Haematological, Oxidative Stress and Trace Elements Variations Associated with Emaciation in Pre-Slaughter White Fulani Cows

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Haematological, oxidative stress and trace elements variations associated with emaciation in pre-slaughter White Fulani cows

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Abstract

Pre-slaughter White Fulani cows were purposively sampled on the basis of body condition: emaciated (n=37) and non-emaciated (n=37), with the objective of understanding the intricate interplay of oxidative stress, trace elements and haematological variations during emaciation. Blood was drawn from the jugular vein for haematological analysis and accruing serum was used for the evaluation of malondialdehyde (oxidative stress marker), antioxidant enzymes and compounds, serum protein, electrolytes as well as trace elements. Significant ($p < 0.05$) differences between the emaciated and non-emaciated cows were established only in the values of copper and reduced glutathione (GSH), which were lower in emaciated cows (EC). None of the animals had packed cell volume (PCV) below the normal reference range, however values above the normal (> 46%) were seen, suggesting dehydration. The PCV in emaciated cattle was slightly lower than in non-emaciated cows. The mean malondialdehyde concentration in non-emaciated cattle was higher than that in emaciated ones, however antioxidants SOD, catalase, Vitamin C and zinc were slightly higher in non-emaciated cows (NEC). Overall, the results indicate that emaciation in studied White Fulani cows (WFC) displayed a variable redox homeostasis confounded by dehydration and depletion of antioxidants.

Keywords: *Anemia, Antioxidants, Cattle, Emaciation, Malondialdehyde*

Declarations

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Not applicable

Authors' contributions:

All authors contributed to the study. Conception and design were done by [Richard Edem Antia] and [Obokparo Godspower Ohore]. Material preparation, data collection and analysis were performed by [Funmilola Clara Thomas], [Abdulhafiz Oloruntoba Ayanleye], [Eyitayo Solomon Ajibola] and [Samson Adisa Rahman]. The first draft of the manuscript was written by [Funmilola Clara Thomas] and [Fakilahyel Musa Mshelbwala] improved on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval:

The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta Research Ethics Committee (CREC). The necessary permissions and consent were obtained from the Abattoir management under the Ogun State Ministry of Agriculture and Natural resources, Veterinary Services Divisions, before collection of data and blood from pre-slaughter cattle at the Lafenwa abattoir, Abeokuta.

Consent to participate:

Verbal informed consent was obtained prior to use of all animals for the study; including the collection of data and sampling of cattle brought in by individuals to the abattoir.

Consent to publish:

Not applicable

Statement of animal rights:

The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Conflicts of interest/Competing interests:

The authors declare that no competing interests exists.

Introduction

Emaciation is a common pathological condition marked by a depreciation of body condition occasioned by the loss of fat and muscle, diminution of organ size and sometimes edematous effusions sequel to anorexia, starvation or cachexia. It is a leading cause of carcass condemnation at ante- and post mortem meat inspection (Kambarage et al., 1995; Phiri, 2006; Raji et al., 2010) and contributes largely to under-pricing and rejection of carcasses for use as meat (due to organoleptic changes in meat (Pełczyńska, 1987). Significant losses also accrue in other consumer products, at critical points of the beef or diary production value chain due to weight loss (Mesele et al., 2012; Assefa and Tesfay, 2013). Emaciation and cachexia also represent a welfare concern in both humans and animals (Grandin, 2014).

Causes of emaciation are diverse and multifactorial, and generally rooted in either insufficient intake/absorption and or losses of nutrients and energy than is being replaced. Haematological evaluations have long been used as a reliable indicator of the physiological and health status of farm animals (Etim et al., 2014). Oxidative stress (OS), the imbalance in levels of oxidants (radicals or non-radicals) and antioxidants in favour of oxidants, in a biological system that triggers oxidation of biomolecules such as proteins, lipids and DNA that results in cellular, tissue and organ damage, have been implicated in the pathogenesis of several diseases of ruminants (Celi, 2011; Lahera et al., 2006).

