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A Comparative Study on the Haematology, Biochemical Alterations in Serum Composition and Pathologic Changes in the Kidneys of Cachectic and Non-cachectic Cattle in Zaria, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AJN, IOI, KANE and NMU designed, wrote all the protocols and supervised the study. Authors AA and SA conducted all the laboratory experiments and analyses while authors AA and NMU wrote the manuscript. Author IOI proof read the initial draft manuscript before final submission for publication. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to compare the haematological, biochemical and kidney pathological changes in cachectic and non cachectic Zebu cattle in Nigeria.
Study Design: A total of 79 cattle were sampled during the study. The number of cachectic and non-cachectic animals varied depending on the parameters analyzed. It was strictly an abattoir-based study.

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Place and Duration of the Experiment: Samples were collected at the Zaria abattoir in Nigeria for a period of 6 months and analyzed in the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria.
Methodology: Body weight and generalized body condition of the animals were determined. Also, the effect of age and sex on cachexiation was investigated. Whole blood was collected via jugular venipuncture for determination of packed cell volume (PCV), haemoglobin, and total and differential leukocyte counts. Serum was processed from the whole blood to determine electrolyte profiles, alanine and aspartate amino transferases (ALT & AST). Urine composition and pathologic changes in the kidneys of cachectic and non cachectic animals were also determined.
Results: The non-cachectic cattle had higher PCV values compared to the cachectic animals, although the difference was not statistically significant (P>0.05). The mean PCVs of the 2 groups were within normal range. The non-cachectic cattle had slightly lower values of neutrophils compared to the cachectic group, although the difference was not statistically significant (P>0.05). The cachectic group showed slightly higher (P>0.05) concentrations of creatinine and higher concentrations of urea (P>0.05) than the non-cachectic group and urinalysis revealed no aciduria, ketonuria or leucocyturia in both cachectic and non cachectic cattle. Similarly, cachectic cattle had increased alkaline phosphatase activity, ALT and AST compared to the non cachectic animals whose values of these enzymes did not vary significantly. Postmortem examination of the carcasses revealed smooth spherical greyish-brown coloured uroliths (stones) in the kidneys of 11 (15%) of the cachectic cattle. The uroliths weighed between 200-700 mg, with a diameter of 5-10 cm. Chemical examination of the uroliths indicated that they contained substances such as ammonium (+), carbonate (++), uric acid (+ and + +), phosphorus (++) and magnesium (+ and ++). Histopathologically, there was intra glomerular cellular infiltration (predominantly lymphocytes and macrophages) for both cachectic and non-cachectic cattle. The cachectic cattle also showed obliterated Bowman's space and moderate congestion. Nephritis was also observed in the cachectic cattle.
Conclusion: This study is the first to report exhaustively the haematologic, biochemical and pathologic changes in the kidneys of cachectic Zebu cattle presented to the abattoir for slaughter. The study did not investigate the role of season on cachexiation and it is concluded that future studies should focus on the role of seasonal variation on cachexiation.

Keywords: Cachectic cattle; non-cachectic cattle; haematology; seum biochemical composition; pathological changes in kidney.

1. INTRODUCTION

Cachexiation in domestic animals is characterized by chronic excessive wasting of muscle mass (sarcopaenia) and loss of body weight. Loss of muscle mass is usually caused by primary muscle degenerative changes such as the muscular dystrophies, or may be a secondary consequence of cachectic disease processes affecting other tissues, such as renal failure, cancer, burns, sepsis and congestive heart failure [1]. Other common chronic diseases associated with cachexiation include tuberculosis, trypanosomiasis, contagious bovine pleuropneumonia (CBPP), malnutrition and helminthosis [2]. Very little is known about the role of cachexiation in kidney dysfunction in indigenous Nigerian breeds of cattle. In this study, we report for the first time, the haematology, biochemical alterations in the serum composition and pathologic changes in the kidneys of cachectic and non-cachectic cattle presented for slaughter at the abattoir in Zaria, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at the Zaria abattoir in Nigeria. Zaria town is located on Latitude 07 38^0E and Longitude 11 10^0N. Cattle slaughtered at this abattoir were representative of the various herds in Zaria and neighbouring environs [3].

