



## **RESEARCH ARTICLE**

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# Creatine kinase activity in the assessment of muscle injuries following lateral recumbency and standing restraint positions for rumenotomy in Kano-Brown Goats

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### Kano-Brown Keçilerinde rumenotomi için yan yatma ve ayakta durma pozisyonlarını takiben kas yaralanmalarının değerlendirilmesinde kreatin kinaz aktivitesi

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#### Öz

**Amaç:** Bu çalışma kreatin kinaz aktivitesinin profillerini temel alan ayakta rumenotomi için yerel olarak imal edilmiş bir mobil küçük ruminant metal kanalı ve iki geleneksel yan yatma rumenotomi tekniğinin sunduğu karşılaştırmalı avantajları değerlendirmek amacıyla yapıldı.

**Gereç ve Yöntem:** Her iki cinsiyetten rumen yabancı cisim (RFBI) teşhisi konan 24 adet Kano Kahverengi Irkı Keçiden (KBGs) 18 adedi A, B ve D gruplarına ayrıldı. Grup A ve B'deki keçilere yan yama pozisyonundayken sırasıyla rumen cilt klemp fiksasyonu (RSCF) ve kalıcı sütür rumenotomi (SSR) teknikleri uygulandı. D grubundaki keçilere, fabrikasyon mobil küçük ruminant cerrahi metal kanal (MSRSC) kullanılarak ayakta durma pozisyonunda rumenotomi yapıldı. RFBI içermeyen diğer altı keçi (KBG ) kontrol grubunu (Grup C) oluşturdu. Kreatin kinaz aktivitesi ELISA'ya kadar -200 ° C'de saklanan serumu elde etmek için 0, 5, 24, 48, 72. saatlerde ve 1., 2. ve 3. haftalarda juguler damarlardan kan alındı.

**Bulgular:** Grup D'de ortalama kreatin kinaz aktivitesi (388.66 ± 28.11 U / L), karışık cinsiyette grup A'daki değerden (924.55 ± 254.66 U / L) anlamlı olarak daha düşüktü (p <0.05). Grup A'nın erkekleri (1148.09 ± 438.94 U / L), grup B (459.56 ± 84.16 U / L) ve D'den (503.60 ± 23.37 U / L) anlamlı olarak daha yüksekti (p <0.05).

Öneri: Ayakta durma posizyonundaki rumenotomide kreatin kinaz aktivitesinde önemli bir değişiklik olmadı. Bu durum küçükbaş hayvanlarda bu tekniğin benimsenebileceğini ortaya koymaktadır.

Anahtar kelimeler: Kreatin kinaz, rumenotomi, kısıtlama, ELISA, keçi

#### Abstract

**Aim:** The study was to evaluate and establish the comparative advantages offered by two conventional lateral recumbence rumenotomy techniques and a locally fabricated mobile small ruminant chute for standing position rumenotomy based on the profiles of creatine kinase activity.

**Materials and Methods:** Eighteen (of n = 24) Kano-Brown goats (KBGs) of both sexes diagnosed of rumen foreign body impaction (RFBI) were allocated to groups A, B and D. Goats in groups A and B underwent rumen skin clamp fixation (RSCF) and stay suture rumenotomy (SSR) techniques respectively, while on lateral recumbency. Goats in group D underwent rumenotomy while on standing restraint using fabricated mobile small ruminant surgical chute (MSRSC). The six other KBGs that are free of RFBI served as control (Group C). Blood were collected via the jugular veins at Pre, 0, 5, 24, 48, 72 hours and at weeks 1, 2 and 3 to harvest serum that were stored at -20°C until creatine kinase activity ELISA.

**Results:** Mean creatine kinase activity in group D (388.66 ± 28.11 U/L) was significantly (p<0.05) lower than the value in group A (924.55 ± 254.66 U/L) in the mixed-sex. Males of group A (1148.09 ± 438.94 U/L) was significantly higher (p< 0.05) than group B (459.56 ± 84.16 U/L) and D (503.60 ± 23.37 U/L).

**Conclusion:** Standing restraint rumenotomy had no significant alterations in the creatine kinase activity, thus; suggests it could be adopted in small ruminants.