There are substantial evidence that OS is also a significant player in metabolic disorders induced by obesity, overweight or high body condition scores (Bernabucci et al., 2005; Bayomi et al., 2017). The role of OS in anemia has been reported (Salem et al., 2016). Essentially, OS induced injury to circulating erythrocytes (damage to cell membrane leading to leakage and scrambling) resulting in suicidal death or eryptosis, thereby causing to anemia (Bissinger et al., 2019).

The WFC breed represents the largest population of cattle types in Nigeria which are raised mainly for milk production (although yields are relatively poor) and beef under a predominantly nomadic (extensive) system (Kubkomawa, 2017). During the dry season, emaciation is highly prevalent in this breed, as animals are trekked over long distances in search of food and water. Haematological disturbances, notably anemia is also a common feature due to presence of physical, environmental and pathogen stressors coupled with various forms of nutritional deficiencies.

Although emaciation, OS and anaemia are fairly frequent characteristics in these animals, the relationships between these disorders have not been well explored in WFC. Moreover, studies on OS roles in ruminant health and disease is a relatively new area and sparse cognate reports are available on these species in the study region. An understanding of the relationship and interactions between these pathologic processes, could provide an important guide for improved management and recovery protocols of EC, leading to better prognosis and quicker treatment outcomes.

The purpose of this study was, therefore, to determine the values of haematological parameters and serum redox dynamics, including serum proteins, electrolytes and trace elements in emaciated pre-slaughter WFC, compared to NEC, so as to determine optimum therapies that can be given to pre-slaughter EC to improve their body condition and carcass quality prior to slaughtering.

Materials and Methods

Study animals

Cattle were transported from the Northern part of Nigeria to be used for human food, and kept in a pre-slaughter holding pen at the Lafenwa Central Abattoir, Abeokuta, (Coordinates; Latitude: 7° 09' 23.40" N and Longitude: 3° 20' 32.40" E), Ogun State, Southwest Nigeria. Duration from time of arrival time to time of slaughter usually ranges from 3 to 7 days. A group of emaciated (n=37) and non-emaciated (n=37) cows were purposively sampled from these group animals. Consent was obtained from the abattoir authorities under the auspices of the Veterinary Services Division of the Ogun State Ministry of Agriculture, and individual owners of the cows for the animal studies and sample collection. Sampled cows had a score of 1 and 3 on a body condition scale of 1 to 5 (with 1 being severely emaciated, 3 - good body condition and 5 - obese.

Sample Collection

Approximately 8 ml of blood was collected from the jugular vein into both heparinized and plain bottles from each animal for hematological and biochemical analyses respectively. The samples were stored on ice park and immediately transported to the laboratory for analysis. Serum was harvested from blood and stored at -20°C until analyzed.

Haematology

The packed cell volume (PCV, %) and haemoglobin concentration (g/dl) were determined using the microhaematocrit and cyanomethaemoglobin methods as described by Lepherd et al. (2009) and Srivastava et al. (2014) respectively. The total white blood cells (WBC) and differential white blood cell counts (neutrophils, lymphocytes, eosinophils, monocytes and basophils) ($\times 10^9$/L) and erythrocyte ($\times 10^{12}$/L) counts were evaluated by haemocytometry (Bain et al., 2016). Erythrocytic indices including mean cell volume (MCV) (fl), mean corpuscular haemoglobin concentration (MCHC) (g/dl) and mean corpuscular haemoglobin (MCH) (pg) were determined by calculations (Latimer, 2011).

Serum Analysis

Serum concentration of malondialdehyde (MDA) was determined according to the method of Buege & Aust (1978) as described by Gasso et al. (2016). The antioxidant reduced glutathione (GSH) was measured by a colorimetric method described in Tian et al. (2010). Antioxidant enzymes: catalase, superoxide dismutase (SOD) and glutathione -S- transferase (GST) activities in the serum were assayed according to the methods of Shangari and O'Brien (2006), Marklund & Marklund (1974) and Habig et al. (1974) respectively, as described by Bauché et al. (1994). Ascorbate (vitamin C) and tocopherol (vitamin E) concentrations were determined by colorimetric and spectrophotometric techniques respectively, using a Randox® Ascorbate kit and as described by Rutkowski and Grzegorczyk (2007). Trace elements in serum including copper, iron, selenium and zinc, were evaluated by atomic absorption spectroscopy (AAS) (Atomic absorption spectrometer, Shimadzu Asc-6100, Japan) as described by Kubaszewski et al. (2014). Electrolytes: sodium, potassium and chloride were measured by spectrophotometry using the respective Teco® diagnostic kits. Total protein and albumin were determined using Randox® kits, while globulin concentration and albumin/globulin (A:G) ratio was calculated by substracting albumin concentration from total protein values and ratio between albumin and globulin concnetrations, respectively.