2.2 Sampling

A total of 79 cattle were sampled during the study. The number of cachectic and non-cachectic animals varied depending on the parameters analyzed.

2.3 Assessment of Body Weight, Age and General Body Condition

Weights of the animals and their ages were determined using conventional procedures [4]. Body condition scores were evaluated as described previously [2,5].

2.4 Determination of Haematological Parameters

Packed cell volume (PCV), total and differential leucocyte counts were determined as described elsewhere [6].

2.5 Determination of Biochemical Changes

Serum was prepared from whole blood and stored at -20°C until analyzed [7]. Concentrations of creatinine, urea, total protein, albumin, bilirubin, glucose and electrolytes such as sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), phosphorous (P), bicarbonate (HCO_3^-), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) in the serum were measured using commercial test kits and digital spectrophotometer [8].

Urine sample collection and analysis. Urine specimens (20 mL) were collected aseptically from the urinary bladder immediately after slaughter into sterile sample bottles. The fresh samples were analyzed both chemically (using reagent stripes) and grossly for the presence of abnormal findings [9].

2.6 Determination of Effect of Age and Sex on Cachexiation

The relationship between age, sex and cachexiation was investigated. A total of 66 female and 13 male animals were investigated in the study. None of the animals examined was less than 3 years old.

2.7 Postmortem Examination for Gross and Histopathologic Changes

Kidneys were sectioned longitudinally to ascertain the presence of kidney stones and any other gross Lesions. Histopathologic lessons were investigated using tissue specimens collected, fixed in 10% normal buffered formalin and stained with H and E [10,11].

2.8 Statistical Analysis

Data from the study was computed as mean ± SD, analyzed using Student's t- test [12] and values of $P<0.05$ were significant.

3. RESULTS

3.1 Haematologic Findings

Haematologic parameters of the cachectic and non-cachectic cattle investigated (mean ± SEM) are presented in Table 1. The non-cachectic cattle had higher PCV values compared to the cachectic group, although the difference was not statistically significant ($P>0.05$). Mean PCV values of the two groups were within the normal range. Haemoglobin concentrations of the former were higher than the later, although the differences were not significant ($P>0.05$). Mean total leucocyte counts for the two groups were similar and within normal range. The non-cachectic cattle had slightly lower values of neutrophils compared to the cachectic group, although the difference was not statistically significant ($P>0.05$). The mean neutrophil values of the two groups were within the reference interval. Lymphocyte values were slightly higher for the non-cachectic, compared to the cachectic group ($P<0.05$), although both values were within normal range. Eosinophil and basophil counts were slightly higher in the cachectic compared to the non-cachectic group, although the difference was not significant ($P>0.05$). Both values were within the normal reference interval (Table 1).

3.2 Serum Biochemical Changes

Changes in serum biochemistry, electrolyte profiles, metabolite concentrations, liver enzyme activities, urine composition and other biochemical parameters are presented in Tables 2-7. The activity of AST was higher in the cachectic group compared to the non-cachectic, although the difference was not statistically significant ($P>0.05$) (Table 4). Moreover, both the means and the ranges were lower than the reference interval (Table 4). The same finding was observed for ALT except that the difference was statistically significant ($P<0.05$). The cachectic group exhibited higher concentrations of glucose than the non-cachectic group. Although, there was no statistically significant difference ($P>0.05$) between the groups (Table 2), values much higher than the reference intervals (2.3-4.1) were recorded as 9.5 mMol/L and 6.6 mMol/L respectively for cachectic and non-cachectic cattle respectively (Table 2).

