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Comparative evaluation of serum amyloid A concentration in Red Sokoto bucks neutered using different procedures

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Abstract

In this study, the changes in concentrations of serum amyloid A (SAA) induced by orchidectomy, Burdizzo castration, and *in-situ* spermatic cord ligation in Red Sokoto bucks were evaluated. Sixteen (16) Red Sokoto bucks, 6 months to one year old and weighing between 11kg and 12 kg, were randomly divided into 4 groups (A-D), each comprising 4 bucks. Bucks in group A were castrated using the Burdizzo method, B *in-situ* spermatic cord ligation, and C orchidectomy while group D served as control. Blood was collected from each buck, serum was harvested and analyzed for SAA at 0, 4, 8, 12, 15, 20, 24, 36, 48 and 72 hours post-castration. Results revealed significant increase (p=0.028) in the levels of SAA in all castrated goats from 4 hours post-castration, reaching peak concentration at 20 hours post-castration with the highest recorded in goats castrated by orchidectomy (63.34 ± 1.49 pg/mL; 120.44 ± 3.74 pg/mL), followed by *in-situ* spermatic cord ligation (25.08 ± 2.19 pg/mL; 109.77 ± 2.97 pg/mL) and then Burdizzo (61.36 ± 2.63 pg/mL; 87.29 ± 3.92 pg/mL). This was followed by a significant (p=0.041) decrease by 24 hours post-castration and non-significant (p=0.101) fluctuations up to 72 hours post-castration in all castrated bucks. In conclusion, all the castration methods induced changes in serum concentration of SAA, which was less marked in Burdizzo and *in-situ* spermatic cord ligation compared to orchidectomy.

Keywords: Burdizzo, Castration, *In-situ* spermatic cord ligation, Orchidectomy, Serum amyloid A

Introduction

Small ruminants constitute one of the livestock groups that plays a major role in food sufficiency, and socio-economic as well as economic development of many countries of the world (Luikart *et al*., 2001). Goats are an important source of income generation and economic sustenance and constitute a very important part of the rural and urban economy with a lot of households keeping goats (Duku et al., 2011) Generally, goats are endowed with special attributes such as heat tolerance, disease resistance, short generation interval, and high reproduction rate (Oguoma, 2003; Lebbie, 2004). As multipurpose animals, goats provide meat, milk, hides, skins, and manure (Adam *et al*., 2010).

Castration of male animals is the removal of the testicles or making the testicles nonfunctional (Abid

& Al-Baghdady, 2013). Castration is performed to prevent the production of androgens and spermatogenesis (Gilbert & Fubini, 2004), to prevent unwanted mating and mounting and accompanying injuries or to treat testicular or inguinal pathology (Price *et al*., 2005; Edwards, 2008), to decrease aggressiveness and to make the animal docile for easy management (Stafford, 2007). In food animals, castration can improve the quality and taste of meat, enhance feed efficiency and weight gain (Thompson, 2000; Anderson, 2007), and reduce the goaty smell in the meat (Merkel & Dawson, 2008).

Several castration techniques have been adopted depending on the animal species, age, purpose of castration, testicular anatomy, and surgeon´s choice (Merkel & Dawson, 2008). Generally, castration methods are classified as either surgical or non-surgical (Fasseha, 2019). The surgical methods include orchidectomy, the use of Burdizzo, and emasculatomes (Fasseha, 2019). Surgical methods are more invasive and possibly more painful than non-surgical ones (Merkel & Dawson, 2008). Non-surgical methods of castration involve the use of chemicals and hormones to render the animal sterile (Fesseha, 2019). The procedure is believed to result in ischaemia with subsequent atrophy and necrosis of the testicle (Stilwell *et al.,* 2008). All the methods of castration (orchidectomy, Burdizzo castration, and elastrator banding) can cause pain (Stafford, 2007). This has been shown to produce physiological, neuroendocrine, and behavioral changes indicative of pain and distress (Pang *et al*., 2006; Stillwell *et al*., 2008; Currah *et al*., 2009) Animals exhibit pain responses after castration such as struggling, kicking the hind legs, tail swishing, foot stamping, head-turning, restlessness, stilted gait, reduced activity, increased recumbency, abnormal standing posture, reduced interest in the environment and reduced grazing or feed intake (Fisher *et al.,* 2001).

