a confounding factor that may result in difficulties in the interpretation of oxidative stress biomarker response patterns (Vinagre *et al*., 2014).

The dynamics of oxidative stress biomarkers in goats is a relatively new field of research in small animal health. There is a paucity of information on the levels of oxidative stress biomarkers in small ruminant animals, especially in tropical rearing systems and climatic conditions. This study was therefore designed to carry out an exploratory investigation of the effect of stages of rainy season (early and late rainy season) on oxidative stress biomarkers in WAD goats raised in both intensive and semi-intensive production systems.

Materials and Methods

The experiment was carried out at the Small Ruminant Unit of the Directorate of University Farm, Federal University of Agriculture, Abeokuta (UNAAB), Ogun State. The site is located in the rain forest vegetation zone of south-western Nigeria at latitude 7° 13’ 49.46’’ N, longitude 3° 26’ 11.98’’ E and altitude of 76 m above the sea level. The climate is humid, with a mean annual rainfall of 1037 mm and mean temperature and humidity of 34.7 °C and 83%, respectively (Google Earth 6.0, 2012).

A total of 24 growing WAD goat bucks, weighing an average of 10.5 $\pm$ 0.78 kg, were used in this experiment. They were purchased from villages around the university. The experimental pens were cleaned and disinfected with Morigad®, one week prior to the arrival of the experimental animals. After purchase, the animals were quarantined for three weeks, during which they were washed with 1% coumaphos (Asumol 50 Bayer, Germany) solution to kill ecto-parasites. The animals were vaccinated against *Peste des Petit Ruminants* (PPR) using tissue culture rinderpest vaccine obtained from National Veterinary Research Institute, VOM, Nigeria. The experiment was carried out from May to October 2013. Data for the early rainy season were collected in June and July and data for late rainy season were collected in September and October. The average temperature in June and July ranged between 25.5 and 27.15 °C and in September and October it ranged between 24.84 and 25.65 °C. The total rainfall ranges between 53.7 and 202.6 mm for the early rainy season while it is between 94.4 and 139 mm for the late rainy season. The 16-week experimental trial consisted of two simultaneous experiments, in which goats were reared under intensive and semi-intensive management systems. Under the intensive management system, the animals were housed individually in a pen (1.5 m x 1.5 m) made of a raised slated floor with corrugated iron sheet roofing. Those on semi-intensive were raised in a fenced paddock (37.00 m x 18.23 m) sown with natural pasture.

The 24 WAD bucks were randomly divided into two groups of 12 animals and allocated to the intensive or the semi-intensive system. Each animal constituted an experimental replicate under each management system. In the intensive management system, the goats were offered wilted *Megathyrsus maximus ad libitum*, while those on the semi-intensive system were allowed to graze for 6 hours per day (9:00 am–3.00 pm) in a fenced paddock sown with natural pasture *(Commelina erecta, Axonopus compressus, Ageratum conyzoides, Sida corymbosa, Chromolaena odorata*, *Heterophyllum indicum, Acalypha ciliata, Andropogon tectorium, Plastostoma africanum, Physalis angulata, Hyptis lanceolata* and *Eleusine indica)*. Both groups were offered a concentrate diet at 4 % of their bodyweight daily at 3.00 pm.

Water was provided *ad libitum.* The concentrate diet was made up of cassava peel (3.53 %), wheat offal (17.7 %), whole maize (34.33 %), maize bran (26 %), palm kernel cake (PKC, 16.67 %), bone meal (0.50 %), oyster shell (0.50 %), and salt (0.50 %) obtained from UNAAB Leventis Agro Allied, Kotopo, Abeokuta, Ogun State. The ingredients were milled into a coarse form and mixed together to form a concentrate diet containing 12 % crude protein. All procedures used in this experiment were in accordance with the ethical standards of the College of Animal Science and Livestock Production Committee on Animal Experimentation. The experiment proceeded after the approval of the proposal by the animal welfare specialist at the college (ethical clearance number COLANIM/APH/PG/0080).

