JOURNAL OF VETERINARY AND APPLIED SCIENCES
2023 VOL. 13(2): 260 – 272
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Evaluation of the haematology and serum biochemistry profile of albino rats fed diets composed of cowhide processed by various singeing methods

Fatima Sanusi[1] *, Abigail C. Abwage[2], Babatunde S. Okediran[1], Yusuf K. Suleiman[1], Yusuf Idris[1]

[1] Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria.

[2] Department of Forestry and Wildlife Management, Faculty of Agriculture, Taraba State University, Jalingo. Taraba State, Nigeria.

==========

Abstract

Singeing cowhide is the first step in producing hide, a staple food component in Nigeria. This study investigated the haematological and serum biochemical alterations associated with feeding albino rats with diets composed of cowhide processed by various singeing methods. Thirty male rats randomly allocated into six groups (A – F) of five rats each, were used for the study. Group A rats served as the control that was not given feed with singed hide. Rats in Groups B – F were fed on diets with cowhide processed as follows: B – Shaving only; C – Shaving after tenderization with detergent; D – Singeing with petrol fire and washing with detergent; E – Singeing with kerosene fire and washing with detergent, F – Singeing with spent tyre fire and washing with detergent. The cowhide inclusion in diet was at the ratio of 40% hides: 60% commercial feed. The rat groups were fed *ad lib* with their group-specific diets for four weeks, after which blood samples were collected for haematology and serum biochemistry evaluations. Results showed that there were no significant variations ($p > 0.05$) in the mean body weights of the rat groups all through the experimental period. Rats in Group D had significantly ($p < 0.05$) lower red blood cell counts, haemoglobin concentration and packed cell volume, and significantly ($p < 0.05$) higher total white blood cell (TWBC), neutrophil and lymphocyte counts. Group F rats had significantly ($p < 0.05$) higher TWBC and lymphocyte counts, while Groups C and E rats had significantly ($p < 0.05$) lower TWBC, neutrophil and lymphocyte counts. Groups C, D, E and F rats had a significantly lower ($p < 0.05$) serum total cholesterol, while Group E alone had significantly ($p < 0.05$) higher serum creatinine levels. Groups E and F also had significantly higher ($p < 0.05$) serum glutathione peroxidase activity. It was concluded that feeding albino rats with cowhide processed by certain singeing methods as used in the study led to significant alterations in some of the haematology and serum biochemical parameters.

Keywords: Singeing, Cowhide, Haematology; Serum Biochemistry, Petrol, Kerosene, Spent tyre.

***Correspondence:** Fatima Sanusi; E-mail: sanusi.f@unilorin.edu.ng Phone: +2348067650602

Article History: Initial submission received: May 22, 2023; Final revised form received: October 19, 2023; Accepted for publication: November 06, 2023; Published: November 21, 2023.

Introduction

Cowhide is a very valuable raw material for the leather industries, and also serves as a good source of animal protein in some parts of the world, where it is also used as a substitute for flesh meat (Marti *et al*., 2012; Ekenma *et al*., 2015). Foods made from processed cowhides are extremely popular in Nigeria. Hence, singed cowhide, popularly known as "Ponmo" or "Kanda," is of great economic importance in this part of the world. It contributes to solving the food security problem, serves as an essential part of the human diet by providing essential proteins and minerals, and it is a viable alternative to consuming red meat (Ekenma *et al*., 2015; Alturiqi and Albedair, 2012).

Singeing is a process by which the fur on the skin of a carcass is removed, making the hide ready for human consumption and evoking flavours in the meat that are acceptable to consumers (Food and Agriculture Organization, 1985). However, some singeing methods/procedures have been questioned in recent times and some has been reported to make the product unwholesome and thus a risk to human health (Egwuonwu *et al*., 2019; Concha *et al.,* 2013). The traditional method of singeing cowhide is by burning the fur off the carcass or hide in an open fire using firewood. However, this method in recent times been modified by the use of some petroleum products (kerosene, petrol, engine oil etc.) as fuel for the fire (Obiri-Danso *et al*., 2008). Local butchers at abattoirs and slaughterhouses in recent times are now in favour of using vehicle scrap tyres, plastics, and polystyrene materials for burning off the fur (Ekenma *et al*., 2015). These preferred alternatives, according to the butchers, are much more affordable and efficient, produce more flame with less heat, and are able to selectively burn off the fur from the animal carcass without cracking the hide (Adam *et al*., 2013; Obiri-Danso *et al*., 2008). However, it has been reported that heavy metals are released during the burning of hides with these materials, thereby contaminating the meat and polluting the environment (Okiei *et al*., 2009).