The acute phase response (APR) refers to a nonspecific and complex reaction of an animal that includes changes in concentration of numerous liver-derived plasma proteins, called acute phase proteins (APPs), (Gonzalez *et al*., 2010). APPs are a group of blood proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma, or stress and they are classified as positive or negative depending on the increase or decrease in the serum concentration, respectively, during the APR (Ceciliani *et al*., 2002). The APR is classically known to produce two biological responses: 1) fever and 2) alterations in liver metabolism resulting in the release of APPs such as serum amyloid A from hepatocytes (Carroll & Forsberg, 2007). Positive APPs such as haptoglobin, C-reactive protein, serum amyloid A, ceruloplasmin, fibrinogen, and alpha 1-acid glycoprotein, increase in concentration in response to inflammation and stress, while the "negative" APPs decrease in concentration in response to inflammation and include proteins like albumin and transferrin (Murata *et al*., 2004). In response to proinflammatory cytokines, positive APPs increase within a few hours, peak within 24 to 48 hours, and remain elevated as long as inflammatory stimuli persist (Dhainaut *et al*., 2001). In general, the role of acute phase proteins is to enhance protective host functions by minimizing tissue damage and enhancing repair processes after infection, trauma, or stress (Crisman *et al*., 2008).

Serum amyloid-A (SAA) has several roles that are associated with immunomodulation and downregulation of inflammatory responses (Ceciliani *et al*., 2002; Petersen & Heegaard, 2004). Positive APPs such as Serum Amyloid A, show a pronounced increase in plasma concentration by at least 25% during an inflammatory and stress response and a rapid decline after the resolution of such (Ceciliani *et al*., 2002). Quantification of serum amyloid A concentration in plasma or serum can provide valuable diagnostic information in the detection, prognosis, and monitoring of disease in several animal species (Eckersall, 2000). There is the paucity of data and information on the effects of orchidectomy, Burdizzo castration, and *in-situ* spermatic cord ligation in Red Sokoto bucks. Hence in this study, the changes induced by orchidectomy, Burdizzo castration, and *in-situ* spermatic cord ligation in Red Sokoto bucks were evaluated to identify the procedure that elicits the least stress and inflammatory response.

Materials and Methods

Ethical considerations

This study involved the use of bucks and ethical approval was granted by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2022/047).

Experimental animals

A total of sixteen Red Sokoto bucks, six months to one year old and weighing 11 kg to 12 kg, were used for the study. The bucks were purchased from the livestock market in Giwa Local Government Area of Kaduna State and kept in the small ruminant pens of the Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria. Before the arrival of

the bucks, the pens were cleaned, disinfected, and treated with insecticide. The bucks were stabilized and acclimatized for 2 weeks, during which routine examination was carried out for any sign of ill health. Also, clinical evaluations were conducted on each animal to determine their baseline data. The animals were fed with groundnut hay, bean husks, and maize offal, twice a day; water was provided *ad libitum*.

The animals were randomly allocated into four groups (A, B, C, and D) each comprising four bucks as follows; A (Burdizzo castration), B (*in-situ* spermatic cord ligation), C (orchidectomy), and D (control).

Experimental procedures

Burdizzo castration method: This was performed using the Burdizzo castrator (Agri Health castrator; Agri Health Limited, Ireland) as described by Olaifa & Opara (2011). After proper restraint of each buck, the hind limbs were spread apart and the scrotal area was exposed to the surgeon. Castration was achieved by applying the Burdizzo laterally onto the scrotal neck. The first finger and thumb were used to hold the cord laterally in the scrotal neck, while the second hand slowly directed the position of the jaws until they were about 8-10 mm apart to grip the skin and cord firmly. The surgeon ordered and maintained rapid closure for 15-30 seconds while ensuring that the cord was properly crushed (Plate I).

