OXIDATIVE STRESS AND HAEMATOLOGICAL PROFILES OF GOATS REARED UNDER DIFFERENT MANAGEMENT SYSTEMS

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ABSTRACT. This study was done to assess oxidative stress and haematological parameters of twenty male goats aged between eight and 22 months of the West African Dwarf breed reared under two management systems. Ten bucks were reared under an intensive system, and another 10 bucks reared under an extensive system were used. The Hb concentration obtained in both groups was significantly (p<0.05) higher in the intensively managed goats (12.39±0.02 gm %) compared to the extensively managed group. The total erythrocyte count in intensive goats, 35.34±1.36×10^6/µl, was significantly (p<0.05) higher than the value recorded in the extensive group (30.01±1.41×10^6/µl). The recorded leucocytes counts in intensive goats of 10.26±0.70×10^3/µl was significantly (p<0.05) lower than 12.38±1.23×10^3/µl recorded in the extensively managed goat, while the calculated value of MCV in intensive goat (9.8±0.73 fl) was significantly (p<0.05) higher than the recorded value in extensive group. The obtained value of MCH was significantly higher (p<0.05) in the extensively managed goats. The recorded neutrophil value of 5.00±0.50×10^3/µl in extensively raised goats was significantly (p<0.05) higher than 3.70±0.20×10^3/µl obtained in the intensively raised group likewise the lymphocyte count of 5.60±0.08 ×10^3/µl in intensively raised goats was significantly (p<0.05) lower than the value obtained in the extensively raised goat. The neutrophil:lymphocyte ratio was significantly higher (p<0.05) in the extensive versus the intensive group viz. 0.75±0.03 as against 0.67±0.04. In the extensive group a higher malondialdehyde (MDA) value of 2.30±0.07 ng/ml was recorded and this was significantly (p<0.05) higher than the corresponding value of 1.28±0.11 ng/ml obtained in the intensively managed goats. The recorded value of superoxide dismutase, catalase and glutathione peroxidase was significantly (p<0.05) higher in the extensive group than those on the intensive group. The fragility test of the extensively managed goats shifts more towards right and was significantly (p<0.05) highest at a sodium chloride concentration of 0.2-0.7% in extensively managed goats. It can be concluded from the present study that the biomarkers of oxidative stress were higher in the extensively than the intensively managed goats.

Keywords: goats, haematological parameters, oxidative stress

INTRODUCTION

Goat (*Capra hircus L.*) production in Nigeria makes a major contribution to the agrarian

economy of the country (Daramola *et al*., 2005). Goats are valued mainly for their meat and milk; the skin, wool and hair also strengthen its economic basis for keeping goats in many parts of the world (Oyeyemi and Akusu, 2002). They are able to thrive as meat producers under conditions to which other production animals are unsuited (Oyeyemi *et al*., 2001). They are by far the most important domesticated small ruminants with a world population of 861.1 million of which 94% are produced in the developing countries (Mahmoud 2010). 29.2 million goats make up the goat population of Nigeria (Ajala *et al*., 1999) and 70 % of which is found in northern Nigeria (Bayer and Otchere, 1984). In Nigeria management of goats is traditionally free range (Ajala, 1995) where goats are allowed to roam freely and browse and to scavenge for food such as scraps, crop residues and agro industrial by-products (Akpa *et al*., 2002). They lack basic housing, rudimentary health care, irregular and adequate nutrition. This system of management is much influenced by by climate, cropping and population density. This predisposes animals to stress (Adenkola and Alilu, 2012) which is likely to elicit a stress response and a subsequent increase in free radical generation (Nazifi *et al*., 2009). This in turn causes oxidative stress which impairs the antioxidant status *in vivo* (Sahin *et al*., 2001; Onmaz *et al*., 2011).