Cachectic animals also had higher serum total protein concentrations than the non-cachectic group (P<0.05) (Table 2). The cachectic group recorded higher concentrations of albumin than the non-cachectic group, although there was no significant difference (P>0.05) between the two groups (Table 2). The lowest value of albumin obtained in both cachectic and non-cachectic groups (14 g/L and 20 g/L) respectively were lower than the reference intervals (27-39 g/L), while the highest values were 70 g/L and 48 g/L respectively (Table 2). The cachectic group showed slightly higher (P>0.05) concentrations of creatinine and higher concentrations of urea (P>0.05) than the non-cachectic group (Table 2). Urinalysis revealed no aciduria, ketonuria or leucocyturia in both cachectic and non cachectic cattle (Table 7).

3.3 Effect of Sex and Age on Cachexiation

There was a statistically significant association between sex and cachexiation (P<0.0001). Female animals were more cachectic than the males. Seventy five (95%)

Table 1. Haematologic parameters (mean ± SEM) in cachectic and non-cachectic cattle examined in Zaria abattoir, Nigeria

Parameters	Cachectic cattle (n= 51)	Non-cachectic cattle (n =14)	Normal range*
Packed cell volume (%)	32.53±8.56[a] (16-61)	36.64±9.19[a] (21-49)	24-46
Haemoglobin (g/dL)	10.89±2.85 [a] (5.30-18.30)	12.19±3.45[a] (7.00-18.30)	8-15
Leucocytes (x10^3/µL)	9.355±2.57 [a] (5.80-14.5)	9.357±1.30[a] (7.00-12.50)	4-12
Band neutrophils (x10^3/µL)	0.117±0.184 [a] (0.0-0.98)	0.138±0.149[a] (0.0-0.47)	0.0-0.2
Neutrophils (x10^3/µL)	3.971±2.265 [a] (1.16-9.8)	3.670±1.488[a] (1.75-8.125)	1.7 – 6.0
Eosinophils (x10^3/µL)	0.249±0.383 [a] (0.0-2.25)	0.331±0.284[a] (0.0-0.90)	0.1 – 1.2
Basophils (x10^3/µL)	0.0037±0.0196 [a] (0.0-0.125)	0.00643±0.0241[a] (0.0-0.09)	0.0 – 0.2
Lymphocytes (x10^3/µL)	4.652±1.456 [a] (0.0-7.8)	4.906±0.1958[a] (3.13-6.11)	1.8 – 8.1
Monocytes (x10^3/µL)	0.1742±0.1959 [a] (0.0-0.81)	0.2023±0.1398[a] (0.0-0.41)	0.1 – 0.7

*Key: Values in brackets are percentages, n = number, * = Jain, 1993, g/dL = grams per decilitre, x = times (multiplication sign), /µl = per micro litre; P>0.05; Values in rows with the same superscripts are comparable (P>0.05)*

Table 2. Serum metabolite concentrations in cachectic and non-cachectic cattle examined at Zaria abattoir, Nigeria

	Cattle		
Metabolites	**Cachectic (n = 65)**	**Non-cachectic (n = 14)**	**Reference values**
Creatinine (µmol/L)	77.94 ±25.93[a] (29-141)	70.43±32.98[a] (37-153)	88.4-176.9**
Urea (mmol/L)	5.23 ± 2.07[a] (2.0-12.0)	4.31 ± 2.08[a] (2.20-8.90)	3.6-8.9**
Total protein (g/L)	68.85 ± 9.94[a] (47-89)	67.43±9.94[a] (47-81)	61-81*
Albumin (g/L)	38.69±9.85[a] (14-70)	37.64±7.85[a] (20-48)	27-39*
Glucose (mmol/L)	5.25±1.45[a] (2.10-9.50)	4.89±1.15[a] (2.90-6.60)	2.3-4.1**

*Key: * = Payne et al., 1970, ** = Sirois, 1995, n = number; P > 0.05; Values in rows with the same superscripts are comparable (P>0.05)*

of the total animals examined were cachectic and 62 (83%) of them were females, while 13 (17%) were males (Table 8). There was no statistically significant association (P>0.05) between age and cachexiation (Table 9).