In-situ spermatic cord ligation procedure: *In-situ* spermatic cord ligation was performed following aseptic preparation of the skin enveloping the spermatic cord (Ponvijay, 2007). Each buck was restrained on the surgical cradle in lateral recumbency, and local anaesthesia was achieved through a linear subcutaneous infiltration of 1 mL of 2% Lidocaine HCl on each lateral aspect of the scrotum. Non-absorbable suture material (Nylon size 2/0), (Anhui Kangning Industries, China) was used for a double external trans-fixing ligation of the entire spermatic cord, 2 cm apart. The procedure was repeated on the other cord (Plate II).

Orchidectomy: Orchidectomy was performed as by modification of the procedure described by Malbrue & Zorilla (2018). The scrotal area was shaved, and scrubbed with soap and water, and chlorhexidine (Saro Life Care Limited, Nigeria) was applied to disinfect. The buck was sedated intramuscularly with 0.05 mg/kg Xylazine (Bioveta, Komenskeho, Czech Republic). A linear subcutaneous infiltration of 2% lidocaine HCl (Afirst Life Science Limited, India) was carried out at 4mg/kg on the lateral aspect of the scrotal sac and was used to achieve local anaesthesia. Each goat was restrained on the surgical cradle in dorsal recumbency. The scrotum was then grasped, and a vertical incision through skin and fascia at the lateral part of the scrotum was made. This allowed the exteriorization of one of the testicles which was stripped off the vaginal tunic with a gauze sponge. The spermatic cord which was covered by a vaginal tunic, was ligated in three places with an absorbable suture ligature (Chromic catgut size 2/0, Anhui Kangning Industries, China). The spermatic cord was cut 1 cm below the ligature and the stump was checked for bleeding. Similarly, the opposite testicle was removed (Plate III).

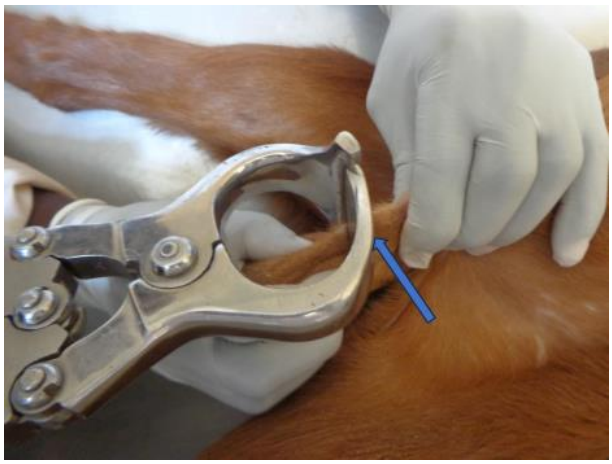

Plate I: Photograph of a Red Sokoto buck undergoing burdizzo castration.
Note the Burdizzo castrator crushing the spermatic cord in the scrotum (Blue arrow)

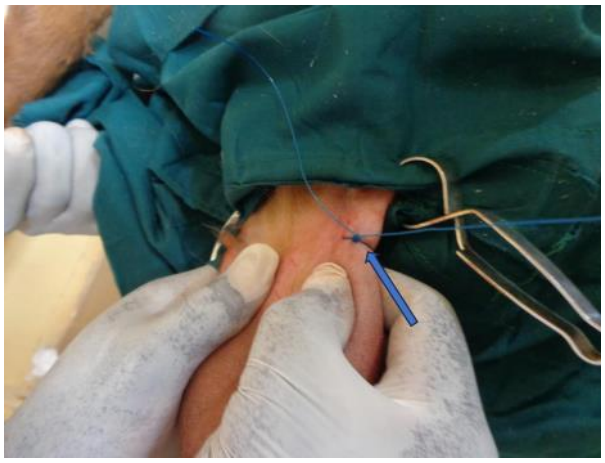

Plate II. Photograph of the scrotum of a Red Sokoto buck undergoing *In-situ* spermatic cord ligation (see blue arrow)

Blood sample collection

Blood sample (3 mL) was collected via jugular venipuncture from each buck pre-castration and at 0, 4, 8, 12, 16, 20, 24-, 36-, 48- and 72-hours post-castration. The collected blood sample was emptied into a labeled tube without anticoagulant, after which serum was harvested using a pipette and stored at -20°C until analyzed for serum amyloid A (SAA) concentration.