Data Analysis

Continuous variables were presented as means and standard deviations. Tests for normality of data distribution was performed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between indices in emaciated versus good body condition animals were carried out using independent samples Student's T-test. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 23.0 (SPSS *Inc*., Chicago, IL, USA).

Results

Sampled cattle were aged two to six years, with a mean of 3.25 years. A few of the cows had minor bruises, branding marks and a small number of ticks, most were apparently healthy except for emaciation and signs of dehydration such as sunken eyes.

Haematology, Oxidative stress, Trace elements and electrolytes in Emaciated Cows

The results of the complete blood counts (mean ± standard deviation) carried out on the blood samples from both EC and NEC and the significance (p-value) levels are shown in Table 1. All values (for both groups) fell within the haematology reference range for cattle (Jackson et al., 2007), except the mean corpuscular haemoglobin (MCH) which was slightly higher than reference range in both groups.

Table 1: Haematological parameters of emaciated (n = 37) versus non-emaciated cattle (n= 37) (mean ± standard deviation)

Parameters	Emaciated	Non-emaciated	P- value
PCV (%)	39.62 ± 8.34	41.22 ± 6.43	0.360
Hb (g/dl)	12.31 ± 1.55	12.33 ± 1.58	0.953
RBC (x 10^{12}/L)	6.91 ± 1.21	6.93 ± 1.09	0.928
MCV (fl)	58.59 ± 8.39	59.88 ± 7.14	0.210
MCH (pg)	18.09 ± 2.36	18. 03 ± 2.49	0.917
MCHC (g/dl)	31. 81 ± 4.73	30.24 ± 3.52	0.108
WBC (x 10^9/L)	9.95 ±1.68	9.97 ± 1.60	0.949
Neutrophil (x 10^9/L)	2.87 ± 0.48	2.83 ± 0.39	0.696
Lymphocytes (x 10^9/L)	6.65 ± 1.19	6.69 ± 1.23	0.883
Eosinophils (x 10^9/L)	0.14 ± 0.08	0.18 ± 0.09	0.110
Basophils (x 10^9/L)	0.13 ± 0.07	0.12 ± 0.07	0.574
Monocytes (x 10^9/L)	0.16 ± 0.09	0.16 ± 0.09	0.899

Oxidative Stress Marker and Antioxidants

Mean and standard deviation values of the OS indicator and antioxidant compounds in EC and NEC as well as p values showing statistical significance are displayed in Table 2. Values of GSH were significantly lower in EC.

Table 2: Oxidative stress marker (MDA) and antioxidant compounds in emaciated (n=30) versus non-emaciated (n=30) cows.

Parameters	Emaciated	Non-emaciated	P-value
MDA (U/L x 10^{-9})	3.85 ± 2.85	4.51 ± 2.72	0.331
Catalase (U/L)	1.97 ± 0.88	2.14 ± 0.89	0.432
SOD (U/L)	0.0085 ± 0.0015	0.009 ± 0.0012	0.127
GSH (U/L)	236.65 ± 23.53	249.95 ± 1.82	0.03*
GST (U/L)	0.021 ± 0.017	0.016 ± 0.009	0.114
Vitamin E (µg/ml)	10. 29 ± 1.28	10.18 ± 1.50	0.730
Vitamin C (mg/dl)	3.73 ± 0.88	4.06 ± 0.77	0.105

Electrolytes and Trace elements

The results of serum electrolytes and trace elements (mean ± standard deviation) as well as p values showing statistical significance between EC and NEC cattle are depicted in Table 3.