The deleterious effects of heavy metals such as lead and arsenic has been extensively reported (Mensah *et al, 2019;* Ekenma *et al,* 2015; Okafor *et al, 2012)*. Volatile organic compounds (VOCs), particulate matter, and polycyclic aromatic hydrocarbons (PAHs) are also released into the environment through the combustion of such petroleum products. These toxic heavy metals are not easily degradable; they tend to bio-accumulate increasingly in organs and tissues, with possible adverse effects on the central nervous system (Isangedighi and David*,* 2019). Some of the heavy metals have been reported to be carcinogenic, nephrotoxic, neurotoxic and haematotoxic (Ercal, *et al.,* 2001; Egwuonwu *et al*., 2019). Despite these numerous documented adverse effects of heavy metals on health, there had been no reports in available literature on the effects of the consumption of hides singed by burning off the fur with petroleum products, on the health of the consumers. This present study evaluated the effects of the inclusion of cowhide singed by various methods on the haematology and serum biochemistry profile of albino rats.

Materials and Methods

Experimental Animals and Ethics: Thirty male albino rats were used for this study. The average weight of the rats was 152 ± 3.50 g at the onset of the experiment. These rats were kept in metallic cages under room temperature and conditions. They were provided with drinking water and commercial laboratory animal feed (Topfeed®, Lagos, Nigeria), with the following declared composition: Fat/oil – 6%, Crude fibre – 5%, Calcium – 1%, Phosphorus – 0.4%, Lysine – 0.85%, Methionine – 0.35%, Salt – 0.3%, Crude

Protein – 18%, Metabolizable Energy – 2900 Kcal/kg, all through the study. The rats were acclimatized for two weeks before the start of the experiment. All experimental protocols carried out on the animals were in accordance with the internationally accepted principles for laboratory animal use and care, and were approved by the Ethics Committee on Laboratory Animal Use of the Faculty of Veterinary Medicine, University of Ilorin, Nigeria (Approval Reference Number: UIL/FVERC/ 022/2021).

Cowhide used for the study: Samples of the hide of un-singed carcass used for the study were purchased from the Abubakar Sola Saraki Memorial, Abattoir, Akerebiata, Ilorin, Kwara State, Nigeria. The abattoir is located along latitude 8.52667N and longitude 4.55284E. The hide purchased was from a heifer of *Bos indicus spp* (Red Bororo). After purchase, the hide was packed in moisture-proof polythene bags and then transported elsewhere for singeing.

Processing of Hides Sample: The hides that were purchased were processed using five common methods of de-hairing cowhides. The transported hides were cut into five portions for the different de-hairing processes. One out of the five portions of the hide was de-haired by shaving using an electric shaver. A second portion was washed with detergent before de-hairing by shaving. The last three portions of the hide were singed by burning off the fur with fire made from petrol, kerosene, or spent tyre, respectively After the singeing process, the hides were air dried and pulverized using an electrical grinder and preserved in air-tight containers to be added as inclusions in the rats' diets.

Laboratory Animal Experimental Design: The 30 albino rats were randomly assigned to six groups of five each (Groups A, B, C, D, E and F). Group A served as the control and was fed on 100% commercial rat feed (Topfeed®, Lagos, Nigeria) only. The other groups (Groups B, C, D, E, and F) were fed on diets composed of 60% rat feed and 40% processed hide, as follows: Group B – Hide de-haired by shaving only; Group C – Hide shaved after tenderization with detergent; Group D – Hide from which hair was burnt off with petrol fire and later washed with detergent, Group E – Hide from which hair was burnt off with kerosene fire and later washed with detergent, Group F – Hide from which hair was burnt off with fire made from spent tyre and later washed off with detergent (Figures 1 and 2). The rats were fed *ad libitum* with their group-specific diets for four weeks, after which blood samples were collected from them for haematology and serum biochemistry evaluations. Body weights were also measured weekly.

Figure 1. Cowhide processed by shaving only or shaving after detergent washing.

Figure 2. Cowhide processed by singeing with fire made from petrol, kerosene or used tyre, and later washed with detergent.

Body weight and feed consumption: Body weights of the individual rats in each group were measured using an electronic weighing balance. The body weight was measured twice during the acclimation period, as well as at the beginning of the study (day 0), and weekly thereafter.

Collection of Blood Samples for hematology and biochemical analysis: At the end of the four weeks experimental period, the rats were sacrificed via jugular venesection after a mild anaesthesia with ether. Blood samples were subsequently collected and dispensed into sample bottles containing the anticoagulant EDTA (for haematology) and plain test tubes (for serum biochemistry).

Haematology: The blood samples in the sample bottles containing EDTA were subjected to haematological analysis using the Perlong HA6000 Auto Haematology Analyzer (Perlong Medical Equipment, China).

Serum preparation: The aliquots of blood samples that were dispensed into plain test tubes were centrifuged at 4000 revolutions per minute for 10 minutes to separate the sera from the clotted blood components. The sera were then decanted and refrigerated in Eppendorf tubes for further analyses.