At 07h00 on each day of blood collection, 5 ml blood was collected directly from jugular vein of each animal using hypodermic needle and syringe. This was done at the beginning and subsequently at two-week intervals throughout the 16-week experiment. The blood (5 mL) was released into sample bottles containing lithium oxalate as an anticoagulant and shaken thoroughly to ensure proper mixing of the blood with the anti-coagulant. Albumin, bilirubin, uric acid, superoxide dismutase (SOD), thiobarbituric acid substance (TBARS), glutathione, and protein thiol were determined in blood immediately after collection. Albumin concentration was determined in the blood as described by Maier *et al*. (2006) and Shin *et al*. (2009). Bilirubin was assayed as described by Nedredal *et al.* (2009) and Beppu *et al.* (2009). Superoxide dismutase (SOD) activity in blood serum was determined by the method of Janknegt *et al* (2007), thiobarbituric acid substance was determined using the method of Buege & Aust (1978), and glutathione was determined according to the procedures of Wang *et al*., (2007) while the thiol was measured by spectrophotometry (412 nm) using DTNB (5, 5′-dithiobis 2-nitrobenzoic acid) as described by Faure & Lafond (1995).

The data were analysed separately for each management system (intensive and semi-intensive system) to circumvent possible confounding between rearing system and diet. Concentration of the biomarkers of oxidative stress in WAD goats was analysed by the ANOVA for repeated measures procedures of SAS (2010). The overall means for early and late rainy season were separated using the T-test as contained in the same statistical package.

Results

From the repeated measures analysis of intensively managed goats, statistical significance (*P* values) of the effect of main factors (stage of rainy season and weeks) and their interaction are presented in Table 1. Stage of rainy season had no effect (P >0.05) on most of the oxidative stress biomarkers, except bilirubin and uric acid. Week differed significantly in albumin, bilirubin, uric acid, TBARS and GSH concentration, but did not affect SOD and thiol concentration. Albumin, bilirubin, uric acid and TBARS concentration were statistically different (P <0.05) by the interaction between the stage of rainy season and weeks. During the early rainy season, bilirubin concentration significantly increased with time from the commencement of the experiment, reaching a maximum at week 4, before declining until the end of the experiment (Figure 1). However, during the late rainy season bilirubin concentration significantly decreased from the beginning of the experiment to the second week of the study, then increased slightly till the end of the experiment.

Table 1 Statistical significance (*P* values) of main effects of season and weeks, and their interaction on blood parameters in intensively managed goats

Parameters	Season	Week	Season x Week
Albumin (g/dl)	NS	*	*
Bilirubin (mg/dl)	*	*	*
Uric acid (mg/dl)	*	*	*
TBARS (µM MDA equivalent)	NS	*	*
SOD (units/Mol)	NS	NS	NS
GSH (µM)	NS	*	NS
Thiol (µM)	NS	NS	NS

*P <0.05
NS: Not significant
TBARS: thiobarbituric acid substance, SOD: superoxide dismutase, GSH: glutathione
g/dl: gram per decilitre, mg/dl: milligram per decilitre, units/Mol: units per mole, µM: micrometre

In the late rainy season, uric acid concentration significantly decreased from the onset of the experiment till the fourth week, in contrast to the early rainy season when uric acid levels increased from the beginning to the end of the experiment (Figure 2).

