

PHYSIOLOGICAL VARIATIONS AND OXIDATIVE STATUS IN WEST AFRICAN DWARF MALE SHEEP AND GOAT IN SEMI INTENSIVE MANAGEMENT SYSTEM

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ABSTRACT

In a study to evaluate variations in physiological indices of West African Dwarf (WAD) rams and bucks, 16 apparently healthy WAD rams and bucks each age range between 8-12 months were selected from a flock managed in a semi intensive system. The average body weight of 12.76 ± 4.09 kg and 12.08 ± 3.26 rams and bucks, respectively. Fastened blood samples were collected from all animals into plain and heparinised bottles for haematology and serum separation, respectively. Serum was obtained using standard procedures and assayed for serum biochemical, lipid peroxidation, total antioxidant activity, catalase and superoxide dismutase using standard procedures, data obtained were subjected to T-test. The result revealed that haematological parameters were not significantly ($p > 0.05$) different between both rams and bucks. The value for glucose (6.68 ± 3.56 vs 1.72 ± 0.76 mmol/L), total protein (92.49 ± 16.99 vs 19.19 ± 8.27 g/L), albumin (13.41 ± 5.25 vs 2.58 ± 0.29 g/dL) and high density lipoprotein (10.28 ± 3.43 vs 1.66 ± 0.26 mmol/L) were significantly ($p < 0.05$) higher in rams compared to bucks. Total antioxidant activity (127.35 ± 36.37 vs 13.04 ± 3.06 mmol/litre), catalase (199.77 ± 130.23 vs 57.93 ± 12.05 nmoles of H_2O_2 consumed/min/mg protein) and superoxide dismutase (0.65 ± 0.33 vs 0.49 ± 0.08 U/min/mg protein) were statistically ($p < 0.05$) higher in rams than in bucks. In conclusion, rams had higher glucose, protein, albumin, and high density lipoprotein. Rams had superior antioxidant defense over bucks and thus had lower susceptibility to oxidative stress compared to bucks.

Keywords: Serum metabolites, Free radicals, Antioxidant enzyme, West African dwarf, Ram, Buck

INTRODUCTION

Ruminants are useful livestock assets in that they depend to a greater extent on plant materials that are of less value to humans as feed resources. Despite this, their low performance is a major challenge to ruminant production in the tropics. The West African Dwarf (WAD) goats are found in the region south of latitude $14^\circ N$ across West Africa in the coastal area, which is humid and favours high prevalence of diseases (Adeloye, 1998). The harsh environmental conditions of the tropics in which the animals are exposed to are further

aggravated by the seasonal fluctuations in quantity and quality of most of these feed resources which they depend on. The effect of these imbalances may enhance, aggravate or have negative consequences on the overall wellbeing of the animal. In the last decade, the evaluation of oxidative stress (the imbalance between the production of free radicals and the efficiency of antioxidant defence systems in the body) has become increasingly important in ruminant health and animal production as complementary tools in evaluation of the nutritional and metabolic status of the animals (Mohebbi-Fani *et al.*, 2012).

Oxidative stress results from increased production of free radicals and reactive oxygen species, and a decrease in antioxidant defense (Trevisan *et al.*, 2001; Williams *et al.*, 2004). External factors such as oxygen exposure, resuscitation (Sunil Kumar *et al.*, 2011), heat, trauma, ultrasound, infections, radiations and toxins can lead to increased free radicals and other ROS and may lead to oxidative stress (Halliwell *et al.*, 1992).

There are few reports of the implication of oxidative stress in ruminants. Erythrocyte TBARS concentration increased in heat exposed cattle and buffalo (Kumar *et al.*, 2007) and Holstein cows (Bernabucci *et al.*, 2002). The increase of thiobarbituric acid reactive substance TBARS immediately before and after calving confirmed that cows during the transition period were under oxidative stress (Chandra and Aggarwal, 2009). TBARS concentration was also higher during summer in prepartum crossbred cows compared to winter season as reported by Chandra and Aggarwal (2009). Aengwanich *et al.* (2011) and Ganaie *et al.* (2013) reported that higher concentration of TBARS in cattle reared with no shade compared to artificial and tree shade animals. The production of reactive oxygen species (ROS) is both essential and detrimental to life; hence, numerous studies indicate that ROS play an important role in normal sperm function and that an imbalance in ROS production, overproduction and/or degradation under-scavenging by antioxidants may have serious adverse effects on sperm (Akiyama, 1999). De Lamirande and Gagnon (1992) suggested that ROS were responsible for the loss of spermatozoal motility through decreased phosphorylation of axonemal proteins required for sperm movement.