Table 3. Serum electrolyte concentrations in cachectic and non-cachectic cattle examined at Zaria abattoir, Nigeria

	Cattle		
Parameters	**Cachectic (n=65)**	**Non-cachectic (n= 14)**	**Reference values***
Na^+ (mmol/L)	136.9±6.38[a] (122-148)	134.9±4.21[a] (128-142)	141-155
K^+ (mmol/L)	4.09±0.75[a] (2.90-6.90)	4.22±0.59[a] (3.0-5.2)	3.9-5.2
Ca^{2+} (mmol/L)	2.36±0.13[a] (2.04-2.66)	2.31±0.19[a] (2.06-2.62)	2.1-2.8
Cl^- (mmol/L)	101.5±5.87[a] (86-121)	100.6±5.64[a] (86-109)	95-120
P (mmol/L)	1.06±0.31[a] (0.54-1.84)	1.16±0.26[a] (0.71-1.54)	1.4-2.5
HCO_3^- (mmol/L)	21.12±3.97[a] (10-40)	21.7±3.09[a] (14-26)	21-29

*Key: n = number, *Sirois, 1995, Na^+ = sodium, K^+ = potassium, Ca^{2+} = calcium, Cl^- = chloride, P = phosphorus, HCO_3^- = bicarbonate; P > 0.05; Values in rows with the same superscripts did not differ significantly (P>0.05)*

Table 4. Serum liver enzymes activities in cachectic and non-cachectic cattle examined at Zaria Abattoir, Nigeria

	Cattle		
Parameters	**Cachectic (n=65)**	**Non-cachectic (n=14)**	**Reference values***
Aspartate amino-transferase (U/L)	15.62±9.74[a] (3-39)	14.79±8.49[a] (4-37)	45-110
Alanine amino-transferase (U/L)	10.83±10.98[a] (2-55)	4.93±3.56[b] (2-16)	6.9-35
Alkaline phosphatase (U/L)	49.57±17.2 [a] (7-97)	59.21±30.09[a] (7-105)	0-38

*Key: [a] = P<0.05, *Sirois, 1995, n= number; Values in rows with different superscripts differ significantly (P<0.05)*

Table 5. Proteinuria in cachectic and non-cachectic cattle examined at Zaria abattoir, Nigeria

	Number of cattle having proteinuria (% in brackets)		
Urine Protein (g/L)	**Cachectic (n=49)**	**Non-cachectic (n=13)**	**Total (n=62)**
0.3	12 (19.4) [a]	3(4.8) [a]	15 (24.2)
1.0	3 (4.8) [a]	3 (4.8) [a]	6 (9.7)
5.0	6 (9.7) [a]	3 (4.8) [a]	9 (14.5)
10.0	2 (3.2) [a]	2 (3.2) [a]	4 (6.5)
Total	23 (37.1)[a]	11 (17.7)[b]	34(54.8)

Key: values in brackets are percentages; g/L = grams per litre; n = number; P < 0.05, X^2 =5.889; df = 1; Values in columns with different superscripts differ significantly (P<0.05)

Table 6. Haematuria in cachectic and non-cachectic cattle examined at Zaria abattoir, Nigeria

	Number of cattle having haematuria (% in brackets)		
Erythrocytes / µl	**Cachectic (n=49)**	**Non-cachectic (n=13)**	**Total (n=62)**
10	3(4.8) [a]	0 (0) [a]	3 (4.8)
50	6 (9.7) [a]	2 (3.2) [a]	8(12.9)
250	3 (4.8) [a]	0 (0) [a]	3 (4.8)
Total	12 (19.4) [a]	2 (3.2) [a]	14 (22.6)

Key: values in brackets are percentages, /µL = per micro litre, n = number; P > 0.05; X^2 = 0.4872; df = 1; Values in columns with the same superscripts are comparable (P>0.05)