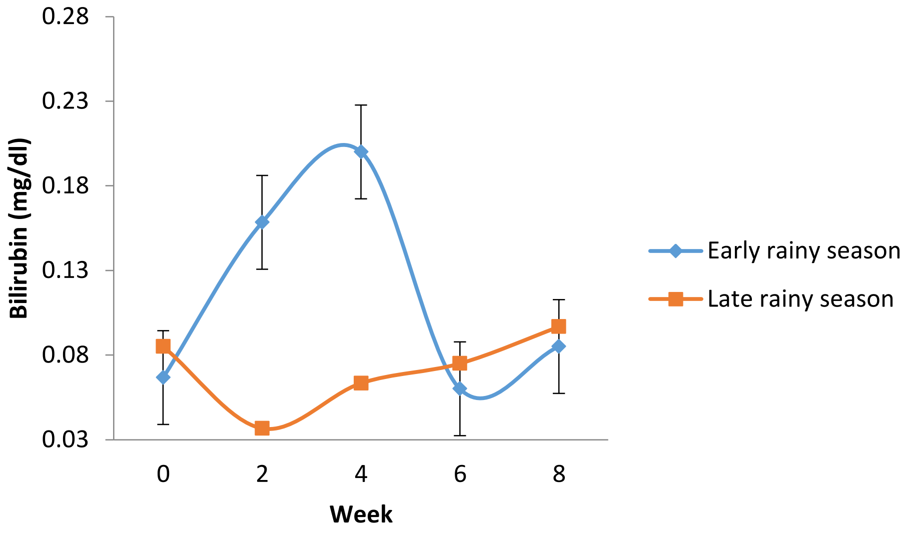

Figure 1 Effect of stage of rainy season on mean values of bilirubin concentration in intensively raised goats

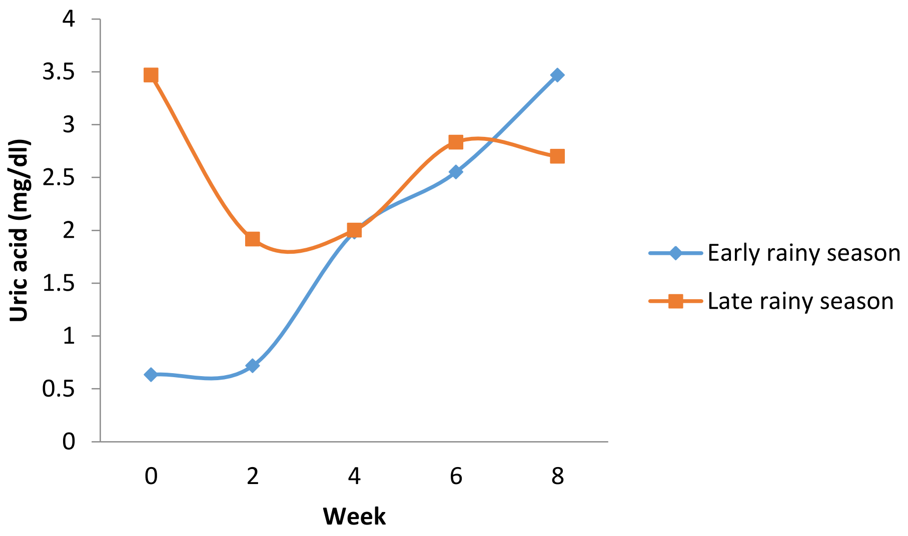

Figure 2 Effect of stage of rainy season on mean values of uric acid concentration in intensively raised goats

Table 2 shows the *P* values of main effects of season and weeks and their interaction on blood parameters in semi-intensively managed goats as obtained from repeated measures analysis. Bilirubin, uric acid, TBARS and GSH concentration differed (P <0.05) by stage of rainy season. However, albumin, SOD and thiol concentration were not statistically different (P >0.05). Week influenced (P <0.05) most of the oxidative stress biomarkers except albumin and thiol concentration. Stage of rainy season and week interaction significantly influenced albumin, uric acid, SOD, GSH, and thiol concentration in the blood.