Keywords: Creatine kinase, rumenotomy, restraint, ELISA, goats

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#### Introduction

Animals would naturally be non-compliant with physical restraint and the handlers react by using more restraint force, imperilling the animal to fight harder (Hassan and Nwannenna 2009, Bennett 2014). Goats have always been subjected to lateral recumbency restraint position for surgeries, perhaps they are small enough to be physically restrained, and the believe that their size does not threaten their surgical outcome unlike larger ruminants and equine species (Zavy et al 1992, Grandin and Shivley 2015). The purpose and duration of restraint make physical restraint pound-for-pound, a lot more cumbersome, and relative, such as its impacts on postsurgery response (Bennett 2014). In large animals, modalities for standing restraint for surgeries with sedatives and/ or tranquilizers were reported with great advantages but similar findings are rarely available for small ruminants such as sheep and goats and they are one of the most presented animals to veterinarians for procedures among food animals. When muscles of the body are damaged (diseased or inflamed), the systemic response in the blood is a high creatine kinase (CK) also known as creatine phosphokinase (CPK), a specific indicator of muscle injury or soreness (Prasse's 2011). Provided peak values were established, studies have suggested that serum CK assessment can be a valid indication of the magnitude of muscle damage in postoperative trauma with the recorded rise within 24 hours post-surgery (Lombao et al 2014). Freeman et al (2009) also reported that many studies compared trauma induced by surgery through investigating dissimilar surgical procedures in different tissues. Similar to other animal species, the caprine muscle is a chief cradle of CK bustle and could serve as a reliable biomarker of the extent of muscle fibre damage (Kramer and Carthewt 1985). Goat CK reference range is 104-220 IU/L (Infovets 2018), but reference range for the different breeds of goats are very rare. A modest rise in serum CK levels was recorded post repetitive succinylcholine administration in (Laurence and Norris 1998).

However, approaches may contrast and denote jeopardy, as manipulation of dissimilar body structures could cause varying creatine kinase activity. A substantial relationship has been established amid serum levels of CK and the period and amount of weight on paraspinal muscles exerted by retraction (Motosuneya et al 2006). The extent of soreness and discomfort expressed as stress following surgery is an imperative sequence of minimal scalpel dissections -minimal flesh injury -minimal post-surgical pain -brief hospital admission -improved post-surgical outcome (Kumbhare et al 2007). Likewise, restraint positions for surgery could influence tissue manoeuvrability and subsequent muscle soreness. This study aims to determine the comparative advantages offered by two conventional lateral recumbence rumenotomy techniques and a locally fabricated mobile small ruminant chute for standing position rumenotomy based on the profiles of creatine kinase activity as a guide in the choice of a technique for adoption in Small Ruminant Practice.

#### **Material and Methods**

The Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) assigned ethical approval number ABUCA-UC/2018/054 for this study. Twenty-four male and female Kano-Brown goats (n = 24) of equal number of both sexes were used in this study of which eighteen in equal number of sexes were diagnosed of rumen foreign body impaction (RFBI) through palpation while six others were free of the pathology. The goats aged 16.72 ± 30 months with average bodyweight of 15 ± 0.52 kg were procured from local livestock market. On arrival, the goats were subjected to physical, faecal and haematological examinations and appropriately dewormed with ivermectin (Bremamectin®, Brema pharma GmBH, 34414 Warburg, Germany) at 200 µg/kg S/C. Prophylactic doses of oxytetracycline (Kepro Oxytet® 20% LA inj, Kepro B.V. Magdenburgtraat 17, 7421 ZA Deventer-Holland, www.kepro.nl) at 20 mg/kg IM were administered to each goat against bacterial infections. The animals were adequately fed groundnut husk and a concentrate cocktail of beans-sorghum shafts-maize offal, thrice daily. Clean drinking water was provided ad libitum, except where specified such as few hours before surgery. Following these treatments, the animals were allowed to acclimatize for two weeks prior to commencement of the study. After numbers were assigned to the goats, a random number generator application software was used to assign the goats to respective groups.

Except for group C (control) with six goats comprising of 3 males and females diagnosed to be free of rumen foreign body impaction (RFBI), eighteen goats with the RFBI on palpation were allocated to groups A, B and D. Three goats to be operated daily were fasted 12 hours for feed and 6 hours for water as preoperative dietary measures for the surgery. The KBGs in groups A, B and D were first sedated with xylazine hydrochloride (XYL-M2® VMD NV/sa-Hoge Mauw 900-B-2370 Arendok-Belgium) at 0.025 mg/kg. The animals in groups A and B were put on recumbency via the right lateral position and the left paralumbar fossa of each goat in group D was similarly shaved while in a standing position restraint in the fabricated mobile small ruminant surgical chute (Fig. 2).

