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Comparative Evaluation of the Effects of Burdizzo Castration, *in situ* Spermatic Cord Ligation, and Orchidectomy on the Serum Biochemical Profiles of Red Sokoto Bucks

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ABSTRACT

Since bucks are raised mainly for meat, there is a need for castration to optimize meat production. In this study, we conducted a comparative evaluation of the serum biochemical profiles of red Sokoto bucks following Burdizzo castration, *in situ* spermatic cord ligation, and orchidectomy. Sixteen red Sokoto bucks aged 6 months to 1 year old and 11-12 kg body weight were used for this study. The bucks were randomly divided into 4 groups of 4 bucks each as follows; group A (Burdizzo), B (*in situ* spermatic cord ligation), C (orchidectomy), and D (control). Blood was collected pre-castration, and at immediate, 4, 8, 12, 16, 20, 24, 32, 48, and 72 hours post-castration (HPC) into labeled tubes without anticoagulant, serum was harvested and used for serum biochemical analyses. The outcomes showed non-significant changes in total protein, sodium, chloride, calcium, and potassium levels in all groups of bucks. Glucose levels were significantly increased at immediate PC in groups A, B, and C, followed by a decline from 4 HPC to normal values by 16 HPC (group B) and 20 HPC (group A). The serum creatinine levels increased significantly at immediate PC, peaked at 20 HPC, and declined from 24 HPC. The blood urea nitrogen level increased significantly from immediate PC up to 72 HPC. The activities of superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde levels increased significantly from 4 HPC, peaked at 16 to 20 HPC, and decreased from 24 to 72 HPC. There were less severe serum biochemical changes by Burdizzo castration and *in situ* spermatic cord ligation compared to orchidectomy in the red Sokoto bucks.

Introduction

Castration is an important management practice for goat farmers who want to keep control of their breeding program and improve the breed.[1,2] Castration is the surgical removal or destruction of a male's testes, epididymis, and a portion of each spermatic cord.[3] Non-breeding males and males who are not slaughtered at a young age should be castrated in most cases. Castration is recommended at the earliest possible age because the stress of castration can impair growth in older animals.[4] Castration is recommended in kids when the testicles descend into the scrotum (between a few days and three weeks of age), with no need for sedation or pain relievers.[5] With age, the procedure becomes more difficult and painful, with an increase in the likelihood of complications[5]. However, castration of male goats in the

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late growth stage was thought to result in better muscular growth as mature body weight and size in the castrated male goat became smaller than that of the intact male goat.[5,4]

The castration methods include surgical, chemical, and immunocastration.[6] From a survey, castration was mostly carried out with a ring or band (mechanical castration) because minimal training is required. Surgical castration does require techniques and this method can also cause acute and long-term pain.[7]This suggests that the different castration procedures tend to elicit different levels of responses which might be associated with several serum biochemical changes. Reports have shown outcomes of different levels of castration on serum biochemical parameters in West African dwarf goats.[8,9] but there is a paucity of data regarding these in the red Sokoto breeds. Therefore, the aim of this study was to conduct a comparative evaluation of the serum biochemical profiles of red Sokoto bucks following Burdizzo castration, *in situ* spermatic cord ligation, and orchidectomy.

Materials and Methods

Animals

This study was performed in the Large Animal Surgery Unit, Department of Veterinary Surgery and Radiology, Ahmadu Bello University (A.B.U.) Zaria, Kaduna State, Nigeria.

Ethical approval for this study was obtained from the Ahmadu Bello University Committee on Animal Care and Use (ABUCAUC/2022/047), A.B.U. Zaria.

Sixteen red Sokoto bucks aged 6 months to 1 year old and weighing 11-12 kg were used in this study. The bucks were obtained from Giwa market, Giwa Local Government Area, Kaduna State and kept in the small ruminant pens, Department of Veterinary Surgery and Radiology, A.B.U. Zaria. These pens were cleaned, disinfected, and treated with insecticide prior to the arrival of the bucks. The bucks were then stabilized and acclimatized for 2 weeks during which they were routinely examined and evaluated clinically. Feed and water were provided *ad libitum*. The feed provided was groundnut hay, bean husks, and maize offal.

Following acclimatization, the bucks were randomly divided into four groups of four animals each (A, B, C, and D). Bucks in Group A were castrated using Burdizzo; bucks in Group B were castrated using *in situ* spermatic cord ligation; bucks in Group C were castrated using orchidectomy; and bucks in Group D were not castrated (control).