Biochemical analysis: Total serum protein and albumin concentrations were determined using Randox® commercial Total Protein and Albumin test kits (Randox®, UK), as described by Tietz (1995) and Grant (1987), respectively. Blood urea nitrogen (BUN) and creatinine concentrations were also determined using Randox® BUN and Creatinine test kits as described by Henry (1974). The aspartate and alanine aminotransferases activities were assayed as described by Reitman and Frankel (1957), while serum triglyceride and cholesterol concentrations were determined as described by McGowan et al., (1983) and Wybenga et al. (1970), respectively. The superoxide dismutase activity was analysed using SOD Assay Kit-WST (Sigma-Aldrich, USA) as previously described by Martin, *et al*. (1987), while the malondialdehyde (MDA) concentrations were evaluated using the double heating method as described by Draper and Hadley (1987). The glutathione peroxidase activity was analysed using the Fortress Diagnostic Glutathione Peroxidase Assay Kits (Fortress, Antrim, UK), as described by Paglia and Valentine (1967).

Statistical analysis: The analyses of the data generated from this study were done using Graph Pad Prism Version 5 (San Diego, California, USA). One-way analysis of variance (ANOVA) was used to compare the parameters of the groups, and differences between variant means were separated by Duncan multiple comparison test. Summaries of the results were presented as mean ± standard error of the mean. Probability less than 0.05 was considered significant.

Results

There were no significant variations ($p > 0.05$) among the rat groups in their body weight all through the experimental period (Table 1). The packed cell volume (PCV), red blood cell (RBC) counts and haemoglobin concentration (Hb) of the Group D rats (fed diets containing 40% of hide singed by burning the hairs off with petrol) was significantly lower ($p < 0.05$) that those of all other groups, but there were no significant differences ($p > 0.05$) in the mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) of all the rats groups (Table 2).

The total white blood cell (TWBC) counts of the Group D and F rats were significantly ($p < 0.05$) higher than that of Groups A, C and E rats, while those of the Groups C and E were further significantly ($p < 0.05$) lower than those of all other groups (Table 3). The absolute neutrophil counts for Groups C, E and F rats were significantly ($p < 0.05$) lower than

that of the Groups A, B and D rats, with that of Group D rats being the highest (Table 3). The absolute lymphocyte counts for Groups C and E rats were significantly ($p < 0.05$) lower those of all other groups, while those of Groups D and F were outstandingly significantly ($p < 0.05$) higher than those of all other groups (Table 3). There were no significant differences ($p > 0.05$) between the groups in their absolute eosinophil counts, but the absolute monocyte counts of Groups D and F rats were significantly ($p < 0.05$) higher than those of all other groups (Table 3).

The serum total cholesterol levels of the Groups C, D, E and F rats were significantly ($p < 0.05$) lower than those of the Groups A and B rats, but there were no significant differences ($p > 0.05$) between the groups in their serum levels of triglyceride, low density lipoproteins, total protein, albumin, blood urea nitrogen and alkaline phosphatase (ALP) activity (Table 4). The serum creatinine levels of Group E rats was significantly ($p < 0.05$) higher than those of all other rat groups (Table 4). The serum AST activity of the Groups B, D and E rats were significantly ($p < 0.05$) lower than those of Groups A and F, while the serum ALT activity of Groups D and F were significantly lower than that of the Group E rats (Table 4).

There were no significant differences ($p < 0.05$) between the groups in their serum levels of sodium, potassium, chloride and calcium (Table 5). There were also no significant ($p > 0.05$) differences between all the groups in their serum superoxide dismutase (SOD) activity (Table 6). The serum glutathione peroxidase (GPx) activity of the Group E and F rats were significantly ($p < 0.05$) higher than those of all other groups (Table 6). The serum malondialdehyde (MDA) level of rats in Groups B, C, E and F was significantly lower than that of the Group A rats (Table 6).

Table 1. Body weights of rat groups* fed diets with 40% inclusion of cowhide singed by various methods.

Experimental period (days)	Mean body weights (g), with standard error in brackets					
	Group A	*Group B*	*Group C*	*Group D*	*Group E*	*Group F*
Day 0	148.02 (2.32)	150.01 (1.14)	150.04 (2.22)	149.11 (2.02)	149.05 (2.45)	152.05 (2.15)
Day 7	149.05 (2.45)	150.06 (2.42)	150.06 (2.12)	148.11 (2.32)	150.05 (2.45)	152.25 (2.45)
Day 14	149.10 (2.21)	152.02 (2.02)	151.01 (2.21)	149.32 (2.12)	149.05 (2.45)	151.05 (3.45)
Day 21	150.11 (2.11)	152.67 (3.32)	150.06 (2.41)	149.02 (2.32)	150.05 (2.45)	151.45 (2.45)
Day 28	150.17 (2.01)	154.92 (2.21)	151.02 (2.30)	148.02 (2.12)	150.05 (2.65)	152.05 (1.15)

No significant variations ($p > 0.05$) between the mean body weights of the rat groups all through.

* Rat Groups: Group A – Rats fed commercial rat feed only (Control Group – No cowhide in diet); Group B – Rats fed diet with 40% cowhide de-haired by shaving only; Group C – Rats fed diet with 40% cowhide de-haired by shaving after tenderization with detergent; Group D – Rats fed diet with 40% cowhide from which hair was burnt off with petrol fire and later washed with detergent, Group E – Rats fed diet with 40% cowhide from which hair was burnt off with kerosene fire and later washed with detergent; Group F – Rats fed diet with 40% cowhide from which hair was burnt off with fire made from spent tyre and later washed off with detergent.