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and the system's ability to readily detoxify the reactive intermediates, or to repair the resulting damage, to a wide range of molecular species including lipo proteins, nucleic acids (McCord, 2000), as cellular damage (Rao *et al*., 2006). Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. A stressful condition leads to the excessive production of radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system (Drooge, 2002; Miao and St Clair, 2009).

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and the system's ability to readily detoxify the reactive intermediates, or to repair the resulting damage, to a wide range of molecular species including lipo proteins, nucleic acids (Mccord, 2000) as cellular damage (Rao *et al*., 2006). Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. A stressful condition leads to the excessive production of radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system (Drooge, 2002; Miao and St Clair, 2009). The relationship between stress and oxidative insults is well demonstrated and implicated in decreased production (Jackson, 2009). The protective mechanisms, evolved to cope with the reactive oxygen metabolites, depend primarily on the synergism between several endogenous and dietary antioxidants (Atalay *et al*., 2012). Excess or uncontrolled production of reactive oxygen species will eventually lead to oxidative stress, defined as a stage where production of ROS and pro oxidants overwhelms the antioxidant defence mechanisms of the cells and tissues (Sies, 1991). The more recent, complementary definition of oxidative stress emphasises the role of disruption of thiol redox circuits,

resulting in imbalance in cell signalling and dysfunctional redox control (Janero, 1990).

Stressors such as road transportation, packing and trekking activate the hypothalamo–pituitary–adrenal axis with the release of glucocorticoids and other hormones that exert various physiological effects with resultant generation of free radicals or ROS that enhance oxidative damage to erythrocyte membranes causing haemoglobin denaturing, cytoskeletal instability and hence haemolysis (McMillian *et al*., 2005, Parker *et al*., 2003).

Of the intensive and semi-intensive systems of goat production in Nigeria, the latter has proved adoptable owing to the comparative advantages it enjoys over the former (Ahamefule *et al*., 2000). There is little information on the haematological and oxidative stress parameters in intensively versus extensively raised goats (Adenkola and Ayo, 2006).

Therefore the aim of this present study was to assess the haematological parameters and associated oxidative stress parameters in goats reared under different management systems.

MATERIALS AND METHOD

Experimental site

The experiment was conducted at the old Government Reserved Area of Makurdi, Nigeria and the Small Ruminant Unit of the Teaching and Research Farm, University of Agriculture Makurdi (07° 41/ N, 08° 37/ E), located in the Southern Guinea Savannah zone of Nigeria.

Experimental Animals and Management

Twenty apparently healthy goats, of the West African dwarf variety, all males, and aged between 8 and 22 months, weighing between 12 to 30 kg were used for this experiment. The animals comprised of two groups, based on the management systems (intensive and extensive). Ten bucks reared intensively system and another 10 bucks extensively were used. The goats under intensive husbandry were kept in a farm at the Old Government Reserved Area of Makurdi, Benue State, while the animals under extensive conditions were kept at the small ruminant unit of the Teaching and Research Farm of the University of Agriculture, Makurdi, Benue State.

The intensive goats were fed with grasses comprising of elephant grass (*Panicum maximum*), gamba grass (*Andropogon gayanus*), legumes mainly (*Centrosema* spp) and a concentrate supplement (16.75% crude protein). Water was provided ad libitum. The animals were kept in a communal pen, with concrete floor and iron sides and asbestos roofing. Between the walls and the roof a space allowed for adequate ventilation. The animals were not restrained inside the pen.

The animals on the extensive system were only allowed free grazing on natural pasture consisting of different species of grasses, legumes and browser plants during the day (9 a.m. to 6 p.m.), and were sheltered at night without any form of supplementation.

The study lasted for 12 weeks. Two weeks prior to the commencement of the experiment the animals were screened for

possible endo and haemo-parasites and were treated accordingly with Albendazole at 7 mg/kg and also with 20 mg/kg of oxytetracycline (20%) (Tridox®, Farvet Laboratories, Handelsweg, Holland).