Table 2 Statistical significance (*P* values) of the effect of season and weeks and their interaction on blood parameters in semi-intensively managed goats

Parameters	Season	Week	Season x Week
Albumin (g/dl)	NS	NS	*
Bilirubin (mg/dl)	*	*	NS
Uric acid (mg/dl)	*	*	*
TBARS (µM MDA equivalent)	*	*	NS
SOD (units/Mol)	NS	*	*
GSH (µM)	*	*	*
Thiol (µM)	NS	NS	*

*P <0.05
NS: not significant
TBARS: thiobarbituric acid substance, SOD: superoxide dismutase, GSH: glutathione
g/dl: gram per decilitre, mg/dl: milligram per decilitre, units/Mol: units per mole, µM: micrometre

Bilirubin concentration was higher (P <0.05) in the early rainy season than late rainy season from the commencement of the experiment till the fourth week (Figure 3). Uric acid concentration significantly decreased from the onset of the experiment till the third week during the late rainy season, while a significant increase in the level of uric acid was observed during the early rainy season (Figure 4). The TBARS concentration in goats during the early rainy season was significantly (P <0.05) lower compared with the late rainy season (Figure 5). GSH concentration in goats was higher during the late rainy season reaching its peak at the sixth week (Figure 6).

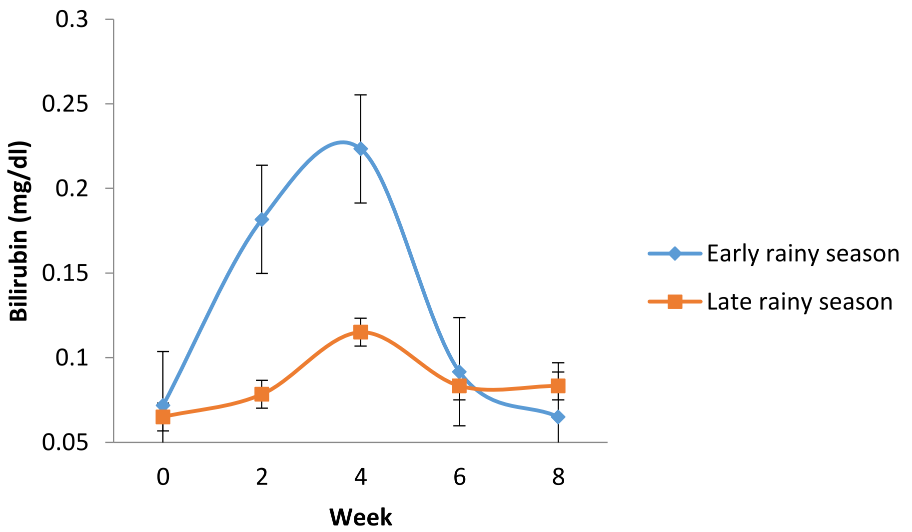

Figure 3 Effect of season on mean values of bilirubin concentration in semi-intensively raised goats

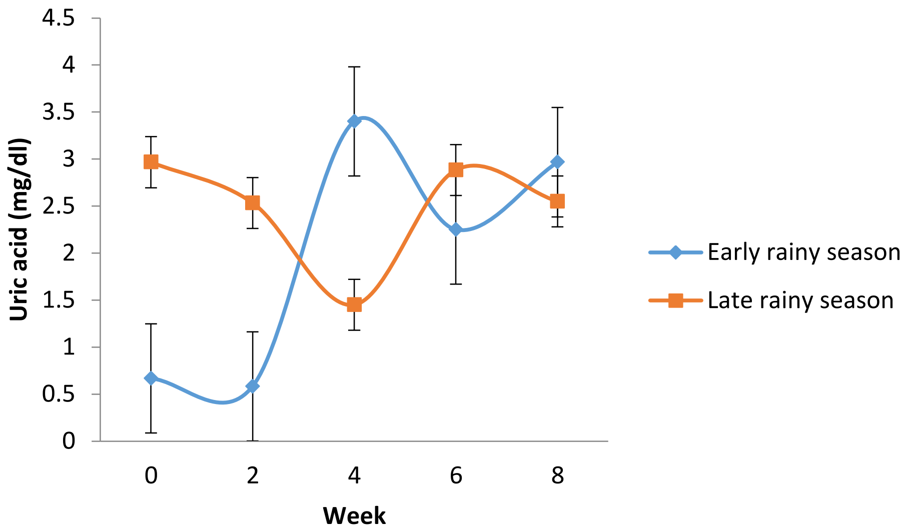

Figure 4 Effect of season on mean values of uric acid concentration in semi-intensively raised goats