Burdizzo Castration Technique

The Burdizzo castrator was used to perform castration.[8] After each buck was properly restrained, the hind limbs were spread apart and the scrotal area was exposed to the surgeon. The Burdizzo was used to castrate the buck by applying it laterally to the scrotal neck. The first and second fingers were used to hold the cord laterally in the scrotal neck, while the second hand gradually directed the position of the jaws until they were about 8-10 mm apart, firmly gripping the skin and cord. The surgeon ordered and maintained rapid closure for 15-30 seconds while ensuring proper cord crush (Figure 1).

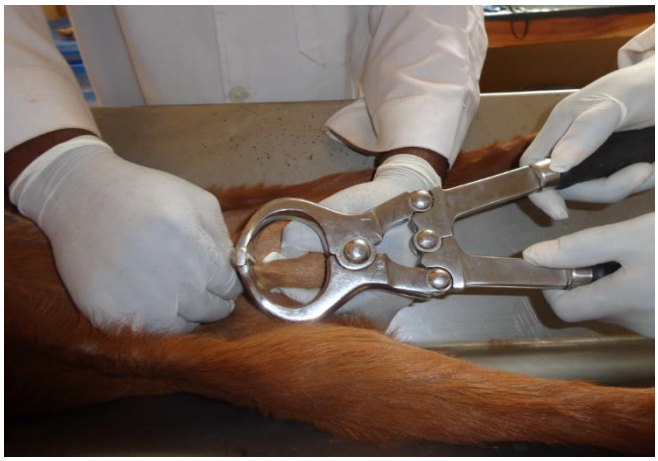

Figure 1. Burdizzo castration in a red Sokoto buck.

In situ Spermatic Cord Ligation Technique

Following aseptic preparation of the skin enveloping the spermatic cord, *in situ* spermatic cord ligation was performed.[10] Each buck was restrained in lateral recumbency on the surgical cradle, and local anesthesia was achieved by a linear subcutaneous infiltration of 1 mL of 2% lidocaine HCl (Kwality Pharmaceutical Ltd., Amristar, India) on each lateral aspect of the scrotum. A double external trans-fixing ligation of the entire spermatic cord, 2 cm apart, was performed using non-absorbable suture material (Nylon size 2-0, Life Care, Anhui Kangning Industries group Co. Ltd., Anhui, China) (Figure 2). The procedure was then repeated on the opposite cord.

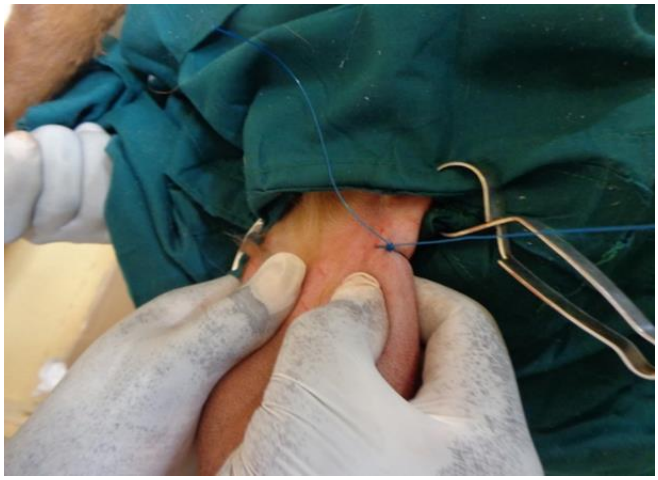

Figure 2. *In situ* spermatic cord ligation in a red Sokoto buck.

Orchidectomy

Orchidectomy was performed using a modified version of the Malbrue and Zorilla procedure.[11] The scrotal area was shaved, scrubbed with soap and water, and disinfected with chlorhexidine (Saro Life Care Limited, Ilorin, Nigeria). 0.05 mg/kg Xylazine (Bioveta, Komenskeho, Czech Republic) was administered intramuscularly to the buck. To achieve local anesthesia, a 5 ml linear subcutaneous infiltration of 2% lidocaine HCl (Kwality Pharmaceutical Ltd., Amristar, India) at the lateral aspect of the scrotal sac was used. Each goat was restrained in dorsal recumbency on the surgical cradle. After grasping the scrotum, a horizontal incision through the skin and fascia at the lateral scrotum was made. The tunica dartos was removed from the vaginal tunic with a gauze sponge after one of the testicles emerged into the incision. An absorbable suture ligature (Chromic catgut size 2-0, Life Care) was used to ligate the spermatic cord, which was then covered by a vaginal tunic (Figure 3). The spermatic cord was severed one centimeter below the ligature, and the stump was examined for bleeding. The opposite testicle was removed in a similar manner.