3.4 Gross and Histopathologic Changes

Physical examination showed the cachectic cattle had prominent ribs, back and shoulders as well as sharp and pointed spines. Postmortem examination revealed smooth spherical greyish-brown coloured uroliths (stones) in the kidneys of 11 (15%) of the cachectic cattle (Plates 1a and b). The uroliths weighed between 200-700 mg, with a diameter of 5-10 cm (Plate 1b). Chemical examination of the uroliths indicated that they contained substances such as ammonium (+), carbonate (++), uric acid (+ and + +), phosphorus (++) and magnesium (+ and ++) (Table 10). Microscopically, there was intra glomerular cellular infiltration (predominantly lymphocytes and macrophages) for both cachectic and non-cachectic cattle. The cachectic cattle also showed obliterated Bowman's space and moderate congestion (Plates 2a and b, 3 and 4). Nephritis was also observed in the cachectic cattle (Plate 3).

Table 7. Other biochemical changes in urine of cachectic and non-cachectic cattle examined at Zaria Abattoir, Nigeria

	Number of cattle (% in brackets)		
	Cachectic (n=49)	**Non-cachectic (n=13)**	**Total (n=62)**
Glucosuria	2(3.2)[a]	0(0)[a]	2(3.2)
Bilirubinuria	18(29.03)[a]	4(6.5)[a]	22(35.5)
Aciduria	0(0)[a]	0(0)[a]	0(0)
Ketonuria	0(0)[a]	0(0)[a]	0(0)
Leucocyturia	0(0)[a]	0(0)[a]	0(0)

Key: Values in brackets are percentages, n = number; P > 0.05; X^2 = 0.1597; df = 1

Table 8. Relationship between sex and cachexiation in cattle examined at Zaria Abattoir, Nigeria

Number of male and female cachectic and non-cachectic cattle (% in bracket)			
Sex	**Cachectic**	**Non-cachectic**	**Total**
Male	3 (3.8)[a]	10 (12.7)[b]	13 (16.4)
Female	62 (78.5)[b]	4 (5.1)[b]	66 (83.5)
Total	65 (82.3)	14 (17.7)	79 (100)

Key: Values in brackets are percentages, X^2 = 37.40, df = 1; P < 0.0001; Values in columns with different superscripts differ significantly (P<0.05)

Table 9. Relationship between age and cachexiation in cattle examined at Zaria Abattoir, Nigeria

Age of cachectic and non-cachectic cattle (% in bracket)			
Age (years)	**Cachectic**	**Non-cachectic**	**Total**
3-3.5[a]	24 (30.4)[a]	8 (10.1)[a]	32 (40.5)
Above 3.5[a]	41 (51.9)[a]	6 (7.6)[a]	47 (59.5)
Total	65 (82.3)	14 (17.7)	79 (100)

Key: Values in brackets are percentages; X^2 = 1.954; df = 1; P>0.05; Values in columns with the same superscripts did not differ significantly (P>0.05)

Table 10. Composition of renal calculi found in some of the cachectic cattle examined at Zaria abattoir, Nigeria

S/N	C/N	NH_3	Carbonate	Calcium oxalate	Uric acid	HPO_4	Mg
1	SJ4	+	++	-	+	++	++
2	SJ9	+	++	-	++	++	++
3	SK5	+	++	-	+	++	+
4	SK8	+	++	-	+	++	+

Key: S/N = serial number; C/N = clinic number; NH_3 = ammonia; HPO_4 = phosphorus; Mg = magnesium; + = presence in trace amount; ++ = presence in larger amount; - = not present

Plate 1a. Urinary calculi (white arrows) observed in cachectic cattle at Zaria abattoir, Nigeria

Plate 1b. Urinary calculi found in cachectic cattle at Zaria abattoir, Nigeria

4. DISCUSSION

Haematuria has been associated with cachexiation in a previous report [9]. The reduced packed cell volume and haemoglobin concentrations observed in the cachectic, compared to the non-chacetic cattle in the present study may be because of increased loss of intact or haemolyzed red blood cells in the urine of cachectic cattle in agreement with Adamu et al. [9].