Five mls of blood was taken aseptically from the jugular vein of an animal using a 5 ml syringe and 18 gauge × 1½. For determination of haematological parameters (2 ml) was immediately poured inside a sample bottle containing an anticoagulant, sodium salt of ethylene diaminetetra-acetic acid (EDTA) at the rate of 2 mg/ml of blood (Oyewale, 1992; Adenkola and Ayo, 2009). The remaining blood in the samples was transferred immediately on an ice pack to the Physiology laboratory, Department of Physiology, Pharmacology and Biochemistry where it was immediately centrifuged at 1,500 ×*g* for 15 minutes. The resultant serum was harvested for determination of the following:

- Malondialdehyde (using the double-heating method of Draper and Hadley (1990) as modified by Altuntas *et al*. (2002)),
- Catalase, superoxide dismutase, glutathione peroxidase, (using a spectrophotometry techinique (Ozyurt *et al*., 2007))
- Alanine transaminase (ALT), aspartate transaminase (AST), urea, total cholesterol (were determined as described by Cheesbrough (1991).
- Total erythrocyte sialic acid and erythrocyte free sialic acid values (were determined using the procedures of Schauer and Kamerling (1997)).

Blood meant for haematological parameters was used to determine

- Packed cell volume (PCV),
- Haemoglobin concentration (Hb),
- Total erythrocyte count (TCs),
- Total leukocyte count (TLC) and differential leucogram as described by (Schalm *et al*., 1975),
- Erythrocyte osmotic fragility (Faulkner and King, 1970).
- Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated (Schalm, 1975).

Ethical Considerations

The handling of animals was carried out humanely in accordance with the guidelines, governing the welfare of research animals by the University of Agriculture, Makurdi as approved by the Ethics Research Committee.

Statistical Analysis

The data obtained were expressed as mean ±standard error of the mean (mean±SEM), and they were subjected to statistical analysis using Student's *t*-test. Values of $p<0.05$ were considered significant.

RESULTS

The recorded PCV value of 37.10±1.49% in intensively managed goats was not significantly ($p<0.05$) different from 34.10±3.28% recorded in the extensive group.

The Hb concentration obtained in both groups was significantly (p<0.05) higher in the intensively managed goats (12.39±0.02 gm %).

The total erythrocyte count in the intensively raised group (35.34±1.36×10^6/µl was significantly (p< 0.05) higher than the value recorded in extensive group (30.01±1.41×10^6/µl).

The recorded leucocyte count (wbc) in the intensive group 10.26±0.70×10^3/µl was significantly (p<0.05) lower than 12.38±1.23×10^3/µl recorded in the extensively managed goats.

The calculated value of mean cell volume (MCV) in intensively raised goats (9.8±0.73 fl) was significantly (p<0.05) higher than the recorded value in the extensive group. The obtained value of mean cell haemoglobin (MCH) was significantly higher (p<0.05) in the extensively managed goats.

The recorded neutrophil value of 5.00±0.50×10^3/µl in the extensively raised group was significantly (p<0.05) higher than 3.70±0.20×10^3/µl obtained in the intensive group. Likewise a mean lymphocyte count of 5.60±0.08×10^3/µl was significantly (p<0.05) lower than the value obtained in the extensively farmed goats.

The neutrophil:lymphocyte ratio was significantly higher (p<0.05) in the extensive goats with a value of 0.75±0.03 as against 0.67±0.04 in the intensive goats (Table 1).