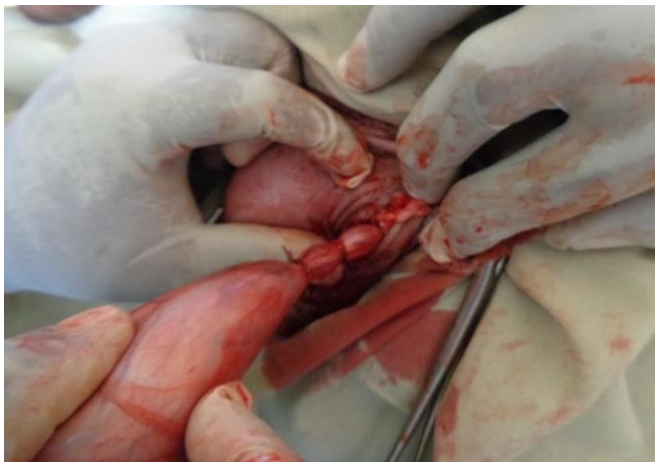

Figure 3. Orchidectomy in a red Sokoto buck.

Blood Sample Collection

Blood (3 ml) was collected from each buck via jugular venipuncture pre-castration, immediate (0 hours), 4, 8, 12, 16, 20, 24, 32, 48, and 72 hours post-castration. The blood was dispensed into a labeled tube without anticoagulant, and allowed to clot at room temperature for two hours before centrifugation (Infitek Co. Ltd., centrifuge model no CFG-4ZA,Jinan, Shandong, China) at 4000 rpm for 3 minutes. After the centrifugation, sera was harvested and emptied into a micro-vial tube and stored at -20 °C until the analysis was carried out.

Serum Biochemical Analyses

Serum total protein, creatinine, blood urea nitrogen, and glucose levels were determined using a serum auto-analyzer (Mindray auto analyzer model no BC 2800, Shenzhen, China). The sera were analyzed for the activity of superoxide dismutase (SOD) was determined using the method of Fridovich.[12] Catalase (CAT) activity was measured using the method of Abebi.[13] The activity of glutathione peroxidase (GPx) was measured as described by Rajagopalan *et al*.[14] Malondialdehyde (MDA) concentration was determined using the method described by Okhawa *et al*.[15] with slight modification by Atawodi *et al*.[16]

Data Analysis

The obtained data were represented graphically as the mean and standard error of the mean (mean ± SEM). Graph pad prism version 5.0 (San Diego California, USA) was used to perform a one-way analysis of variance (ANOVA) with Tukey's post hoc test on the data. The level of significance was set at $p \leq 0.05$.

Results

Total Protein and Glucose Levels

Serum total protein levels showed no significant ($p > 0.05$) difference in all groups of bucks up to 72 HPC but were non-significantly lower in groups A, B, and C compared to the control (Figure 4). There was a significant ($p < 0.05$) increase in glucose levels at immediate post-castration (0 HPC) in groups A (82.25 ± 3.64 mg/dl), B (99.50 ± 11.99 mg/dl) and C (104.25 ± 3.59 mg/dl) compared to control (66.00 ± 2.08 mg/dl). This was followed by a significant ($p < 0.05$) decline from 4 HPC (groups A and B) to pre-castration values of 16 HPC (group B) and 20 HPC (group A). In group C, there was a significant ($p < 0.05$) decline in glucose level from 8 to 20 HPC (Figure 5).

Creatinine Level

The serum creatinine level significantly ($p < 0.05$) increased at 0 HPC and peaked at 20 HPC in all castrated bucks with the highest recorded in group C (1.70 ± 0.06 mg/dl; 2.63 ± 0.43 mg/dl), followed by groups B (1.35 ± 0.06 mg/dl; 2.15 ± 0.06 mg/dl) and A (1.28 ± 0.05 mg/dl; 1.73 ± 0.06 mg/dl) compared to control (1.00 ± 0.09 mg/dl; 0.83 ± 0.06 mg/dl). These were followed by a significant decline from 24 HPC up to 72 HPC in all castrated bucks but these were significantly ($p < 0.05$) higher compared to those of control (Figure 6).