Bilirubin concentration was higher in the urine of cachectic compared to the non-cachectic animals. This may be due to impaired kidney function as some diseases are associated with haemolysis, leading to increased bilirubin production and its subsequent conjugation, part of which is then excreted by the kidney. The observation of glomerulonephritis and reduced mean PCV and Hb concentration in the cachectic group gives more support to our suspicion that kidney diseases accompanied by haemolysis may be partly responsible for the bilirubinuria which was observed.

Protein is usually reabsorbed by the kidney uriniferous tubules. The number of cachectic animals with proteinuria was statistically significantly different from the non-cachectic cattle ($P<0.05$). This may be as a result of necrosis of renal epithelium which was observed histopathologically. The foregoing may likely be responsible for protein loss in the urine in agreement with the report of White et al. [13].

Creatinine and urea levels were higher in the cachectic than non-cachectic cattle, signifying possible impairment of renal function in the former. This tally with the report of Mahmoud [14] who found no significant difference in the serum levels of creatinine and urea between emaciated and control groups of buffaloes. In lactating cows with medium condition scores, Aktas et al. [8] observed significant decreases in serum concentrations of creatinine and urea. The hypophosphataemia observed in cachectic cattle in the present study may have affected creatinine metabolism in muscles leading to a buildup of creatinine, thus agreeing with the report of Radostits et al. [2].

Mean serum total protein was slightly higher in cachectic than non-cachectic cattle. This may be because of severe dehydration [15] observed in the cachectic animals. Mahmoud [14] and Aktas et al. [8] found significant and non significant hypoproteinaemia in emaciated group of buffaloes and lactating cows with fairly low body condition score respectively. High glucose levels were observed in the cachectic animals to collaborate other reports. Mahmoud [14] found high glucose levels in the serum of cachectic cattle, compared to the non-cachectic group. This was attributed to possible impaired pancreatic function with resultant negative effect on insulin production, leading to possible impaired glucose metabolism and its subsequent accumulation in the blood. Aktas et al. [8] found significant increase in mean serum glucose levels in lactating cows with low body condition score. Emaciated cows had significantly higher mean serum glucose levels compared to normal cattle in the same study.

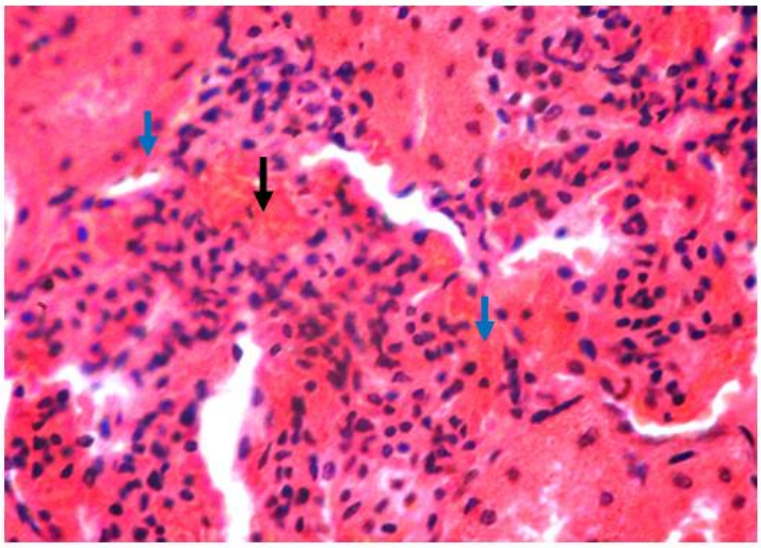

Plate 2a. Photomicrograph of a section of kidney from cachectic cattle, note the obliterated Bowman's space (blue arrows) and moderate congestion (black arrow) (H & E x 1000)