The shaved paralumbar fossa of each goat was aseptically prepared by scrubbing with 0.2 % Chlorhexidine gluconate (Savlon®, Vervaading deur, Johnson and Johnson (Pty) Ltd., London) and smeared with povidone-iodine (Sawke-10%®, Jawa International Limited, Jawa House Compound, Plot 6, Abimbola Estate, Isolo, Lagos, Nigeria) prior to local anaesthesia and surgery. Regional anaesthesia with 2 % lidocaine HCl at 4 mg/kg (NCL Lidocaine®, Syncom Formulations, NCL





Figure 1. Exteriorised rumen in a lateral recumbency restraint for RSCF and SSR techniques in Kano-Brown Goat

Pharm Chem Ind. Ltd., India) was done in an inverted L-block fashion on the left flank, Lee (2012). The Group A goats were subjected to rumen skin clamp fixation technique (RSCF) of rumenotomy. The goats were positioned in a right lateral recumbency and draped (Fig. 1).

A seven centimetre through and through incision as described by Firth et al (1985) was made on the skin via the left mid-flank into the abdominal cavity. Following laparotomy and necessary haemostasis, the rumen dorsal sac was identified and firmly held in place to the skin with towel clamps appropriate for the technique. On the less vascularized region of the rumen dorsal sac, a stab incision to allow the escape of the rumen gas before the incision was extended up to 7 cm with a sharp-blunt pair of scissors was made so that incised rumen edges were fixed to the skin with towel clamps as described by Dehghani and Ghadrdani (1995).

The rumen was reconnoitred and largely, polythene based foreign bodies were removed (Fig. 4) and a solution of 0.9% normal saline (Juhel®, Fabrique par Juhel Nig. Ltd., Awka, Anambra, Nigeria) was poured under pressure to rinse the rumen ingesta. To aid rumen closure, some towel clamps were removed leaving the dorsal and ventral clamps in pla-



Figure 3. Exteriorisation of the rumen in a standing restraint position rumenotomy using a mobile small ruminant surgical chute in Kano-Brown goat



Figure 2. Presentation of shaved left flank of kano-brown goats restrained in a mobile small ruminant surgical chute for rumenotomy in a standing position

ce. A combination of continuous Lambert and Cushing suture pattern to invert the rumen edges with a number 2 polyglycolic acid sutures (Atramat®, International Farmaceutica, Planta, Mexico). Chromic catgut (LIFECARE®, Anhui Kangning Industrial group Co., Ltd., Tianchang City, Anhui, China) was used in apposing the muscles in 3 layers simple continuous suture pattern. Ford interlocking suture pattern was employed to appose the skin using a metric size 2 nylon suture (LIFECARE®, Anhui Kangning Industrial group Co. Ltd, Tianchang City, Anhui, China) in all the treated groups A, B and D. The goats in group B had stay suture rumenotomy technique that involved preoperative preparations as described for RSCF up to the point of skin incisions and differed at the time of rumen manipulations for rumenotomy. The rumen was softly lifted out so that the rumen wall was anchored to the skin with 4 nylon suture knots in a simple interrupted suture pattern. The knots were placed dorsally, ventrally, cranially, and caudally as described by Dehghani and Ghadrdani (1995). The rumen was then incised and its edges grasped with artery forceps and the ruminal cavity were then explored. Ruminal and abdominal closure was also as described for RSCF Dehghani and Ghadrdani (1995). The group D KBGs had small ruminant surgical chute rumenotomy. A goat is fitted into the mobile small ruminant surgical chute and restrained effortlessly (Fig 2).



Figure 4. Evacuation of rumen foreign body in a lateral recumbency restraint position in Kano Brown Goats

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Figure 2. Rumenotomy and the demonstration of rumen foreign bodies evacuation in kano-brown goats restrained in standing position using a mobile small ruminant surgical chute.

The left flank was aseptically scrubbed with chlorhexidine gluconate and painted with povidone-iodine with the goats restrained in the standing restraint position in the MSRSC (Fig. 2). The goats were draped with polythene-based laparotomy sheet (rumen shroud) (Fig. 3).

The Surgeon double gloved for the procedure and incision was made over the skin of the left flank and the rumen was exteriorized through gentle lifting and held in place by the surgeon or an assistant. Rumen shroud was adjusted for a fit while the rumen was still held in place by the surgeon's non-dominant hand. The dorsal rumen sac was identified over the greater curvature and a stab incision was made over a less vascular portion with a scalpel, held in the surgeon's dominant hand. The incision was then extended to 7 cm with a sharp-blunt scissors with the surgeon's dominant hand and the same hand was inserted into the rumen and all foreign bodies were evacuated. During this time, the rumen was pulled away from the abdominal wall to prevent contamination with the rumen ingesta.

