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HEMATOLOGICAL, OXIDATIVE STRESS AND TRACE ELEMENTS VALUES OF EMACIATED AND NON-EMACIATED 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 hematological variations during emaciation. Blood was drawn from the jugular vein for hematological analysis and accruing serum was used for the evaluation of malondialdehyde (oxidative stress marker), antioxidant enzymes and compounds, serum protein levels, 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. None of the animals had a packed cell volume (PCV) below the reference range (24 - 46%), or anemia. Values, however, above the reference were seen, thus 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. Overall, results indicate that emaciation in studied White Fulani cows displayed variable redox homeostasis confounded by dehydration and depletion of antioxidants.

Keywords: anemia, antioxidants, cattle, emaciation, malondialdehyde

INTRODUCTION

The pre-slaughter stage is usually characterized by a highly stressed population of cattle, mostly due to the recent (usually 3-7 days earlier) transportation of animals frequently under harsh conditions over long distances. During this time, emaciation, dehydration, injuries and so on are commonly seen. Emaciation is a common pathological condition marked by the 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 in developing countries (Kambarage *et al*., 1995; Phiri, 2006; Raji, *et al*., 2010)

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and contributes largely to underpricing 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 dairy 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).

Hematological 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 favor 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, has been implicated in the pathogenesis of several diseases of ruminants (Lahera *et al*., 2006; Celi, 2011).

There is 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 circulate erythrocytes (damage to cell membrane leading to leakage and scrambling) resulting in suicidal death or eryptosis, thereby causing anemia (Bissinger *et al*., 2019).

The White Fulani cattle breed represents the largest population of cattle breed in Nigeria which is raised mainly for beef under a predominantly nomadic (extensive) system (Kubkomawa, 2017). Emaciation is highly prevalent in this breed, as animals are trekked over long distances in search of food and water, especially during the dry season. Hematological disturbances, notably anemia, are also a common feature due to the presence of physical, environmental and pathogen stressors coupled with various forms of nutritional deficiencies.

Although emaciation, oxidative stress and hematological changes (anemia) are fairly frequent characteristics in these animals, the relationships between these disorders have not been well explored in White Fulani cattle (Tasew and Duguma, 2012). Moreover, studies on OS roles in ruminant health and disease are a relatively new area and sparse cognate reports are available relative to 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 emaciated cows, leading to better outcomes.

The purpose of this study was to determine the values of hematological parameters and serum redox dynamics, including serum proteins, electrolytes and trace elements in emaciated pre-slaughter White Fulani cows, compared to non-emaciated, so as to ascertain optimum therapies that can be given to pre-slaughter emaciated cattle to improve their body condition and carcass quality prior to slaughtering.

MATERIALS AND METHODS

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 cattle brought into the abattoir, over a period of two weeks. Samples were

collected from the cows during evisceration, which represented approximately 2 to 3 days after arrival at the abattoir. Animals that had visible ribcage, depressed paralumbar fossa, sunken eyes and prominent scapula bones were selected as emaciated, while cows in which these signs were absent were adjudged non-emaciated. Sampled cattle were aged between 2 to 6 years, with a mean of 3.25 years. A few of the cows had minor bruises, branding marks and a small number of ticks, others were apparently healthy except for emaciation and signs of dehydration such as sunken eyes. Between 70 and 100 cattle are brought into the abattoir daily except on weekends, and the studied animals were selected from this population. Consent was obtained from the abattoir authorities under the auspices of the Veterinary Services Division of the 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).

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

The packed cell volume (PCV, %) and hemoglobin concentration (g/dl) were determined using the microhematocrit and cyanomethemoglobin 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 hemocytometry after making blood smears (Bain *et al*., 2016). Erythrocytic indices including mean cell volume (MCV) (fl), mean corpuscular hemoglobin concentration (MCHC) (g/dl) and mean corpuscular hemoglobin (MCH) (pg) was determined by calculations (Latimer, 2011).

The serum concentration of malondialdehyde (MDA) was determined according to the method of Buege and Aust (1978) as described by Gasso *et al*. (2016). The antioxidant-reduced glutathione (GSH) was measured by a colorimetric method described in the study of 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 described by Shangari and O'Brien (2006), Marklund and 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, such as 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 was calculated by substracting albumin concentration from total protein values.

Continuous variables were presented as means and standard deviations. Tests for normality of data distribution were 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 Students *t*-test. Correlations between trace elements and oxidant/antioxidants were tested using Pearson's correlation tests. 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

The results of complete blood counts (mean ± standard deviation) carried out on the blood samples from both emaciated and non-emaciated cattle indicated no significant differences in parameters between these groups, also all values (for both groups) fell within the hematology reference range for cattle (Jackson and Cockcroft, 2007), except the mean corpuscular hemoglobin (MCH) which was slightly higher than reference range in both groups (Table 1).

Mean and standard deviation values of the oxidative stress indicator and antioxidant compounds in emaciated and good condition cattle as well as *P*-values showing statistical significance between emaciation versus non-emaciated cattle are displayed in Table 2. Values of GSH were significantly lower in emaciated cows.

The results of serum electrolytes and trace elements (mean ± standard deviation), as well as *P*-values showing statistical significance between emaciation and non-emaciated cattle, are depicted in Table 2. A significantly higher Cu concentration was observed in non-emaciated cows, while other analytes were not significantly varied as shown in Table 3. However, there was a trend of lower values of the elements in emaciated animals.

Serum protein profile in both emaciated versus non-emaciated cattle (mean ± standard deviation) along with *P*-values is shown in Figure 1. Total protein was higher in non-emaciated cows, although the results were not significant on statistical evaluation.