Blood Urea Nitrogen Level

There were significantly ($p < 0.05$) increased blood urea nitrogen (BUN) levels in all castrated bucks from 0 to 72 HPC with the highest in group C (22.25 ± 1.25 mg/dl; 43.75 ± 0.48 mg/dl), followed by groups B (20.25 ± 0.48 mg/dl; 31.50 ± 1.19 mg/dl) and A (18.50 ± 1.04 mg/dl; 26.75 ± 0.85 mg/dl) compared to control (17.75 ± 0.48 mg/dl; 16.75 ± 0.63 mg/dl) (Figure 7).

Superoxide Dismutase Activity

The activity of superoxide dismutase significantly (p < 0.05) increased from 0 HPC and peaked 16 HPC in group C (9.70 ± 0.23 U/mg; 16.85 ± 0.79 U/mg). This increase was observed from 4 HPC to a peak at 16 HPC in groups A (9.83 ± 0.32 U/mg; 14.60 ± 0.47 U/mg) and B (10.50 ± 0.52 U/mg; 14.60 ± 0.47 U/mg). This was followed by a significant (p < 0.05) decrease from 24 to 72 HPC in groups A, B, and C (Figure 8).

Catalase Activity

The activities of catalase showed a significant (p < 0.05) increase from 0 to 16 HPC in groups A (10.75 ± 0.64 U/mg; 13.68 ± 0.88 U/mg), B (9.08 ± 0.31 U/mg; 14.05 ± 1.35 U/mg) and C (9.98 ± 0.46 U/mg; 19.00 ± 1.45 U/mg) compared to control (4.75 ± 0.46 U/mg; 5.28 ± 1.00 U/mg). This was followed by a significant (p < 0.05) decrease from 20 to 72 HPC in groups A, B, and C, but these were significantly higher than in the control (Figure 9).

Glutathione Peroxidase Activity

From 0 to 16 HPC, there was a significant increase (p < 0.05) in glutathione peroxidase (GPx) activity followed by a decline from 20 to 72 HPC in groups B and C. In group A, a significant increase (p < 0.05) in GPx activity was observed from 4 to 20 HPC, followed by a decline up to 72 HPC. The activities of GPx were significantly higher (p < 0.05) in all castrated bucks than in the control (Figure 10).

Malondialdehyde Level

The level of malondialdehyde (MDA) significantly (p < 0.05) increased from 4 to 16 HPC in all castrated bucks with the highest observed in groups C and B. These were followed by significant (p < 0.05) decreases up to 72 HPC in all the castrated bucks, but were significantly (p < 0.05) higher than that of the control (Figure 11).

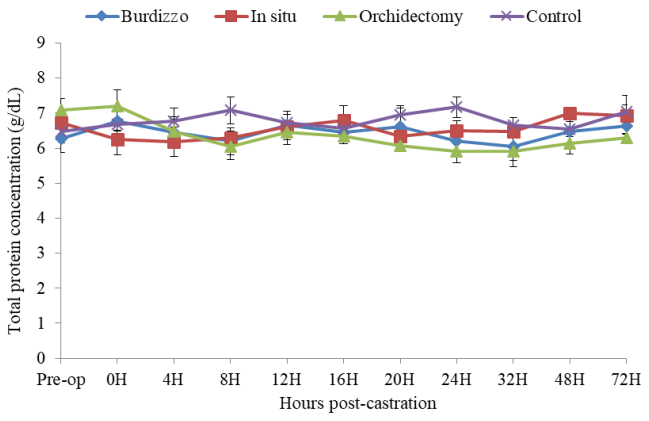

Figure 4. Serum total protein levels of red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy.

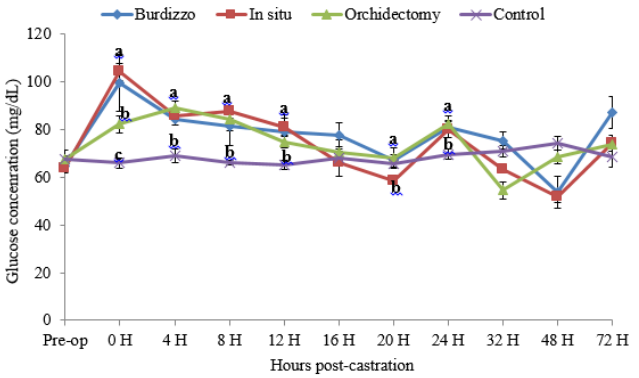

Figure 5. Glucose levels of red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at p < 0.05.