In the extensive group a higher malondialdehyde (MDA) value of 2.30±0.07 ng/ml was recorded and this was significantly (p<0.05) higher than the

Table 1. Haematological Parameters of Goats Reared Under Intensive and Extensive Management System

	Intensive Goats	Extensive Goats
Packed Cell Volume (%)	37.10 ± 1.49	34.10 ± 3.28
Haemoglobin Concentration (gm %)	12.39 ± 0.02	11.36 ± 0.43
Total Erythrocyte Count (×10^6/µl)	35.34 ± 1.36*	30.01 ± 1.41
Mean Corpuscular Volume (fl)	9.8 ± 0.73	10 ± 0.49+*
Mean Corpuscular Haemoglobin (pg)	3.3 ± 0.24	3.5 ± 0.16*
Mean Corpuscular Haemoglobin Concentration (g/dl)	33 ± 0.00	33 ± 0.00
Total Leucocyte Count (×10^3/µl)	10.26 ± 0.70	12.38 ± 1.23*
Neutrophil (×10^3/µl)	3.70 ± 0.20	5.00 ± 0.50*
Eosinophil (×10^3/µl)	4.4 ± 0.65	4.0 ± 0.65
Basophil (×10^3/µl)	X	X
Lymphocyte (×10^3/µl)	5.60 ± 0.08	6.9 ± 0.75*
Monocyte (×10^3/µl)	0.16 ± 0.16	0.23 ± 0.05
Neutrophil : Lymphocyte	0.67 ± 0.04	0.75 ± 0.03*

Values in the table with asterisk are significantly (P < 0.05) higher

corresponding value of 1.28±0.11 ng/ml obtained in the intensively managed goats.

The observed values of superoxide dismutase, catalase and glutathione peroxidase were was significantly (p<0.05) higher in the extensive group than those on the intensive group.

The obtained transaminase enzyme of aspartate amino transferase was significantly higher (p<0.05) in the extensive group with a value of 42.40±1.86 IU/L.

The recorded value for urea and total cholesterol were not significantly (p>0.05) different in the two groups.

The total sialic acid value was not significantly (p>0.05) different in the groups, while the free sialic acid value of 33.12±1.31 mg/ml obtained in the extensive group was significantly (p<0.05) higher than that of 21.58±0.58 mg/ml in the intensive goats.

The fragiligram of the extensively managed goats shows a shift towards the right and was significantly (p<0.05) highest at sodium chloride concentration of 0.2-0.7% in extensively managed goats. A higher percentage haemolysis, 97.83±1.26% was obtained at 0.1 NaCl concentration in the extensive group which was significantly (p<0.05) higher than the corresponding value of 83.99±1.20% in the intensive group while the lowest percent haemolysis was obtained at 0.85 sodium chloride with a value of 0.85% and was significantly (p<0.05) lower than 6.56±0.56% obtained in the extensively managed goats (Figure 1).

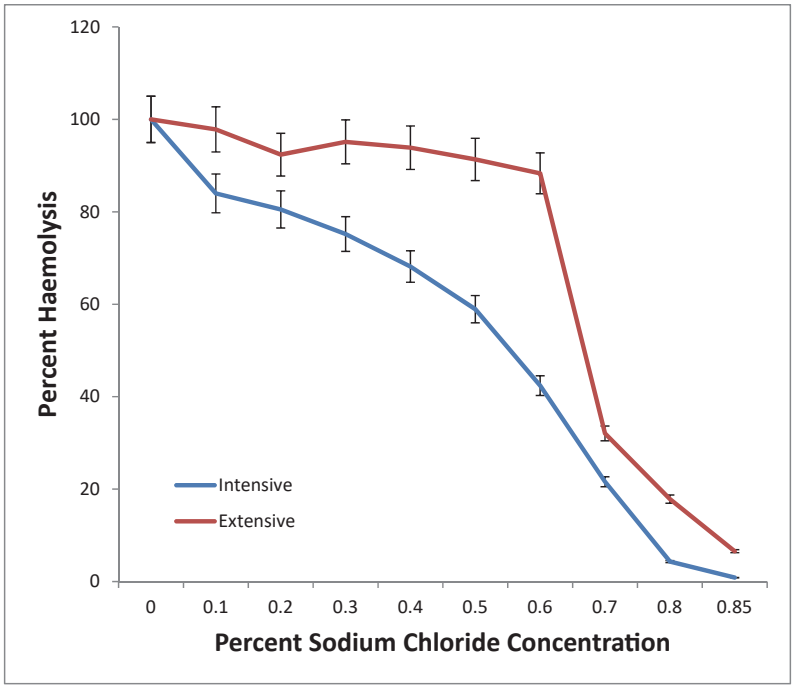

Figure 1. Erythrocyte Osmotic Fragility of Goats Reared Under Intensive and Extensive Management System