Table 1. Hematological parameters of emaciated (n = 37) versus non-emaciated cows (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

Table 2: Oxidative stress marker MDA and antioxidant compounds in emaciated versus non-emaciated 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	0.030*
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

*Significantly different at $P<0.05$.

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

Parameters	Emaciated	Non-emaciated	*P*-value
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

*Significantly different at $P<0.05$.

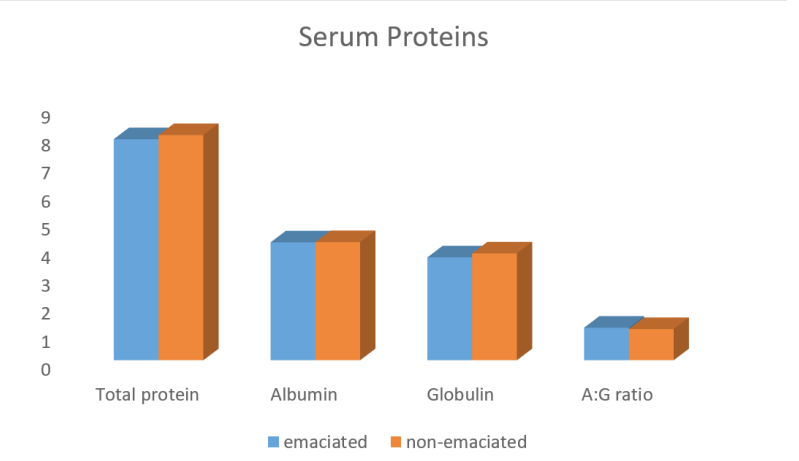

Figure 1. Serum total protein, albumin, globulin and A:G ratio (g/dL) values of emaciated versus non-emaciated pre-slaughter White Fulani cows.

DISCUSSION

The present study has revealed the variations in hematological parameters, an oxidative stress marker, some enzymatic and non-enzymatic antioxidants, trace elements, electrolytes and protein profiles of serum in emaciated and non-emaciated pre-slaughter white Fulani cows.

Several studies have reported significant associations between anemia (Igbokwe *et al*., 2012; Akhaine *et al*., 2021), as well as leukopenia (Sivajothi *et al*., 2015) and leukocytosis (Langenmayer *et al*., 2015; Ihedioha *et al*., 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 the transport of cattle over long distances are key factors that could precipitate dehydration (Rakib *et al*., 2016). Signs of dehydration which were observed as mainly sunken eyes and tent in the skin were observed in all the pre-slaughter animals but 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 (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 hematology agree closely with the 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 in emaciated cows) were 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).

The presence of dehydration in both groups of cows (due to the conditions in the abattoir) has contributed to causing the PCV values to be high, thereby masking the presence of anemias in this study, which is expected to be common in emaciated cows.

Good condition White Fulani cattle 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 mirror this finding. Contrary to the report of Aro (2019), who observed pre-slaughter leukocytosis due to monocytosis in cattle, we did not observe abnormal ranges in the leucocyte parameters in either emaciated or good condition pre-slaughter cows.

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 emaciated cows' group. Previous reports have shown positive relationships between good body condition and oxidative stress (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 elements zinc were also slightly higher in non-emaciated cows, 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 non-emaciated cows, favor the opposing opinion that the non-emaciated cows, had a better redox balance than emaciated ones. Only GST, vitamin E and selenium were slightly higher in emaciated cows than in good body condition ones, and OS is also reported to be involved in the process of cachexia (an advanced stage of emaciation) (Ábrigo *et al*., 2018), therefore it is difficult to make conclusions on which group of animals have an optimum oxidant-antioxidant balance.

The tendency of higher total protein concentration in non-emaciated cows compared to emaciated is expected as emaciation is associated with depletion of nutrients in the body, including proteins (Igbokwe *et al*., 2012).

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 emaciated cows 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 the 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, although dehydration would have contrary effects which may have been responsible for not very significant differences between the groups. According to Onmaz *et al*. (2019), depletion of these elements and antioxidants are likely causes of pica in beef cattle.

In studies by França *et al*. (2007), it was shown that emaciation and dehydration often occur together and that these two conditions can increase OS. These findings were observed in cattle that were recently transported (Knowles, 1999).

It can therefore be speculated that findings that implicate a tendency towards a pro-oxidant status in emaciated cows, may have been contributed by dehydration which was more commonly noticed (although not graded) among the emaciated cows in this study.

Serum electrolytes; Na, Cl were generally (both groups) lower than that reported by Olayemi *et al*. (2001), while K was higher on the average in the present study and specifically slightly higher in emaciated cows. Loss of electrolytes in the extracellular fluid along with water in the dehydration process is a possible explanation for this occurrence.

The values of Fe, Cu and Na were higher in this study than the ranges reported by Asif *et al*. (1996), for cattle in different physiological states. The observation that Fe and Cu in the good body condition cows were slightly higher in the non-emaciated cows' group, may be related to the higher demand for Fe and Cu, and the need for these elements to be recruited into several other biological processes, for example, erythropoiesis, aside from just the antioxidant system, in emaciated animals (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, emaciated and non-emaciated pre-slaughter White Fulani cows showed minor differences in hematology, oxidant/antioxidants, protein and trace elements patterns. Both groups of cows can be said to be prone to oxidative stress through dehydration on one hand, and emaciation (depletion of antioxidants) and dehydration on the other hand. Higher body condition is related to enhanced OS as revealed by several studies (Gheise *et al*., 2017; Laubenthal *et al*., 2017) and results of MDA (higher in non-emaciated cows)

in the present study. It would be interesting to investigate which contributes greater to the generation of OS between high body condition and emaciation-dehydration. 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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