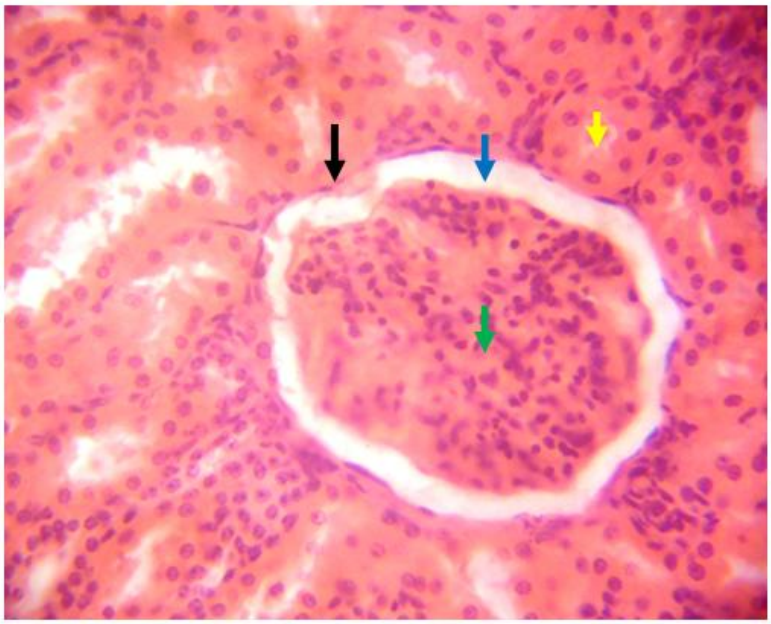

Plate 2b. Photomicrograph of a section of kidney from non-cachectic cattle, note the glomerulus (green arrow), renal tubules (yellow arrow), intact Bowman's capsule (black arrow) and clear bowman's space (blue arrow) (H & E x 800)

Transaminases (transferases) (i.e. ALT and AST) increased in the serum of cachectic compared to non-cachectic cattle signifying possible impaired liver function. The increase in alkaline phosphatase activity observed may be as a result of possible ongoing liver, placental, kidney, bone or gastrointestinal mucosal disease. Hypophosphataemia was observed in the cachectic group and was attributed to the process of cachexiation. This is because inorganic phosphorus is being utilized in the formation of protein and tissue enzymes after being withdrawn from the plasma inorganic phosphorus [2].

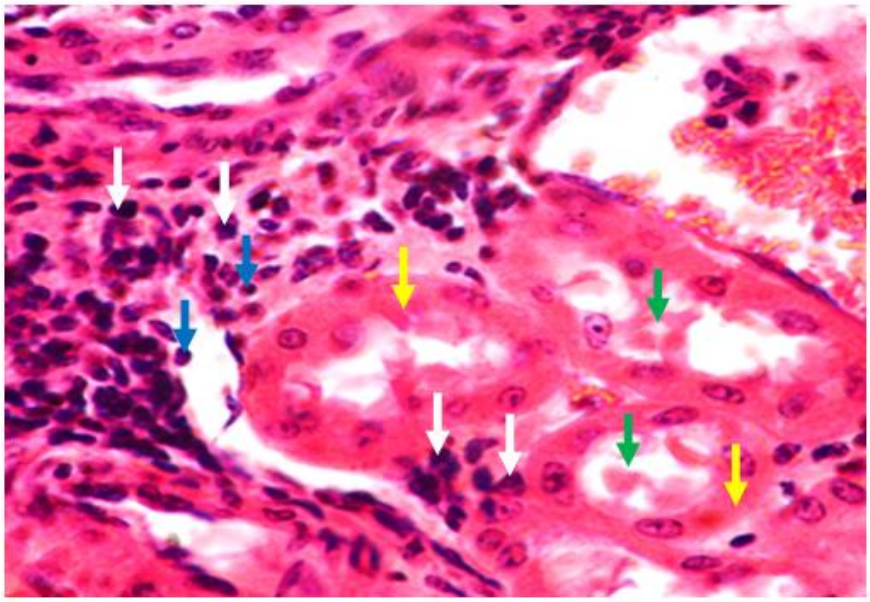

Plate 3. Photomicrograph of a section of kidney from cachectic cattle, note the nephritis with moderate cellular infiltrations, predominantly lymphocytes (blue arrows) and macrophages (white arrows), necrosis of renal tubular epithelium (yellow arrows) and intra tubular proteinaceous deposits (green arrows) (H & E x 1000)