Table 2. The erythrocytic profile of rat groups* fed diets with 40% inclusion of cowhide singed by various methods.

Erythrocytic parameters	*Means of erythrocytic parameters ± standard error.*					
	Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
PCV (%)	35.0 ± 2.89 [a]	41.0 ± 0.58 [a]	34.7 ± 1.45 [a]	24.7 ± 2.60 [b]	37.0 ± 1.73 [a]	35.0 ± 4.04 [a]
RBC counts (10^6/µL)	5.9 ± 0.41 [a]	6.5 ± 0.21 [a]	5.6 ± 0.29 [a]	4.0 ± 0.46 [b]	6.03 ± 0.20 [a]	5.7 ± 0.72 [a]
Hb (g/dL)	11.4 ± 1.94 [a]	13.7 ± 0.20 [a]	11.4 ± 0.52 [a]	8.3 ± 0.85 [b]	12.3 ± 0.58 [a]	11.9 ± 1.39 [a]
MCV (fL)	60.2 ± 0.78	62.6 ± 1.05	61.7 ± 0.58	58.8 ± 0.87	61.8 ± 0.87	61.1 ± 0.64
MCH (pg)	22.7 ± 0.20	21.0 ± 0.35	21.4 ± 0.11	22.9 ± 0.26	21.3 ± 0.33	23.1 ± 0.20
MCHC (g/dL)	33.2 ± 0.09	33.4 ± 0.01	32.9 ± 0.12	33.5 ± 0.12	33.2 ± 0.02	33.5 ± 0.03

[a, b] Mean values with different superscripts in a row indicate significant ($p < 0.05$) difference between the groups. [RBC – Red blood cell; PCV – Packed cell volume; Hb – Haemoglobin; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration].

Table 3. The leukocytic profile of rat groups* fed diets with 40% inclusion of cowhide singed by various methods.

Leukocytic parameters	*Means of leukocytic parameters ± standard error.*					
	Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
TWBC count (10^3/µL)	8.2 ± 0.48 [a]	9.3 ± 0.15 [ad]	4.9 ± 1.24 [b]	15.0 ± 0.35 [c]	3.8 ± 0.88 [b]	11.2 ± 1.79 [d]
Neutrophil (10^3/µL)	1.34 ± 0.11 [a]	1.40 ± 0.38 [a]	0.74 ± 0.14 [b]	2.70 ± 0.01 [c]	0.56 ± 0.06 [b]	0.71 ± 0.10 [b]
Lymphocyte (10^3/µL)	6.72 ± 0.14 [a]	7.47 ± 0.38 [a]	3.92 ± 0.14 [b]	10.20 ± 0.26 [c]	2.66 ± 0.06 [d]	9.74 ± 0.16 [c]
Eosinophils (10^3/µL)	0.10 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.00
Monocytes (10^3/µL)	0.10 ± 0.00 [a]	0.10 ± 0.00 [a]	0.10 ± 0.00 [a]	0.32 ± 0.06 [b]	0.09 ± 0.03 [a]	0.59 ± 0.05 [c]

[a, b, c, d] Mean values with different superscripts in a row indicate significant ($p < 0.05$) difference between the groups. [TWBC - Total white blood cell].

* Rat Groups: Group A – Rats fed commercial rat feed only (Control Group – No cowhide in diet); Group B – Rats fed diet with 40% cowhide de-haired by shaving only; Group C – Rats fed diet with 40% cowhide de-haired by shaving after tenderization with detergent; Group D – Rats fed diet with 40% cowhide from which hair was burnt off with petrol fire and later washed with detergent, Group E – Rats fed diet with 40% cowhide from which hair was burnt off with kerosene fire and later washed with detergent; Group F – Rats fed diet with 40% cowhide from which hair was burnt off with fire made from spent tyre and later washed off with detergent.

Table 4. The serum biochemistry profile of rat groups* fed diets with 40% inclusion of cowhide singed by various methods.