DISCUSSION

The higher erythrocyte count recorded in the intensive group may result from good veterinary a lower parasite challenge, and adequate feed containing the necessary vitamins and minerals for optimal haemoglobin formation (Adenkola *et al*., 2009; Adenkola and Tuleun, 2011). It has been demonstrated that animals on a high protein diet had higher total iron binding capacity than those receiving a low protein diet. Iron plays a positive role in erythropoesis. Intensively managed goats had their feed supplemented with a high quality protein concentrate unlike the goats under the extensive system. This increase in PCV observed is probably due to increased erythropoiesis and absence of external and internal haemo-parasites as a result of good veterinary care.

The MCV is the average volume of individual RBC (Dacie and Lewis, 1995) and the MCV of the extensive groups were higher than those of intensive group. Considering the fact that SAVR determines the intra-erythrocytic pressure (Kumar, 2002), when in a hypotonic solution, the increase in intra-erythrocytic pressure observed in the erythrocytes of extensive goat will be higher. Hence, any discrepancy in erythrocyte osmotic fragility among the two groups may be associated to changes in plasma membrane.What sort of changes are referred to significantly higher values of mean corpuscular volume (MCV) indicates macrocytosis (Latimer *et al*., 2004). Increased MCV may also be observed in regenerative anaemia due to hemolysis and haemorrhages (Awodi *et al*., 2005; Chineke *et al*., 2006).

Total WBC counts and differentials observed in this study compared well with values obtained in goats elsewhere (Sandabe and Yahi, 2000). The higher values of the WBC observed may be attributed to the challenges encountered by extensively managed goats from endoparasites and ectoparasites when on free range coupled with environmental and physiological stress this probably stimulates a leucoctytosis specifically a neutrophilia ascribed to inflammation, acute and chronic stress (Dellmann and Brown, 1987). However, the values obtained in this study fell within the normal broad range recorded for WAD goats (Opara *et al*., 2010). The leukocytosis far from indicating suppressed immunity may reflect a good immunity which effectively triggers a white cell reaction in extensive goats, since according to Schalm (1975), changes in total and differential leukocyte count and erythrocyte parameters and indices are pointers to various disease processes even at subclinical levels.

It was reported that, like other ruminants there are more lymphocytes than neutrophils in circulation (Olusanya *et al*., 1976) which was similar to our findings in this study. The high value of neutrophil:lymphocyte ratio observed in extensive goat agrees with the established fact that the parameter is a good indicator of stress in goats (Rajion *et al*., 2001)

Erythrocyte osmotic fragility (EOF) is the measurement of the ease with which an erythrocyte lyses in hypotonic solution and is expressed in terms of the concentration of saline solution in which haemolysis

occurred (Kumar, 2002). The erythrocyte is surrounded by bi-lipid membrane layers and lipids are one of the most susceptible substrates to free radicals damage and are biomarkers of lipid peroxidation, considered to be the best indicators of oxidative stress (Georgieva, 2005). Malondialdehyde (MDA) is one of the several low molecular-weight end products formed during the radical induced decomposition of poly-unsaturated fatty acids (Janero, 1990). MDA readily reacts with thiobarbituric acid producing a red pigment which can be easily measured by spectrophotometry in the form of thiobarbituric acid reactive substances (TBARS) (Janero, 1990). The increased MDA concentration obtained in the extensive goats occurred, apparently, as a result of free-radical induced lipid peroxidation of the erythrocyte membranes, which impairs cell integrity and results in cell destruction (Hanzawa and Wantanabe, 2000). This may be associated with a difference in metabolic rate among the goats kept in the two different management systems used for this study.