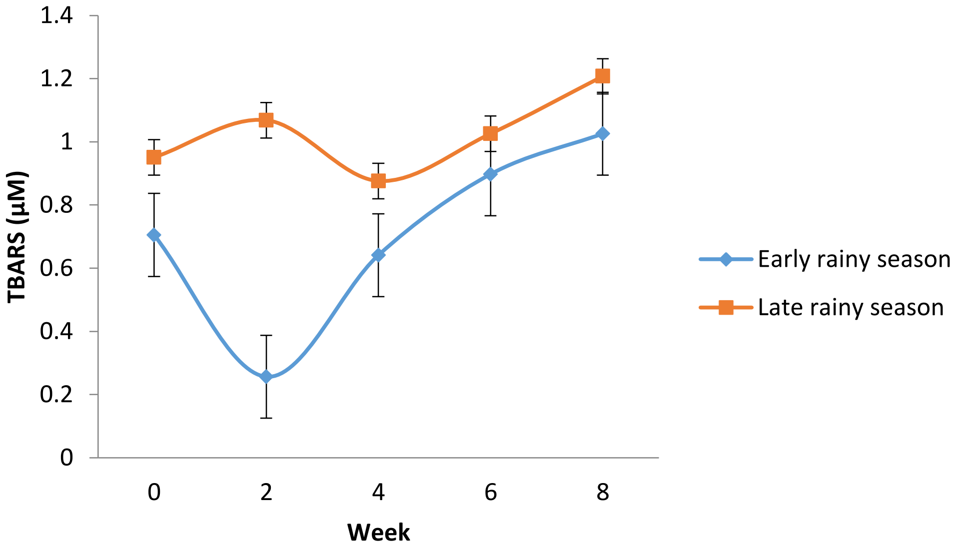

Figure 5 Effect of season on mean values of thiobarbituric acid concentration in semi-intensively raised goats

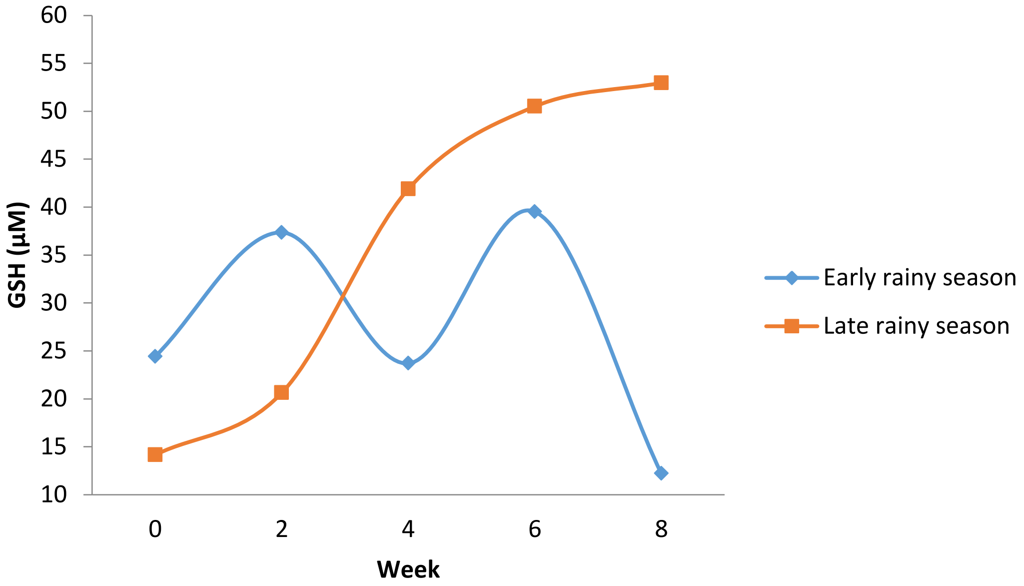

Figure 6 Effect of season on mean values of GSH concentration in semi-intensively raised goats