Serum biochemistry parameters	*Means of serum biochemistry parameters, with standard error in brackets*					
	Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
Total Cholesterol (mmol/L)	13.00 [a] (1.00)	13.50 [a] (0.30)	3.20 [b] (0.20)	2.80 [b] (1.45)	3.30 [b] (0.27)	3.10 [b] (0.20)
Triglycerides (mmol/L)	0.90 (0.35)	1.10 (0.21)	0.90 (0.09)	0.80 (74)	0.80 (0.89)	0.70 (0.00)
LDL (mmol/L)	1.90 (0.40)	1.50 (0.15)	1.90 (0.49)	1.50 (0.50)	1.40 (0.20)	1.50 (0.10)
Total Protein (g/dl)	6.05 (0.35)	5.23 (0.38)	5.73 (0.47)	5.50 (0.64)	5.97 (0.18)	6.05 (0.15)
Albumin (g/dl)	3.30 (0.30)	2.77 (0.12)	2.87 (0.15)	3.10 (0.15)	3.06 (0.12)	3.25 (0.15)
Creatinine (µmol/L)	52.00 [a] (6.00)	50.00 [a] (3.46)	47.00 [a] (3.60)	53.00 [a] (3.40)	60.30 [b] (1.20)	55.00 [a] (1.00)
BUN (mg/dl)	4.75 (.05)	4.30 (0.95)	5.20 (0.03)	4.60 (0.57)	4.90 (0.24)	4.10 (0.15)
AST (IU/L)	28.0 [a] (1.00)	22.0 [b] (0.57)	26.0 [ab] (2.00)	20.0 [b] (3.22)	20.0 [b] (1.15)	28.5 [a] (2.50)
ALP (IU/L)	24.5 (1.50)	29.7 (3.36)	26.0 (4.04)	22.0 (4.53)	28.6 (2.40)	20.0 (1.00)
ALT (IU/L)	13.0 [ab] (1.00)	9.67 [ab] (1.86)	10.3 [ab] (0.88)	9.00 [a] (0.05)	14.7 [b] (0.67)	9.00 [a] (1.00)

[a, b] Mean values with different superscripts in a row indicate significant ($p < 0.05$) difference between the groups. [ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; ALP – Alkaline phosphatase; BUN – Blood urea nitrogen; LDL – Low density lipoprotein.

* Rat Groups: Group A – Rats fed commercial rat feed only (Control Group – No cowhide in diet); Group B – Rats fed diet with 40% cowhide de-haired by shaving only; Group C – Rats fed diet with 40% cowhide de-haired by shaving after tenderization with detergent; Group D – Rats fed diet with 40% cowhide from which hair was burnt off with petrol fire and later washed with detergent, Group E – Rats fed diet with 40% cowhide from which hair was burnt off with kerosene fire and later washed with detergent; Group F – Rats fed diet with 40% cowhide from which hair was burnt off with fire made from spent tyre and later washed off with detergent.

Table 5. The serum electrolyte profile of rat groups* fed diets with 40% inclusion of cowhide singed by various methods.

Serum electrolytes	*Means of serum levels of electrolytes, with standard error in brackets.*					
	Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
Sodium (mEq/L)	128.0 (1.50)	134.0 (3.52)	128.0 (1.67)	140.0 (1.50)	136.0 (1.33)	139.0 (0.50)
Potassium (mEq/L)	6.10 (0.00)	7.33 (0.54)	5.80 (1.10)	5.50 (0.54)	6.43 (0.68)	7.00 (0.40)
Chloride (mEq/L)	117 (2.00)	110 (2.40)	105 (8.17)	92.0 (0.09)	109 (4.04)	117 (3.00)
Calcium (mmol/L)	2.19 (0.05)	2.14 (0.09)	2.15 (0.09)	2.2 (0.04)	2.2 (0.01)	2.3 (0.08)

No significant variations ($p > 0.05$) between the mean serum electrolytes levels of the rat groups.

Table 6. The serum levels of oxidative stress markers of rat groups* fed diets with 40% inclusion of cowhide singed by various methods.

Serum oxidative stress markers	*Means of serum levels of oxidative stress markers, with standard error in brackets*					
	Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
Superoxide dismutase (IU/ml)	174.0 (4.00)	151.0 (2.08)	166.0 (1.00)	151.0 (7.00)	167.0 (11.9)	177.0 (38.5)
Glutathione peroxidase (IU/L)	2706.0 [a] (237.0)	2227.0 [a] (165.0)	2710.0 [a] (841.0)	2414.0 [a] (39.0)	3059.0 [b] (81.0)	3528.0 [c] (21.0)
Malondialdehyde (nmol/ml)	2.30 [a] (0.10)	1.6 [b] (0.10)	1.3 [b] (0.25)	1.9 [ab] (0.05)	1.55 [b] (0.35)	1.50 [b] (0.10)

[a, b, c] Mean values with different superscripts in a row indicate significant ($p < 0.05$) difference between the groups.

* Rat Groups: Group A – Rats fed commercial rat feed only (Control Group – No cowhide in diet); Group B – Rats fed diet with 40% cowhide de-haired by shaving only; Group C – Rats fed diet with 40% cowhide de-haired by shaving after tenderization with detergent; Group D – Rats fed diet with 40% cowhide from which hair was burnt off with petrol fire and later washed with detergent, Group E – Rats fed diet with 40% cowhide from which hair was burnt off with kerosene fire and later washed with detergent; Group F – Rats fed diet with 40% cowhide from which hair was burnt off with fire made from spent tyre and later washed off with detergent.

Discussion

Cowhide is a staple meat substitute consumed daily by millions of Nigerians. There is poor control and hygiene enforcement by abattoir and slaughter slab officials who have allowed the use of unconventional and toxic materials such as petroleum product-based fuels and spare tyres as sources of fire for singeing cowhide.

The lack of significant differences between the body weights of rats fed diets with cowhide inclusion when compared to the Group A that was fed diet without cowhide inclusion, implies that cowhide inclusion in the diet led to no significant improvement in body weight, and this finding concurs with earlier reports by Olukitibi *et al* (2017) that showed that cowhide has low caloric and nutritional value. The present finding on body weight of the rats however contrasts with the reports of Ademola *et al*., (2022) which stated that consumption of processed cowhide improved weight gain.