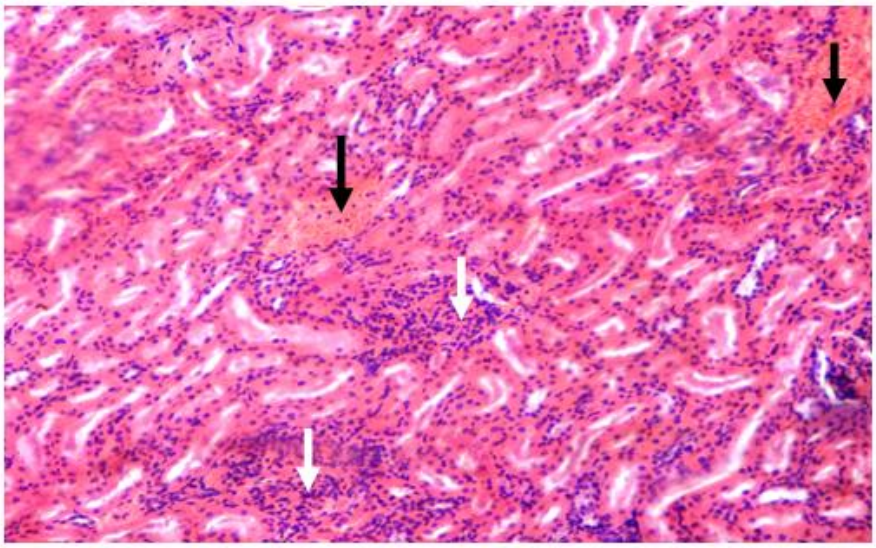

Plate 4. Photomicrograph of a section of kidney from cachectic cattle, note the moderate congestion of inter tubular spaces (black arrows) and diffused inter tubular cellular infiltrations (white arrows) (H & E x 200)

The presence of urinary calculi in the kidneys of cachectic cattle may be probably due to local infection in the kidney. This is because nidus (a group of desquamated epithelial cells or necrotic tissue) may have formed because of local infection in the urinary tract [2]. The renal calculi observed in the study were either due to phosphorus, magnesium, ammonium or carbonate. This is contrary to the report of Hammad et al. [16] who found calcium oxalate crystals as the most common cause of kidney stones in human patients. It is common

knowledge that nutritional sources, for instance high intake of sodium rich foods (e.g. spinach and cocoa) and proteins of animal origin may increase the risk of developing calcium oxalate stones [17].

Nephritis was observed in this study and may be due to on-going microbial infection. Angelopoulou et al. [11] observed thickening of the glomerular basement membrane and the Bowman's capsule, adhesion between Bowman's capsule and the basement membrane, mononuclear cellular infiltration (lymphocytes and macrophages) as well as degeneration and necrosis of the renal tubular epithelium due to Maedi-visna virus infection in the kidney of experimental sheep.

There was a statistically significant association between sex and cachexiation, where the female animals were more cachectic than the males. This is because they maintain pregnancy and undergo stress of lactation. These factors may contribute to loss of weight, especially if there is negative energy balance [18]. However, age was not statistically significantly associated with cachexiation in patients with pulmonary tuberculosis [19].

5. CONCLUSION

In conclusion, this study is the first to demonstrate exhaustively the haematological, biochemical and pathologic changes in the kidneys of cachectic Zebu cattle presented to the abattoir for slaughter. There were derangements in most of the parameters analyzed suggesting that cachexiation is usually accompanied by severe health consequences in cattle. The study did not investigate the role of season on cachexiation and future studies should focus on this.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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