The mean values of overall effect of stage of rainy season on the oxidative stress biomarkers in intensively raised goats from the T-test analysis are presented in Table 3. Stage of rainy season had no influence (*P* >0.05) on all the parameters. Albumin, bilirubin, uric acid, TBARS, SOD, GSH and thiol concentration were not statisticaly affected (*P* >0.05) by stage of rainy season in semi-intensively managed goats (Table 4).

Table 3 Effect of stage of rainy season on oxidative stress biomarkers in intensively raised goats

Parameters	Early Rainy Season	Late Rainy Season	SEM
Albumin (g/dl)	3.60	3.49	0.17
Bilirubin (mg/dl)	0.11	0.07	0.02
Uric acid (mg/dl)	1.87	2.58	0.41
TBARS (µM)	0.75	1.11	0.08
SOD (units/Mol)	61.02	65.50	13.41
GSH (µM)	25.29	28.80	4.44
Thiol (µM)	262.97	246.60	7.60

SEM: standard error of mean
TBARS: thiobarbituric acid substance, SOD: superoxide dismutase, GSH: glutathione
g/dl: gram per decilitre, mg/dl: milligram per decilitre, units/Mol: units per mole, µM: micrometre

Discussion

Management practices are intrinsically linked with animal health, production and welfare. To efficiently deliver high quality animal products, farmers must practise good animal husbandry (Celi & Chauhan, 2013). Most of the results of this study show that under intensive management bilirubin and uric acid concentration were influenced by stage of rainy season. Bilirubin, the end product of heme catabolism in mammals, is generally regarded as potentially a cytotoxic, lipid-soluble waste product that needs to be excreted. It has been demonstrated, using *in vitro* and *in vivo* studies, that bilirubin exhibits potent anti-oxidant properties that prevent the oxidative damage triggered by a wide range of oxidant-related stimuli (Tomaro & Batlle, 2002). Also, uric acid has been reported by Ames *et al*. (1981) to be a powerful antioxidant and a scavenger of

Table 4 Effect of stage of rainy season on oxidative stress biomarkers in semi-intensively raised goats

Parameters	Early Rainy Season	Late Rainy Season	SEM
Albumin (g/dl)	3.46	3.35	0.15
Bilirubin (mg/dl)	0.13	0.09	0.02
Uric acid (mg/dl)	1.97	2.48	0.43
TBARS (µM)	0.66	1.03	0.07
SOD (units/Mol)	39.03	43.81	5.35
GSH (µM)	27.45	36.03	6.35
Thiol (µM)	245.80	245.37	20.10

SEM: standard error of mean
TBARS: thiobarbituric acid substance, SOD: superoxide dismutase, GSH: glutathione
g/dl: gram per decilitre, mg/dl: milligram per decilitre, units/Mol: units per mole, µM: micrometre

singlet oxygen and radicals. At physiological concentrations, urate reduces the oxo-heme oxidant formed by peroxide reaction with haemoglobin, protects erythrocyte ghosts against lipid peroxidation, and protectserythrocytes from peroxidative damage leading to lysis. The increased bilirubin and uric acid levels in goats reared intensively during the early rainy season showed an absence of oxidative stress. Concentrate supplementation with adequate protein and energy have been reported to enhance the physiological performance of ruminant animals (NRC, 2005). As such, the increased level of the biomarkers might be attributed to the higher quality of the grasses available during early rainy season. Seasonal variations affect the availability of nutrients from the soil to forage species (Ezenwa *et al*., 1995). The nutritional values of forage species tend to be low in the late wet season compared with the early wet season (Buxton, 1996). This is as a result of the association between forage nutrient content and the amount of moisture found in the soil in which the forage plants grow (McDowell *et al.,* 1983). According to McDowell *et al*. (1983), concentrations of nutrients in forage plants are dependent on the interaction of a number of factors. These are plant species, soil type, plant age, pasture management and climate. Differences in forage quality between grasses and legumes can be large. This variation during the period of low moisture can lead to nutritional stress in animals owing to low nutrient uptake by plants and subsequent lignification of the forage.