Table 3: Serum electrolytes and trace elements in emaciated (n=30) versus non-emaciated (n=30) cows (mean ± SD)

Parameters	Emaciated	Non-emaciated	P-values
Sodium (mEq/L)	109. 69 ± 9.05	109.95 ± 8.40	0.902
Potassium (mEq/L)	5.93 ± 1.37	5.61 ± 2.23	0.466
Chloride (mEq/L)	85. 53 ± 15.82	86.91 ± 15. 62	0.720
Copper (µg/ml)	0.45 ± 0.18	0.55 ± 0.19	0.032*
Zinc (µg/ml)	1.02 ± 0.39	1.07 ± 0.23	0.530
Iron (µg/ml)	1.92 ± 0.51	2.10 ± 0.51	0.147
Selenium (µg/ml)	0.23 ± 0.12	0.19 ± 0.09	0.116

Serum Total Proteins

Serum protein profile in both EC versus NEC (mean ± standard deviation) along with p values are shown in Table 4.

Table 4: Serum protein in emaciated (n=30) versus non-emaciated (n=30) cows

Parameters	Emaciated cattle	Non-emaciated	P values
Total protein (g/dl)	7.88 ± 0.43	8.02 ± 0.48	0.199
Albumin (g/dl)	4.20 ± 0.33	4.20 ± 0.27	0.897
Globulin (g/dl)	3.66 ± 0.36	3.81 ± 0. 32	0.094
A:G ration	1.14 ± 0.92	1.10 ± 0.84	0.160

DISCUSSION

The present study has revealed the variations in haematological parameters, an OS marker, some enzymatic and non-enzymatic antioxidants, trace elements, electrolytes and protein profiles of serum in EC and non-emaciated pre-slaughter WFC.

Several studies have reported significant associations between anaemia (Igbokwe and Igbokwe, 2012; Akhaine et al., 2021), as well as leukopenia (Sivajothi *et al*., 2015) and leukocytosis (Langenmayer et al., 2015; Ihedioha and Udeani, 2017) and emaciation. The variable relationship between emaciation and parameters indicative of anemia specifically the PCV, may be influenced by the presence of other homeostatic perturbations such as dehydration, which could present a spurious increase in PCV (above reference range of 46%) as observed in some animals sampled in this study. The feed and water restriction, and exposure to high ambient temperature, overcrowding and stress causing diarrheas and nasal discharge associated with transport of cattle over long distances is a key factor that could precipitate dehydration (Rakib *et al*., 2016). In this study, signs of dehydration, mainly sunken eyes and tenting of the skin, were observed more in the emaciated cows. In addition, pre-slaughter animals frequently undergo prolonged duration (up to 24 to 72 hours) without feed and water even in pre-slaughter pens (Personal comm., ; Jarvis *et al*., 1996; Alam *et al*., 2010; Rakib *et al*., 2016). As animals examined in the present study were recently transported, our findings on the haematology agree closely with findings of Rakib et al. (2016) who found elevated levels of PCV and Hb in recently transported cattle intended for slaughter, these values were pointers to dehydration. Furthermore, from this study, RBC counts did not exceed the reference range, therefore it could be concluded that the above-reference –range PCVs seen in 17% of cows (with 53% of these, EC) was not due to polycythemia or even erythrocytosis of splenic contraction.

Previous reports have shown a strong association between emaciation and anemia (Igbokwe *et al*., 2012), who found almost 37% of sampled emaciated animals to have anemia and even in non-emaciated animals, so the results from the present study, where no animal (including emaciated) had anemia (PCV below 24%) was a deviation. This discrepancy may be due to various degrees of dehydration in sampled animals which confounded the detection of anemia *(*Atata *et al*., 2018).

It is probable that the observation of slightly higher hematocrit in EC than the NEC, may be due to pre-existing anemia in some of the cattle of both groups which was masked by dehydration.

White Fulani cattle in good condition have been reported to have higher PCV and lower neutrophil counts compared to a group of cachectic cattle, although the difference was not statistically significant (Aliyu *et al*., 2017). Our results from the present study collaborate this finding. Contrary to the report of Aro (2019), who observed pre-slaughter leukocytosis due to monocytosis in cattle, we did not observe abnormal values in the leucocyte-parameters in either EC or NEC.