There is need for more information on the levels of oxidative stress biomarkers in small ruminant animals, especially in tropical rearing systems and climatic conditions (Yusuf *et al.*, 2017). Jack *et al.* (2016) reported that WAD rams have better oxidative stability than ewes, suggesting that ewes should be protected during oxidative stress prone conditions. Requirement for high semen quality in WAD breeds is necessary to meet breeding goals.

Knowledge of oxidative status of healthy and normal sheep and goat is important to create basis for inference on different production and management condition especially stress prone areas. This will create awareness for mitigation and/or amelioration of oxidative stress for high semen output and quality. These necessitated assessing haematological, serum biochemical, oxidative stress indicators in trypanotolerant and hardy WAD rams and bucks.

MATERIALS AND METHODS

Physiological variations and oxidative status of sixteen West African dwarf rams and bucks each aged 8 – 12 months with average weight of 12.76 ± 4.09 kg and 12.08 ± 3.26 kg, respectively were studied in a completely randomised design. The animals were selected from a flock, quarantined, acclimatized and managed in a semi intensive system. The animals were apparently healthy and of good conformation. The animals were offered concentrate and free choice grazing. Fastened blood sample were collected from the animals into heparinised and plain bottles for haematology and serum separation, respectively. Serum obtained using standard procedures. Haematological assay was done as outlined in Dacie and Lewis (2001). Serum was assayed for glucose (Cooper and McDaniel, 1970), total protein (Reinhold, 1953; Kohn and Allen, 1995), urea and creatinine (Toro and Ackermann, 1975), albumin (Peters *et al.*, 1982), high density lipoprotein, low density lipoprotein, cholesterol and triglycerides (Friedewald *et al.*, 1972; Gowenlock. *et al.*, 1988), alanine amino transferase (Hodder and Rej, 1983), aspartate amino transferase (Hodder and Rej, 1983) and alkaline phosphatase (McComb *et al.*, 1988) using commercial Randox kits.

Serum total antioxidant activities was carried out according to Koracevic *et al.* (2001). Superoxide dismutase (SOD) was estimated by the method of Marklund and Marklund (1974) adopted by Soon and Tan (2002) and catalase was estimated by the method of Beers and Sizer (1952). Serum lipid peroxidation was determined using thiobarbituric acid assay

according to Ohkawa *et al.* (1979). All assays were done as outlined in Jimoh (2016). Data obtained in this study was subjected to T test using statistical analysis software (SAS, 2003).

RESULTS AND DISCUSSION

Serum biochemicals, haematological indices and blood electrolytes are critical physiological indices which have health implications on the diagnosis, prognosis and treatment of livestock diseases especially in animals bred under different management systems (Onasanya *et al.*, 2015). The results of the haematological indices of WAD rams and bucks are shown in Table 1. All haematological indices evaluated were statistically similar ($p > 0.05$) for the two species. This could infer that the animals had similar health status and the values obtained were within normal range reported by Duncan and Prasse (1986).

The range of packed cell volume and haemoglobin obtained in this study was within the range of values reported by Njidda *et al.* (2014) (33.5, 38 and 43.8) and (5.6, 11.11, 12.20) in Yankassa, Ouda and Balami rams in Nigeria. Differences in red blood cell (RBC) counts may be attributable to stress (splenic contraction), hydration status, hormonal influences, dietary/nutritional differences, adaptations to a desert environment or adaptation to a high mountain environment (Borjesson *et al.*, 2000). African animal trypanosomiasis have been reported to be responsible for early sequestration and destruction of erythrocytes by cells of the mononuclear phagocytic series and subsequent anaemia during trypanosomiasis. Erythrocyte peroxidation is one of the factors that have been observed to play an important role in the pathogenesis of anaemia in acute trypanosomiasis in experimental mice infected with *T. brucei* (Onasanya *et al.*, 2015). Trypanosomes and activated phagocytes (macrophages and neutrophils) are known to elaborate sialidases, proteases, reactive oxygen radicals such as O_2 , OH leading to the rapid destruction erythrocyte membranes during infection (Taiwo *et al.*, 2003).

The values of haematological indices of bucks obtained in this study was in agreement with values obtained in WAD bucks and normal range reported by Ewuola *et al.* (2014).