Enzyme-linked immunosorbent assay for determination of serum amyloid A concentration

Each serum was analyzed for SAA using a Goat Serum Amyloid A (SAA) Elisa kit (KTE 50046, Abbkine, Incorporated China), by following the manufacturer's instructions.

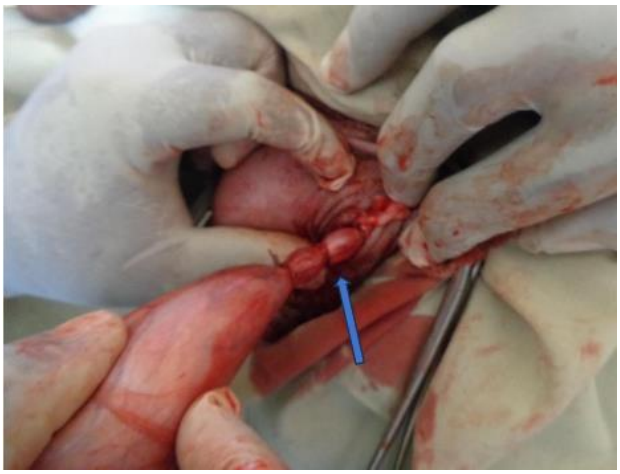

Plate III: Photograph of a Red Sokoto buck undergoing closed castration (Orchidectomy) (see blue arrow)

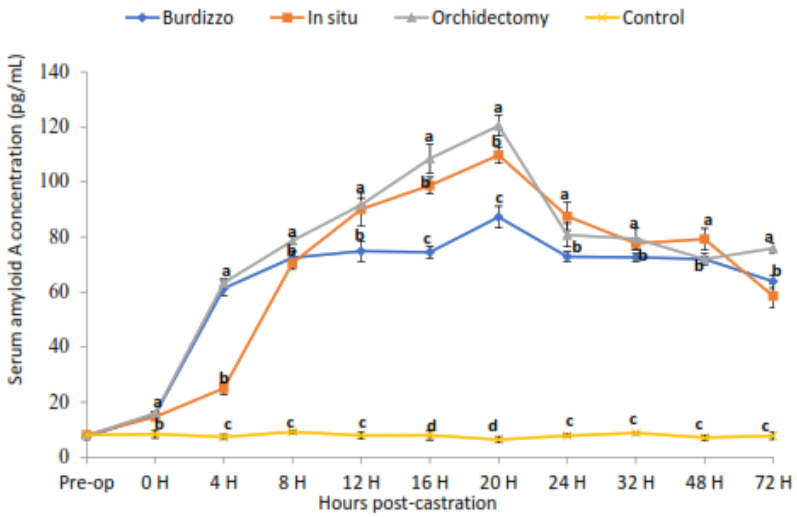

Figure I: Serum amyloid A levels in goats before and after bilateral castration using the Burdizzo method, *in-situ* spermatic cord ligation and orchidectomy. Values with different alphabets in the same hour differ significantly at $p < 0.05$

Data analysis

Data obtained were presented in charts as the mean and standard deviation of the mean (mean ± SD). Data were subjected to one-way analysis of variance (ANOVA) with repeated measures with the Bonferroni *post hoc* test, using Graph pad Prism version 5.0 (San Diego California, USA). Values of $p \leq 0.05$ were considered significant.

Results

Pre-castration result of SAA showed no significant ($p=0.235$) difference in all groups of goats (Figure 1). This was followed by a significant increase ($p=0.028$) in the levels of SAA in all castrated goats from immediate (0 hours) post-castration, reaching peak (0 hours) post-castration, reaching peak concentration at 20 hours post-castration with the highest recorded in goats castrated by orchidectomy (63.34 ± 1.49 pg/mL; 120.44 ± 3.74 pg/mL), followed by *in-situ* spermatic cord ligation (25.08 ± 2.19 pg/mL; 109.77 ± 2.97 pg/mL) and then Burdizzo (61.36 ± 2.63 pg/mL; 87.29 ± 3.92 pg/mL). Thereafter, there was a significant ($p=0.041$) decrease by 24 hours post-castration and non-significant ($p=0.101$) fluctuations up to 72 hours post-castration (Figure 1).