Exposure to toxic residues of petroleum products used for the burning of cowhide has been shown to cause significant alterations in the haematology of exposed individuals by causing leucocytosis and anaemia (Sajid *et al*., 2020). In addition, exposure to gasoline by ingestion has been shown to have negative effect on the haematopoietic system of individuals exposed, mainly as a result of decreased ability of red blood cells to transport oxygen (Seriki *et al*., 2016); this earlier reports concurs with our current finding of anaemia in the rat group fed diet with inclusion of cowhide de-haired with petrol fire (Group D). Other possible causes of the anaemia recorded in the group fed diets with inclusion of cowhide de-haired with petrol fire may be lead poisoning and exposure to heavy metal pollutants which are commonly found in petrol (Egwuonwu *et al.,* 2019). In Nigeria, hides are usually consumed directly after removal of all hair and/or washing. Removal of the hair from the hide in Nigeria is traditionally done by singeing with fire made from fuel in the form of petrol, kerosene, engine oil and scrap tyre. The burnt hide is usually scraped and washed using detergent to remove the black soothe to give a finished product (Woko *et al*., 2020). Adam et al. (2013) reported that washing of singed goat hide slightly reduce the heavy metal concentrations, but the concentration is still higher than the maximum permissible level (MPL), signifying that washing doesn't make it totally wholesome for consumption

The leukopaenia recorded for Group C and E is thought to possibly be due to depression of leukocyte production by heavy metals and hydrocarbon contaminants in detergents and kerosene. The leucocytosis recorded in the group D and F rats is due probably to petroleum toxicity and deposit of benzene from burnt tyre. (Ita and Udofia 2011). Aromatic compounds such as benzene and its metabolites like benzo-a-pyrene is found in petrol and is also a product of burning tyre, which may cause depression of the hematopoietic system or leucocytosis (Begeman *et al*., 1968; Okonkwo *et al*., 2018). The alterations in the leukogram of the rat groups fed diets with cowhide that were processed by singeing with fuel fire and washing with detergent can also be linked to the detergent used for tenderization and for washing off the black smoke residue on the finished cowhide in these groups; possibly due to the presence of toxic residues in detergents, as had earlier been reported by Egwuonwu *et al.* (2019) and Ademola *et al*. (2022). The toxic effects and toxicity of the detergents have been reported by several earlier studies, mainly the anionic detergents (Warne and Schifko, 1999; Dehelean *et al*., 2004; Idowu *et al*., 2017). It is thought that consumers that boil the finished cowhide product further and discard the water to eliminate unwanted residues may successfully reduce the risk of exposure to the toxic

residues from the detergent while others who consume the cowhide finished product directly may stand a higher risk of consuming the finished cowhide along with the toxic residues from detergent (Bielawski, 1990; Okiei *et al.,* 2009). The significantly lower neutrophil and lymphocyte counts recorded for the Group C and E rats fed diets with cowhide washed with detergent and cowhide singed with kerosene fire before washing with detergent, respectively suggests that consumption of cowhide processed in these ways may be associated with immunosuppression because neutrophils and lymphocytes are responsible for cellular and humoral immunity (Janeway *et al*., 2001; Marshal *et al*., 2018). On the other hand, the significantly higher levels of neutrophils in the Group D rats fed diets with cowhide processed by singeing with petrol fire suggests toxicity (Haschek *et al*., 2009).

Hypocholesterolaemia has been associated with some health problems. Earlier research highlighting low cholesterol level is limited. However, research has linked low serum cholesterol levels to cancer (Song *et al*., 2000). It is therefore possible that if the outcome of this present study can be extrapolated to humans, it can be speculated that consistent consumption of cowhide singed by burning off the hair with petrol, kerosene, and used tyre and also washing with detergent may predispose the consumers to hypocholesterolemia and possibly cancer. The significantly higher serum creatinine levels recorded for the Group E rats fed diets with cowhide singed with kerosene fire before washing with detergent suggests that consumption of such cowhides may predispose the consumer to development of kidney failure, because serum creatinine is a well-known marker of kidney dysfunction (Isles and Paterson, 1996; Gounden *et al*., 2023). The variations recorded for the AST and ALT were within the reference ranges for these parameters, and this were not considered of much pathological significance, while the lack of significant variations among the groups in their levels of serum electrolytes implied that the consumption of cowhides as used for the study did not significantly affect electrolyte dynamics in the blood of the albino rats.

Glutathione peroxidase (GPx) is an antioxidant enzyme whose biochemical functions include preventing lipid peroxidation and maintaining intracellular homeostasis by scavenging for free radicals (Mulgund *et al*., 2015). GPx has been implicated in the development and prevention of many common and complex diseases, including cancer and cardiovascular disease (Lubos *et al*., 2011). Increased or excess GPx may be present in response to oxidative damage and inflammation leading to enhanced survival of transformed cells. The increase in serum GPx in the groups fed diets containing cowhide singed with kerosene and used tyre fire in this case may be a preventive or protective response to the consumption of the cowhides singed with fire from petrol or used tyre (Brigelius-Flohe and Kipp, 2009, Lubos *et al*., 2011). The reason for the significantly lower levels of MDA in all the groups fed cowhide is not known.