Increased metabolic activity in the cell will result in elevation in the production of free radicals (Brunet-Rossinni, 2004). Due to the higher level of polyunsaturated fatty acids in their plasma membrane and intracellular oxygen and haemoglobin content, erythrocytes tend to be quite sensitive to oxidative stress (Aguirre *et al*., 1998). Peroxidation of unsaturated chain of membrane lipids increases the susceptibility of erythrocyte to osmotic haemolysis (Brzezinska-Slebodzinska, 2001) because of membrane fluidity and ultimate destruction of the bilayer integrity of erythrocyte membrane (Azeez *et al*., 2012). This probably accounted for increased haemolysis seen in extensive goats over that in intensively managed goats. The result of the present study demonstrated clearly that EOF

Table 2. Serum Biochemical Parameters of Goats Reared Under Intensive and Extensive Management System

	Intensive Goats	Extensive Goats
Malondialdehyde (ng/ml)	1.28±0.11	2.30±0.07*
Superoxide dismutase (µg/ml)	2.38±0.10	3.32±0.08*
Catalase (µg/ml)	51.00±1.18	55.20±0.86*
Glutathione peroxidase (µg/ml)	38.40±2.50	46.20±1.46*
Aspartate amino transferase (IU/L)	38.40±2.50	42.40±1.86*
Alanine amino transferase (IU/L)	46.6±1.86	45.00±2.98
Urea (mg/100 ml)	3.72±0.23	4.34±0.37
Total cholesterol (mg/100 ml)	2.68±0.17	2.92±0.13
Total sialic acid (mg/ml)	31.32±1.22	29.20±2.86
Free sialic acid (mg/ml)	21.58±0.58	33.12±1.31*

Values in the table with asterisk are significantly ($p<0.05$) higher.

may serve as a biomarker of stress due to management stress.

A stressful condition leads to the excessive production of free radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system. Generation of free radicals is an integral feature of normal cellular functions. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). The cellular antioxidants defense system consists of superoxide dismutase, catalase, and glutathione peroxidase. The shift of the delicate balance between free radicals and cellular antioxidants defense system in favour of free radicals might lead to development of oxidative stress (Elsayed and Bendich, 2001). The result of this study indicative of biomarkers of oxidative stress as shown in Tables 2 showed higher values of malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase in goats managed under extensive system than those managed under intensive system. Malondialdehyde is a by-product of lipid peroxidation indicative of free radicals while catalase, glutathione peroxidase and superoxide dismutase are antioxidant enzymes that quench free radicals and are measured as biomarkers of oxidative stress. Higher biomarkers of oxidative stress concentrations recorded in the extensive group may be due to the stressful conditions associated with the extensive system of management. Animals under extensive management move about in search for food and water with very little attention to their health needs. High level of aerobic energy production usually accompanies stressful conditions with resultant increase in glucocorticoids secretion and activation of adrenergic pathways. These pathways are involved in the stimulation and generation of free radicals resulting in lipid peroxidation with malondialdehyde which is higher in the goat under extensive system of management, being one of the by-products of lipid peroxidation (Nazifi *et al*., 2009) and this leads to an increase in activities of antioxidant enzymes.

Enzymes of aspartate alanine amino transferase, alanine amino transferase generally appear in muscular tissue under excessive stress, especially when the muscular tissue was damaged (Ozyurrt *et al*., 2007). This probably is why a higher value of aspartate alanine amino transferase is recorded in extensive goats which are constantly on the move from one location to the other.

CONCLUSION

It can be concluded from this study that the biomarkers of oxidative stress were higher in the extensively managed than the intensively managed goats indicating that that extensive management system exerts enormous stress on animals. It is important that small holder farmers particularly in the developing world should educated through robust agricultural extension programs to embrace intensive system of managing goats to improve their profit margins.

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