Discussion

Serum Amyloid A (SAA) is one of the positive and major acute phase proteins (APPs) in goats and APPs were suggested to be biomarkers of inflammation, infection, stress, and trauma in human and veterinary medicine (Eckersall, 2008; 2010). The APR is a systemic and dynamic process that includes a wide range of pathophysiological responses, such as fever, leukocytosis, hormone alterations, and muscle protein depletion that combine to minimize tissue damage while enhancing the repair process (Petersen & Heegaard, 2004). In this study, the concentration of serum amyloid A (SAA) increased significantly in all castrated goats from immediate (0 hours) post-castration, reaching peak concentration at 20 hours post-castration with the highest recorded in goats castrated by orchidectomy, followed by *in-situ* spermatic cord ligation and then Burdizzo. These findings were consistent with the study of Saidu *et al*. (2017) who reported an increased SAA concentration in Sahel goats that

were subjected to rumenotomy. Also, the concentration of acute-phase proteins, such as haptoglobin, and SAA was reported to increase as part of the inflammatory cascade associated with tissue damage or in response to a stressor, an increase in plasma haptoglobin and SAA concentration was reported within 1–3 days after castration in young animals (Earley & Crowe 2002; Ting *et al*., 2003; Pang *et al*. 2006). Meléndez *et al*. (2017) observed that calves that underwent knife castration (orchidectomy) had greater concentrations of SAA compared to calves that passed through sham castration and banding, calves' samples were collected in a 7-day period with a greater swelling score observed in knife-castrated calves. Furthermore, Horadagoda *et al*. (1999) found that SAA was elevated more by acute inflammatory cases rather than chronic inflammatory cases after clinical and postmortem examinations in 81 cattle. Furthermore, in cows, stress-related factors along with damage to the reproductive tract were shown to be responsible for increasing the concentrations of SAA, thus suggesting its possible use to monitor the onset of an inflammatory response (Chan *et al*., 2010).

The increase in SAA level is due to the stress responses induced by damage to tissues during the procedure. It is suggested that trauma, via surgery, was documented to increase SAA concentrations (Yamamoto, 1993; Dabrowski *et al*., 2009; Saidu *et al*., 2017). Animals with trauma (ear/tail bites), as well as arthritis, also have been shown to demonstrate high levels of SAA, C-reactive protein (CRP), and Haptoglobin (HP) (Parra *et al*., 2006). In addition, an experimental surgery conducted in animals was reported to result in transient increases in SAA and HP (Jacobsen *et al*., 2006). The increased SAA concentration might have also resulted from the rapid synthesis of sialoproteins and an increase in globulin release from damaged tissues induced by the castration (Citil *et al*., 2004). Positive acute phase proteins like SAA increase within a few hours, peak within 24 to 48 hours, and remain elevated as long as the inflammatory stimulus persists (Dhainaut *et al*., 2001). This is also attributable to the beginning of a stressful and inflammatory process that the animal is passing through as a result of the procedure. The highest increase observed in goats castrated by orchidectomy suggests that the procedure exerted a more painful experience which led to a stressful impact with resultant tissue damage than *in-situ* spermatic cord ligation and Burdizzo castration. Orchidectomy has been reported to cause more acute pain (Molony *et al*., 1995; Meléndez *et al*., 2017). Castration can elicit stressful conditions which can cause an imbalance between oxidants and antioxidants in favor of oxidants at the cellular and individual levels creating the status of oxidative stress (Lee *et al*., 2018).

In conclusion, all the methods of castration elicited stress and inflammatory reactions and this was evident by increased serum concentration of SAA. Due to the rapid increase after the onset of SAA, SAA is considered to be a sensitive, early diagnostic, and prognostic indicator of inflammation and stress in goats undergoing husbandry procedures like orchidectomy, *in-situ* spermatic cord ligation, and Burdizzo castration. However, Burdizzo and *in-situ* spermatic cord ligation provoked less stress, and inflammatory reaction in the animals compared to the orchidectomy technique.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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