Conclusion and Recommendations: Based on the outcome of the study, it was concluded that cowhide processed by washing with detergent and singed using fire made from petrol, kerosene and/or used tyre, as used in the study, led to variable alterations in haematology and serum biochemistry of the rats, such as anaemia, leucocytosis, leukopaenia, hypocholesterolemia, and high level serum levels of GPx depending on the group, but the rat group fed cowhide processed by shaving alone showed no obvious abnormalities in its haematology and serum biochemistry profile. Shaving alone is recommended for the processing of cowhide meant for human consumption.

Acknowledgment:

The authors wish to express gratitude to the University of Ilorin and the Department of Veterinary Physiology and Biochemistry, University of Ilorin specifically, for granting access to the Animal House and Laboratory facilities. We also acknowledge Dr Ahmad Ibrahim Al-Mustapha and Dr Basiru Afisu for pre-viewing the manuscript.

Funding Statement:

This research received no specific funding from any funding individual or agency in the public, private or non-profit sectors.

Conflict of Interest

The Author(s) declares no conflict of interest.

References

Adam I, Okyere D and Teye M (2013). Assessment of heavy metal residues in hides of goats singed with tyres, and the effect of boiling on the heavy metal concentrations in the hides. *Journal of Veterinary Advances*, 3 (5): 165 – 169.

Ademola AS, Kayode BI, Motolani AM and Muyideen I (2022). Consumers' perception, nutritional and mineral composition of processed cowhide (ponmo) as affected by different processing methods. *Al-Qadisiyah Journal for Agriculture Sciences*, 12(1): 65 – 69.

Alturiqi AS and Albedair LA (2012). Evaluation of some heavy metals in certain fish, meat and meat products in Saudi Arabian markets. *The Egyptian Journal of Aquatic Research*, *38*(1), 45 – 49.

Begeman CR and Colucci JM (1968). Benzo(a)pyrene in gasoline partially persists in automobile exhaust. *Science,* 161(3838): 271.

Bielawski J (1990). Two types of haemolytic activity of detergents. *Biochimica et Biophysica Acta*, *1035*(2): 214 – 217.

Brigelius-Flohé R and Kipp A (2009). Glutathione peroxidases in different stages of carcinogenesis. *Biochimica et Biophysica Acta*, *1790*(11): 1555 – 1568.

Concha G, Eneroth H, Hallström H and Sand S (2013). Contaminants and minerals in foods for infants and young children. National Food Agency, Uppsala, Sweden.

Dehelean C, Năstase V, Dragomirescu A, Hegheş A and Dinte E (2004). Skin toxicity of sodium lauryl sulfate as evidenced in an animal model. *Revista Medico-Chirurgicala a Societatii de Medici si Naturalisti din Lasi*, *108*(1): 169 – 172.

Draper HH and Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, *186*: 421 – 431.

Egwuonwu FC, Abubakar A and Hamzah RU (2019). Effect of different dehairing methods on the concentrations of some heavy metals in cow tail sold in Minna Abattoir. *GSC Biological and Pharmaceutical Sciences,* 8(3): 79 – 84.

Ekenma K, Anelon NJ and Ottah AA (2015). Determination of the presence and concentration of heavy metal in cowhides singed in Nsukka abattoir. *Journal of Veterinary Medicine and Animal Health*, *7*(1): 9 – 17.

Ercal N, Gurer-Orhan H and Aykin-Burns N (2001). Toxic metals and oxidative stress. Part I: Mechanisms involved in metal-induced oxidative damage. *Current Topics in Medicinal Chemistry*, *1*(6): 529 – 539.

Food and Agriculture Organization (FAO) (1985). Manual of the Slaughter of Small Ruminants in developing countries: Slaughtering practices and techniques. FAO Animal Production and Health Paper No. 49.

Gounden V, Bhatt H and Jialal I (2023). Renal function tests. StatPearls Publishing, Treasure Island, USA.

Grant GH (1987). Amino acids and Proteins. In: Tietz NW (Ed.), Fundamentals of Clinical Chemistry. WB Saunders Company, Philadelphia, USA, pp. 328 – 329.

Haschek WM, Riusseaux CG and Wallig MA (2009) Fundamentals of Toxicologic Pathology, 2nd edition. Elsevier Inc., USA.

Henry RJ (1974). Colorimetric determination of creatinine in serum: Clinical Chemistry: Principles and Techniques. 2nd edition, Harper and Row, New York, pp. 525 – 529.

Idowu EO, Adewumi AA and Babalola AE (2017). Effects of detergent (alkyl benzene sulfonate) on the haematology of *Clarias gariepinus*. IOSR Journal of Agriculture and Veterinary Science, 10(3): 69 – 75.