The higher levels of bilirubin and uric acid during the early rainy season in semi-intensively reared goats indicate the absence of oxidative stress during this period. Elevated levels of bilirubin and uric acid serve as potential antioxidants. The decrease noticed during the late rainy season suggests that at some point during the transition period between the early and late rainy seasons, oxidative stress may have set in. This can be attributed to change in environmental temperature and change in the quality of the forage because as pasture plants mature, intake and digestibility decline. Heat stress increases the oxidative status of an animal, leading to enhanced production of free radicals that decrease the antioxidant defence system (Trevisan *et al*., 2001). An increase in TBARS was noticed during the early rainy season. This can be attributed to the lack of specificity of the TBARS assay because no increase was observed for SOD in this period. According to Alessio (2000), TBARS can react with saturated and unsaturated non-functional aldehydes, carbohydrates and prostaglandins, which would affect its stability.

Higher levels of glutathione observed in goats in early rainy season can be attributed to adequate forage of good quality, coupled with the supplemented concentrates, which allowed the goats to meet their nutrient requirements. Semi-intensive management systems are usually employed in the tropics to allow goats to exhibit their natural behaviour as browsers, allowing them to pick nutritious plants when grazing. This improves their physiological performance as their nutrient requirements are met because pf their selectivity.

Conclusion

It could be concluded from the results of this study that antioxidant levels increased during the early stage of rainy season in intensively and semi-intensively managed goats. Additionally, the levels of the antioxidant increased with time in the early stage of the rainy season. The non-significant overall means can serve as reference values for the WAD goats raised in intensive or semi-intensive production systems.

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Authors' Contributions

AOY and OSS were in charge of project design and implementation, AOY and VM were in charge of writing the manuscript. AOY and RS were in charge of data analysis and interpretation.

Conflict of Interest Declaration

The authors declared that there is no conflict of interest in the course of this research.

References

Ajala, M.K., Lamidi, O.S. & Otaru, S.M., 2008. Peri-urban small ruminant production in northern Guinea savannah, Nigeria. Asian J. Anim. Vet. Adv. 3(3), 138-146.

Alessio, H.M., 2000. In: Handbook of oxidants and antioxidants in exercise. Eds: Hanninen, O., Packer, L. & Sen, C.K., Elsevier, Amsterdam, pp. 115-128.

Ames, B.N., Cathcart, R., Schwiers, E. & Hochstein, P., 1981. Uric acid provides an antioxidant defense in humans against oxidant-and radical-caused aging and cancer: A hypothesis. Proceedings of the National Academic of Sciences, 78(11), pp.6858-6862.

Aziz, M.A., 2010. Present status of the world goat populations and their productivity. World 861(1078.2)

Beppu, F., Niwano, Y., Tsukui, T., Hosokawa, M. & Miyashita, K., 2009. Single and repeated oral dose toxicity study of fucoxanthin (FX), a marine carotenoid, in mice. J. Toxicol. Sci. 34(5), 501-510.

Buege, J.A. & Aust, S.D., 1978. [30] Microsomal lipid peroxidation. Meth. Enzymol., 52, 302-310.

Buxton, D.R., 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Technol. 59(1), 37-49.

Celi, P., 2011. Biomarkers of oxidative stress in ruminant medicine. Immunopharmacol. Immunotoxicol. 33(2), 233-240.

Celi, P. & Chauhan, S.S., 2013. Oxidative stress management in farm animals: Opportunities and challenges. In: Proceedings of the 4th International Conference on Sustainable Animal Agriculture for Developing Countries (SAADC), pp. 95-109.