Results of serum biochemical indices of WAD rams and bucks showed a significant increase ($p < 0.05$) in the glucose, total protein, albumin and HDL in ram compared to buck (Table 2). Animals reared under extensive systems tend to have lower glucose levels compared to those reared under intensive systems (Onasanya *et al.*, 2015). The study reported that sedentary nature of the animals under intensive care may probably cause the increase levels of blood glucose, while the animals under extensive care might have used appreciable levels of their blood glucose to meet the energy demand of physical and ranging activities. Goats are browse animals, and engage in more physical activity than sheep, this accounted for the higher serum glucose in sheep. Total protein is an important serum indicator of protein metabolism in animals and this is most often associated with increased protein intake. Several researchers (Okoruwa, 2014; Okoruwa *et al.* 2014; Olafadehan *et al.* 2014) had attributed increased serum total protein and albumin to increased protein intake in nutritional studies. However, Erickson and Poole (2006) reported that dehydration as a result of stress conditions such as increased breathing rate was responsible for increased serum total protein and albumin. The range of values obtained for serum glucose and protein in this study were higher than values reported by Njidda *et al.* (2014) in breeds of ram in northern Nigeria. Yokus *et al.* (2006) reported that fluid loss could account for increased albumin, higher serum protein and albumin in sheep may be attributed to high fluid composition/blood volume relative to their metabolic body size compared to goats. Furthermore, the damage of biological macromolecules such as lipids, proteins, carbohydrates and DNA due to the accumulation of ROS which is an indication of oxidative stress (Sunil Kumar *et al.*, 2011) may have also resulted in increased serum total protein, albumin and HDL in rams.

Table 1: Haematological indices of male WAD sheep and goats in semi intensive management system

Parameters	Ram	Buck
Packed cell volume (L)	0.36 ± 0.06	0.32 ± 0.71
Haemoglobin (mmol/l)	7.42 ± 1.27	6.54 ± 1.53
Red Blood cell (x10 ¹² /l)	11.15 ± 1.00	10.85 ± 1.49
Mean cell volume (fl)	32.22 ± 5.14	29.36 ± 3.85
Mean cell haemoglobin (fmol)	0.67 ± 0.10	0.60 ± 0.08
Mean cell haemoglobin concentration (x10 g/l)	20.69 ± 0.36	20.30 ± 0.59
White blood cell (x10 ⁹ /l)	10.60 ± 0.72	8.79 ± 1.90
Lymphocytes (x10 ⁹ /l)	6.63 ± 1.46	5.85 ± 0.55
Neutrophils (x10 ⁹ /l)	3.47 ± 0.81	2.40 ± 0.31
Monocytes (x10 ⁸ /l)	1.77 ± 0.76	1.97 ± 0.28
Eosinophils (x10 ⁸ /l)	3.27 ± 1.64	3.41 ± 1.13

Mean ± SEM on the same row without letter superscript are not significantly different ($p > 0.05$)

Table 2: Serum biochemistry of male WAD sheep and goats in semi intensive management system

Parameters	Ram	Buck
Glucose (mmol/L)	6.68 ± 3.56 ^a	1.72 ± 0.76 ^b
Total protein (g/L)	92.49 ± 16.99 ^a	19.19 ± 8.27 ^b
Albumin (g/dL)	13.41 ± 5.25 ^a	2.58 ± 0.29 ^b
High density lipoprotein (mmol/L)	10.28 ± 3.43 ^a	1.66 ± 0.26 ^b
Cholesterol (mmol/L)	1.32 ± 1.08 ^a	1.10 ± 0.60 ^a
Triglycerides (mmol/L)	0.62 ± 0.16 ^a	0.61 ± 0.24 ^a
Alkaline phosphatase (μ/L)	2580.55 ± 632.11 ^a	1453.40 ± 408.75 ^a
Creatinine (μmol/L)	193.14 ± 68.68 ^a	129.80 ± 67.26 ^a
Alanine amino transferase (iu/l)	2.35 ± 0.73 ^a	3.45 ± 1.54 ^a
Aspartate amino transferase (iu/l)	14.63 ± 3.96 ^a	15.00 ± 5.73 ^a
Urea (mg/dL)	157.08 ± 28.39 ^a	176.12 ± 30.57 ^a

Mean ± SEM on the same row with different letter superscript are significantly different ($p < 0.05$), Mean ± SEM on the same row with similar letter superscript are not significantly different ($p > 0.05$)

However, cholesterol, triglycerides, ALP, creatinine, ALT, AST and urea were not significantly ($p > 0.05$) affected in both rams and bucks. ALT activities were also reported to be affected only by reproductive status in sheep and goat (Yokus *et al.*, 2006).