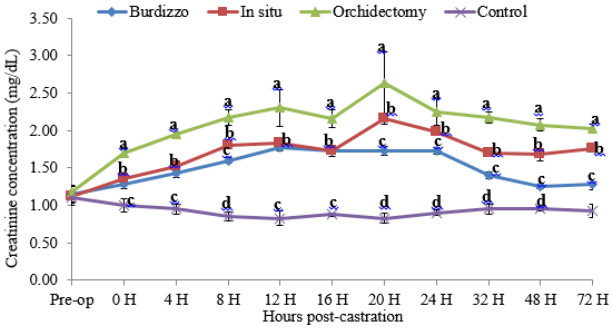

Figure 6. Serum creatinine levels of red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at p < 0.05.

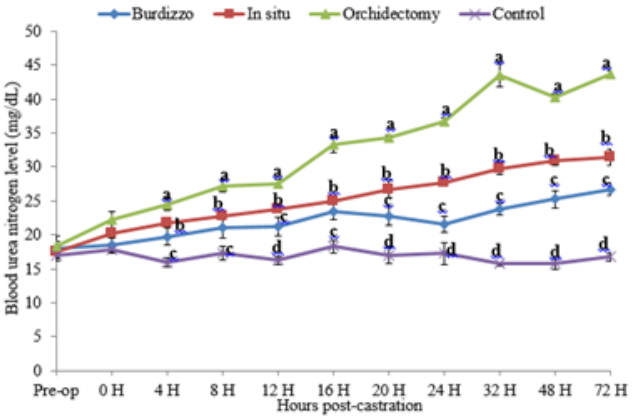

Figure 7. Blood urea nitrogen levels of red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at p < 0.05.

Discussion

This study showed non-significant decrease in serum total protein of all castrated red Sokoto bucks (groups A, B and C). In contrast, the studies of Oyeyemi *et al.* (2000)[17] and Olaifa and Opara (2011)[8] reported a significant decrease in serum total protein following castration in West African dwarf bucks. Differences in the breed of goats might be responsible for this variation. The decrease in serum total protein following castration might be due to the procedure as surgery and trauma

were suggested to cause decreased protein synthesis and increased protein catabolism as a result of pain.[18] In addition, reduction in acute phase proteins might be another possible mechanism as this indicated that the animal might be under pain and stress.[19] However, the decrease (though not statistically significant) in serum total protein was more in bucks castrated by orchidectomy (group C) thus suggesting possible loss of protein via blood loss.

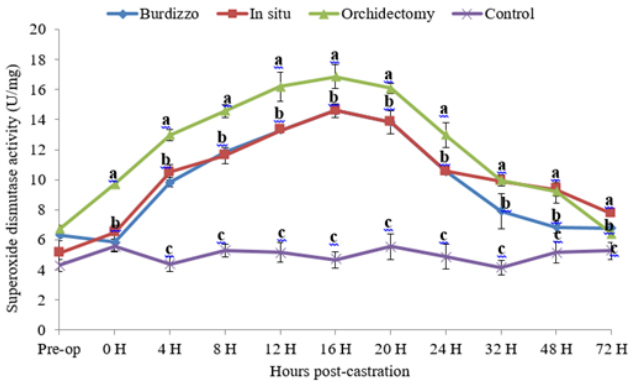

Figure 8. Superoxide dismutase activities in red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at $p < 0.05$.

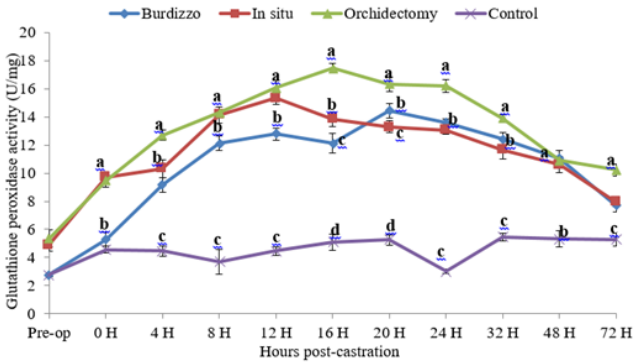

Figure 9. Glutathione peroxidase activities in red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at $p < 0.05$.