Ezenwa, I., Reynolds, L., Aken'Ova, M.E., Atta-Krah, A.N. & Cobbina, J., 1995. Cutting management of alley cropped leucaena/gliricidia-Guinea grass mixtures for forage production in southwestern Nigeria. Agrofor. Syst. 29(1), 9-20.

Faure, P. & Lafond, J.L., 1995. Measurement of plasma sulfhydryl and carbonyl groups as a possible indicator of protein oxidation. In: Analysis of free radicals in biological systems. Birkhäuser Basel, pp. 237-248.

Google Earth for Desktop. Google Earth. Google. Retrieved 22 February 2012

Janknegt, P.J., Rijstenbil, J.W., Van de Poll, W.H., Gechev, T.S. & Buma, A.G., 2007. A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae. J. Photochem. Photobiol. B, Biol. 87(3), 218-226

Maier, S.M., Gross, J.K., Hamlin, K.L., Maier, J.L., Workman, J.L., Kim-Howard, X.R., Schoeb, T.R. & Farris, A.D., 2007. Proteinuria of nonautoimmune origin in wild-type FVB/NJ mice. Comp. Med. 57(3), 255-266.

McDowell, L.R., Conrad, G.H., Ellis, G.L., & Loosli, J.K., 1983. Minerals for grazing ruminants in tropical regions. University of Florida Gainesville. Florida. 13:121-128.

Nedredal, G.I., Amiot, B.P., Nyberg, P., Luebke-Wheeler, J., Lillegard, J.B., McKenzie, T.J. & Nyberg, S.L., 2009. Optimization of mass transfer for toxin removal and immunoprotection of hepatocytes in a bioartificial liver. Biotechnol. Bioeng. 104(5), 995.

Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Welham, S.J., Kane, A.F., Gilmour, A.R., Thompson, R., Webster, R. & Wilson, G.T., 2005. The Guide to GenStat Release 8, Part 2: Statistics. VSN International.

Powers, S.K. & Jackson, M.J., 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol. Rev. 88(4), 1243-1276.

Radovanović D, Aleksandrović M, & Ranković G., 2004. The effects of water polo training on aerobic power and pulmonary function in 11- and 12-year -ld boys. Acta Fac. Med. Naiss. 21 (3): 137-141

Shin, S.Y., Choi, G.S., Park, H.S., Lee, K.H., Kim, S.W. & Cho, J.S., 2009. Immunological investigation in the adenoid tissues from children with chronic rhinosinusitis. JAMA Otolaryngol. Head Neck Surg. 141(1), 91-96.

Thornton, P.K., 2010. Livestock production: Recent trends, future prospects. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1554), 2853-2867. http://doi.org/10.1098/rstb.2010.0134

Tomaro, M.L. & del C Batlle, A.M., 2002. Bilirubin: Its role in cytoprotection against oxidative stress. Int. J. Biochem. Cell Biol. 34(3), 216-220.

Trevisan M., Browne, R., Ram, M., Muti, P., Freudenheim, J, Carosella, A.N. & Armstrong, D., 2001. Correlates of markers of oxidative status in the general population. Am. J. Epidemiol. 154: 348-356.

Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J. & Diniz, M.S., 2014. Effect of increasing temperature in the differential activity of oxidative stress biomarkers in various tissues of the rock goby Gobius paganellus. Mar. Environ. Res. 97, 10-14.

Wang, X.J., Sun, Z., Chen, W., Eblin, K.E., Gandolfi, J.A. & Zhang, D.D., 2007. Nrf2 protects human bladder urothelial cells from arsenite and monomethylarsonous acid toxicity. Toxicol. Appl. Phamacol. 225(2), pp.206-213.

Weaver, S., 2005. Sheep: Small-scale sheep keeping for pleasure and profit 3. Burroughs Irvine, Calif 92618.