Isangedighi IA and David GS (2019). Heavy metals contamination in fish: effects on human health. *Journal of Aquatic Science and Marine Biology*, 2(4): 7 – 12.

Isles CG and Paterson JR (1996). Serum creatinine and urea: make the most of these simple tests. British Journal of Hospital Medicine, 55(8): 513 – 516.

Ita SO and Udofia UA (2011). Comparative study of some haematological parameters in rats following ingestion of crude oil (Nigerian Bonny Light), petrol, kerosene and diesel. *Asian Journal of Biological Sciences*, 4(6): 498 – 505.

Janeway CA Jr, Travers P, Walport M (2001) Immunobiology: The Immune system in Health and Disease, 5th edition. Garland Science, New York.

Lubos E, Loscalzo J and Handy DE (2011). Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxidants & Redox Signaling*, *15*(7): 1957 – 1997.

Marshal JS, Warrington R, Watson W and Kim HL (2018). An introduction to immunology and immunopathology. *Allergy, Asthma & Clinical immunology*, 14(Suppl. 2): 49

Marti DL, Johnson RJ, Mathews Jr KH (2012). Where's the (not) meat? Byproducts from beef and pork production. *Journal of Current Issues in Globalization*, 5(4): 397.

Martin JP Jr, Dailey M and Sugarman E (1987). Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. *Archives of Biochemistry and Biophysics*, *255*(2): 329 – 336.

McGowan MW, Artiss JD, Strandbergh DR and Zak B (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clinical chemistry*, *29*(3): 538 – 542.

Mensah NJ, Antwi-Akomeah S, Akanlu S, Sixtus BMB and Godfred SE (2019). Residual levels of heavy metal contaminants in cowhides singed with scrap tyre and firewood fuel sources: A comparative study in the Wa Municipality of Ghana. *American Journal of Environmental Science and Technology*, *3*(1): 11 – 21.

Mulgund A, Doshi S and Agarwal A (2015). The role of oxidative stress in endometriosis. In: Handbook of Fertility. Academic Press, USA, pp. 273 – 281.

Obiri-Danso K, Hogarh JN and Antwi-Agyei P (2008). Assessment of contamination of singed hides from cattle and goats by heavy metals in Ghana. *African Journal of Environmental Science and Technology*, *2*(8): 217 – 221.

Okafor CS, Okeke CE, Omuku PE, Okafor NC (2012). Heavy metal contents assessment of cowhide singed with firewood (Bamboo). Biochemistry: An Indian Journal, 6(7): 243 – 245.

Okiei W, Ogunlesi M, Alabi F, Osiughwu B, Sojinrin A (2009). Determination of toxic metal concentrations in flame treated meat products, ponmo. *African Journal of Biochemistry Research*, 3(10): 332 – 339.

Okonkwo FO, Njan AA, Ejike CECC, Nwodo UU and Onwurah INE (2018). Health implications of occupational exposure of butchers to emissions from burning tyres. *Annals of Global Health*, *84*(3): 387 – 396.

Olukitibi TA, Adetuyi FC, Adeleke BS, Abe SC (2017). Isolation and antibiogram of bacteria isolated from processed and unprocessed cow-skin (ponmo) in Ogbese market. *Journal of Advances in Microbiology,* 2(4): 1 – 8.

Paglia DE and Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine*, *70*(1): 158 – 169.

Reitman S and Frankel S (1957). Colorimetric methods for aspartate and alanine aminotransferase. *American Journal of Clinical Pathology* 28: 55 – 60.

Sajid Jabbar A and Ali ET (2020). Impact of petroleum exposure on some hematological indices, interleukin-6, and inflammatory markers of workers at petroleum stations in Basra City. *Journal of Environmental and Public Health*, *2020:* 7693891.

Seriki SA, Adebayo OF, Shafe MO and Bivan ZK (2016). Effect of gasoline poisoning on some hematological parameters. *Annals of Hematology and Oncology*, 3 (43): 1087 – 1090.

Song YM, Sung J and Kim JS (2000). Which cholesterol level is related to the lowest mortality in a population with low mean cholesterol level: a 6.4-year follow-up study of 482,472 Korean men. *American Journal of Epidemiology*, *151*(8): 739 – 747.

Tietz NW (1995). Clinical Guide to Laboratory Tests, 3rd edition. Saunders, Philadelphia, pp. 1096 - 1099.

Warne MS and Schifko AD (1999). Toxicity of laundry detergent components to a freshwater cladoceran and their contribution to detergent toxicity. *Ecotoxicology and Environmental Safety*, *44*(2): 196 – 206.

Woko EC, Ibegbulem CO and Alisi CS (2021). Consumption of cattle hide singed with scrap tyre is detrimental to the liver, heart and kidney of rats. *European Journal of Nutrition & Food Safety*, *13*(6): 29 – 43.

Wybenga DR, Pileggi VJ, Dirstine PH and Di Giorgio J (1970). Direct manual determination of serum total cholesterol with a single stable reagent. *Clinical Chemistry*, *16*(12): 980 – 984.