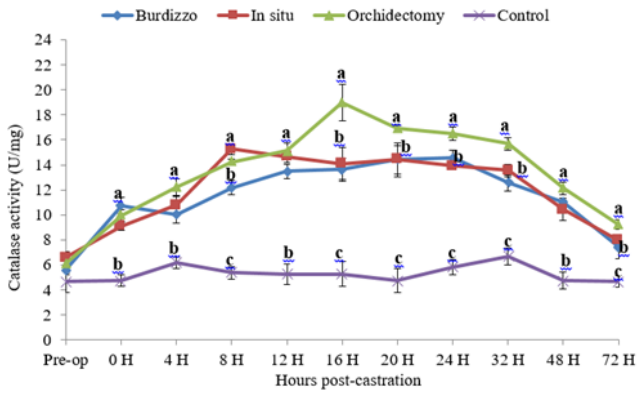

Figure 10. Catalase activities in red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at $p < 0.05$.

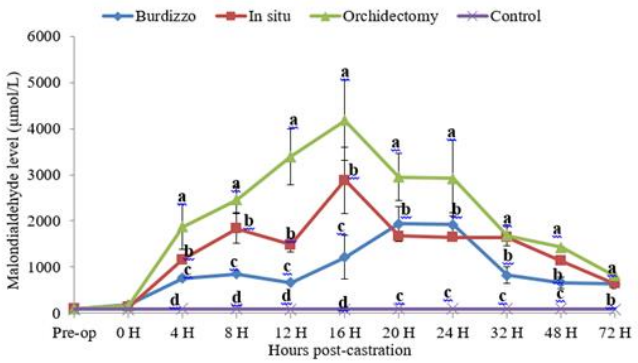

Figure 11. Malondialdehyde levels in red Sokoto bucks before and after bilateral castration using Burdizzo, *in situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at $p < 0.05$.

There was a significant increase in blood glucose at 0 hours across the groups and this might have resulted from the possible acute pain immediately after castration as post-operative pain was reported to elicit a cellular stress response, diminishing the autonomic, somatic, and endocrine reflexes leading to protein break down and consequent hyperglycemia.[20,21] Also, cortisol and catecholamines facilitate glucose production as a result of increased hepatic glycogenolysis and gluconeogenesis. The significant decline by 4 hours post-castration (using the Burdizzo method and *in situ* spermatic cord ligation) might be due to anorexia resulting from pain and also decreased peripheral use of glucose.[22] The return to normal values by 16 hours post-castration (*in situ* spermatic cord ligation) and 20 hours post-castration (Burdizzo method and Orchidectomy) showed that there was an eventual return to normal function as the pain started diminishing.

The significant increase in blood urea nitrogen (BUN) and creatinine levels in all castrated goats in this study was consistent with the reports of Mohr *et al*. (2002)[23] and Olaifa and Opara (2011)[8]. This increase might be due to the fact that urea functions as a source of nitrogen for protein biosynthesis and excess breakdown of protein led to the formation of ammonia from urea in the digestive tract.[24] Creatinine is a non-enzymatic breakdown product of phosphocreatine in the muscle, changes in creatinine observed in this study might have resulted from the breakdown of tissues due to the castration.[25]The increase of BUN and creatinine concentrations in goats castrated by orchidectomy indicated that there was more tissue breakdown than by *in situ* spermatic cord ligation and Burdizzo. In addition, the later pain induced by orchidectomy might have resulted in decreased feed and water intake leading to possible dehydration and subsequent increase in BUN concentration observed in this study.

The activities of glutathione peroxidase (GPx), catalase and superoxide dismutase (SOD), and malondialdehyde (MDA) levels were significantly

increased in all castrated goats with the highest observed in goats castrated by orchidectomy. This increase might be due to the stress induced by the castration procedures leading to the production of free radicals hence, oxidative stress.[26] An increase in reactive oxygen molecules was reported to occur following increased oxidative reactions as seen in intense exercise, pregnancy, stress, injury, and infection.[27] The excessive generation and inadequate removal of free radicals resulted in destructive and irreversible damage to the cell.[28] The increases noticed due to orchidectomy in this study thus indicate that orchidectomy caused the generation of reactive oxygen molecules compared to *in situ* spermatic cord ligation and Burdizzo, hence, was more stressful.

In conclusion, orchidectomy induced more severe serum biochemical changes than the Burdizzo method and *in situ* spermatic cord ligation in red Sokoto bucks. Hence, in the performance of castration in goats, Burdizzo method and *in situ* spermatic cord ligation should be considered to minimize serum biochemical changes.

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Conflict of Interest

There is no conflict of Interest in any form.

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