The slightly higher concentration of the oxidant MDA, in the good condition cows, is a weak indicator of an enhanced oxidative stress level in this group compared to the EC group. Previous reports have shown positive relationships between good body condition and OS (Sordillo, 2013; Bayomi et al., 2017; Gheise et al., 2017). This study's finding on the MDA seems to support their observations.

However, antioxidants (SOD, catalase and vitamin C and the trace element zinc) were also slightly higher in NEC, thereby contradicting the MDA pro-oxidant pointer. Furthermore, values of GSH and copper (also involved in the antioxidant defense) which were significantly ($p < 0.05$) higher in NEC, favour the opposing opinion that the NEC, had a better redox balance than EC. Only GST, vitamin E and selenium were slightly higher in EC than in NEC ones, and OS is also reported to be involved in the process of cachexia (an advanced stage of emaciation) (Ábrigo *et al*., 2018). Therefore, further studies (under more controlled conditions) to ascertain if emaciation with or without dehydration induces greater oxidative stress than obesity, is warranted.

It has been reported that underfeeding in cattle (which is a major cause of emaciation) results in significant depletion of antioxidants (Sansinanea *et al*., 2000), so the ratio of some antioxidants in EC relative to the non-emaciated cows observed in this study may be due to the greater demand for nutrients (for example, amino acids required for synthesis of GSH, Cu and Fe required for other non-antioxidant biosynthetic pathways in the body), in this group. Therefore, the lower PCV, Cu, GSH and Fe in emaciated than in non-emaciated animals may be mirroring the general effect of reduction of nutrients in the body.

Moreover, dehydration occurs frequently with emaciation (and can also lead to emaciation) and has been shown to increase OS (França *et al*., 2007) especially in transported cattle (Knowles, 1999). It can therefore be speculated that findings that implicate a tendency towards a pro-oxidant status in EC, may have been contributed by dehydration which was more commonly noticed (although not graded) among the EC in this study.

Of the serum electrolytes recorded in this study, the mean Na and Cl values were lower in both groups while the mean K value was higher than that reported by Olayemi *et al*. (2001). Sodium and Cl were recorded in the present study were slightly lower, while K, slightly higher in EC. Loss of electrolytes in the extracellular fluid along with water in the dehydration process is possible explanation for this occurrence.

The mean total protein values in this study were close to range reported by Olayemi *et al.* (2001) in extensively raised White Fulani cattle, but lower than in intensively raised ones, probably resulting from reduced nutrient intake. A higher proportion of chronically emaciated cows in the study of Akhaine *et al*. (2021) also displayed hypoproteinemia mainly due hypoalbuminemia.

The values of Fe, Cu and Na were higher in this study than ranges reported by Asif *et al*. (1996), for cattle in different physiological states. The observation that Fe was slightly higher and Cu, significantly higher in the NEC group, may be related to the depletion from higher demand for Fe and Cu in the EC. Also, the need for these elements to be recruited into several other biological processes, for example erythropoiesis, aside from just the antioxidant system, in EC (Mishra *et al.*, 2019). A delicate balance of Fe is required to prevent OS as either deficiencies or excesses have been shown to precipitate the production of free radicals (Knutson *et al*., 2000).

In conclusion, EC and non-emaciated pre-slaughter WFC showed minor differences in haematology, oxidant/antioxidants, protein and trace elements patterns. Both groups of cows can be said to be prone to oxidative stress as dehydration and depletion of antioxidants may contribute to upsetting the redox homeostasis in favour of pro-oxidation in emaciated cows, while the higher the body condition, has been related to enhanced OS in cattle as revealed by several studies (Gheise *et al*., 2017; Laubenthal *et al*., 2017) and results of MDA in the present study. Therefore, in addition to identifying and correcting the primary cause of emaciation in cattle intended for food, guided antioxidant and trace elements supplementation is recommended, during fattening of cattle for better market value. This is necessary to reduce the risks of OS and its associated complications, and the long run effect on carcass and meat quality.

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