The results of the serum oxidative stress indicators of WAD rams and goats revealed that lipid peroxidation was not significantly affected ($p > 0.05$) in rams and bucks, but the total antioxidant activity, catalase and superoxide dismutase were significantly ($p < 0.05$) higher in rams than bucks (Table 3). The increase in these stress indicators implied that the effect of stress was more in rams compared to bucks. Moreover, the increase in oxidative stress indicators could have been as a direct response to the effect of stress. Jack *et al.* (2016) reported that significantly higher serum catalase activity in WAD rams led to apparently higher total antioxidant activity.

According to Puppel *et al.* (2013) free radicals have a crucial role in the development of over 100 diseases affecting all major organs. Tightly controlled ROS generation appears to be one of the central elements in the mechanisms involved in cell function, growth, differentiation and death (Valko *et al.*, 2007). Maintaining physiological equilibrium between intracellular antioxidants levels and production of reactive oxygen species (ROS) is crucial for the survival of the organisms (Garrel *et al.*, 2010; Hayajneh *et al.*, 2016). Although oxidative stress has been implicated in numerous disease (Celi 2008), it can be particularly dangerous because it usually leaves no clinical signs and the condition is diagnosed by means of dedicated analytical methods to evaluate the integrity of the defence mechanisms and the end products of oxidative stress (Celi *et al.*, 2010). Peripartum oxidative stress has also been reported in sheep (Kamiloglu *et al.*, 2006).

Table 3: Serum oxidative stress indicators of male WAD sheep and goats in semi intensive management system

Parameters	Ram	Buck
Total antioxidant activity (mmol/litre)	127.35 ± 36.37 ^a	13.04 ± 3.06 ^b
Catalase (nmoles of H ₂ O ₂ consumed/min/mg protein)	199.77 ± 130.23 ^a	57.93 ± 12.05 ^b
Superoxide dismutase (U/min/mg protein)	0.65 ± 0.33 ^a	0.49 ± 0.08 ^b
Lipid peroxidation (x10 ⁻³ MDA/mg protein)	3.58 ± 2.46 ^a	1.79 ± 1.33 ^a

Mean ± SEM on the same row with different letter superscript are significantly different ($p < 0.05$), Mean ± SEM on the same row with similar letter superscript are not significantly different ($p > 0.05$)

Mohebbi-Fani *et al.* (2012) reported that ewes, sheep (Gaal *et al.*, 1993) and goats (Di Trana *et al.*, 2006) may suffer from oxidative stress in other stages of reproductive / productive cycle that have lower metabolic changes and nutritional requirements. Hajimohammad *et al.* (2015) reported decrease in the levels of SOD, GPX and catalase antioxidant enzymes in salinomycin intoxicated sheep due to liver damage which indicated increased exposure of different organs to oxidative stress products, with consequent loss of the symmetrical structure of lipids in cell membrane, decrease in flexibility, disturbance in water and ion exchange, swallowing and destruction of cells. Yusuf *et al.* (2017) reported that the antioxidant capacity of WAD goats was greater early in the rainy season compared with the late rainy season under both intensive and semi intensive management systems. Increased oxidative conditions within the reproductive tissues along with dietary protein deficiency led to decreased activity of antioxidant enzymes and increased concentrations of TBARS (Mohebbi-Fani *et al.*, 2012). Thus suggesting better management of bucks and ram for better semen output and seminal oxidative stability. Sivakumar *et al.* (2010) reported that supplementing goat with antioxidants reduced the level of oxidative stress (Hayajneh *et al.*, 2016). Antioxidants have an important protective action on the membrane integrity and lipid stability in both seminal plasma and spermatozoa (Jimoh, 2016). There is a positive correlation between oxidative stress and cells life cycle (Hajimohammad *et al.*, 2015). Reactive oxygen species are normal physiological event in various organs including the testis (Jimoh *et al.*, 2017). Paradoxically, the production of ROS is both essential and detrimental to life; hence, numerous studies

indicated that ROS play an important role in normal sperm function and that an imbalance in ROS production overproduction and/or degradation under-scavenging by antioxidants may have serious adverse effects on sperm (Akiyama, 1999; Jimoh, 2016). 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, improves their physiological performance as their nutrient requirements are met because of their selectivity (Yusuf *et al.*, 2017).

Conclusion: It was concluded from this study that WAD rams had better antioxidant defense mechanism compared to bucks. Efforts to improve antioxidant protection of bucks should be emphasised in oxidative stress prone production and management systems as well as climatic conditions. Higher oxidative stability in rams and buck will prolong cell life and protect against tissue and organ damage, which are essential for good semen output.

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