After the evacuation of the rumen ingesta (Fig. 5), the rumen was rinsed with 0.9% normal saline (Juhel®, Fabrique par Juhel Nig. Ltd/ Awka, Anambra, Nigeria) with the rumen gently pulled away from the abdominal cavity. The rumen was closed in two suture patterns, Lambert and Cushing respectively with polyglycolic acid (PGA) suture number 2. The abdominal muscles and skin were closed in the same fashion as in the other techniques with chromic catgut number 2 and the skin with nylon sutures number 2. The group C goats were not subjected to any form of surgery, but were similarly sampled for blood as described in the experimental design. Blood Sample Collection were on the day rumenotomy procedure for the groups A, B and D were performed and just before the surgery, 5 ml of blood was collected via the jugular vein of each goat to establish pre-rumenotomy values.

Subsequently, blood samples were also collected post rumenotomy, at 0, 5, 24, 48 and 72 hours, and subsequently at weeks 1, 2, and 3. The 0 hour is the period immediately post rumenotomy. The blood samples were dispensed into a plain vacutainer tube and allowed to clot for two hours at room temperature before centrifugation for 20 minutes at approximately  $1000 \times g$ . The harvested serum samples were discharged into micro-vials and preserved at a temperature of -20 °C. A commercially prepared ELISA kits procured from Abbkine Scientific, Wuhan, Hubei province, China, www.abbkine.com, Abbkine, Inc© was used to quantify the CK activities via ELISA.

#### Statistical analysis

The statistical package used analyse data obtained in the study was GraphPad Prism version 5.03, (2009), GraphPad Software, San Diego California USA, www.graphpad.com. The M±SE of the variables were calculated by column statistics. A Two Way Repeated Measures ANOVA with Bonferroni post-test was employed to compare between the four groups A, B, C and D. Analyses were considered as significant at p < 0.05.

#### Results

The mean serum CK activity in the mixed-sex group of KBGs of the experimental groups is as provided in Table 1. The mean CK activity of KBGs in groups A, B, C and D prerumenotomy were  $364.73 \pm 8.17$ ,  $285.79 \pm 23.08$ ,  $347.50 \pm 8.82$  and  $336.06 \pm 14.53$  U/L respectively. At 0 hours postrumenotomy, the mean values for the groups were  $579.62 \pm 98.00$ ,  $654.93 \pm 189.93$ ,  $357.74 \pm 16.98$  and  $398.30 \pm 40.17$ U/L, respectively. The difference in the mean serum creatine kinase activities in the different groups was not significant (p > 0.05). The KBG CK activity in group A (924.55 \pm 254.66) U/L) peaked at week 1 post-rumenotomy and was only significantly higher (p < 0.05) than that of group C 388.66  $\pm$ 28.11 U/L.

The female KBGs pre-rumenotomy CK activity for groups A, B, C and D were  $374.47 \pm 14.99$ ,  $254.96 \pm 26.40$ ,  $354.12 \pm 5.56$  and  $339.67 \pm 18.08$ , respectively. These values rose at 0-hour post-rumenotomy to  $492.31 \pm 29.10$ ,  $950.27 \pm 298.67$ ,  $379.83 \pm 56.35$  and  $373.39 \pm 33.72$  U/L for groups A, B, C and D, respectively. However, the CK activities in the experimental groups did not significantly differ (p > 0.05) (Table 2).

In the males of group, A, the mean CK activity (1148.09  $\pm$  438.94 U/L) was significantly higher (p < 0.05) than those of groups B (459.56  $\pm$  84.16 U/L) and D (503.60  $\pm$  23.37 U/L) (Table 3).



Variables	Pre and post-rumenotomy sampling periods									
Enzyme	Group	Pre	0 h	5 h	24 h	48 h	72 h	Week 1	Week 2	Week 3
CK (U/L)	A	364.73 ± 8.17	579.62 ± 98.00	444.04 ± 81.35	708.12 ± 128.68	575.61 ± 84.39	745.48 ± 231.46	924.55 ± 254.66ª	776.78 ± 216.46	610.61 ± 127.78
	В	285.79 ± 23.08	654.93 ± 189.93	698.95 ± 230.92	825.46 ± 334.01	594.46 ± 95.72	668.00 ± 113.88	518.39 ± 71.18	686.50 ± 258.82	404.98 ± 42.37
	С	347.50 ± 8.82	357.74 ± 16.98	342.08 ± 12.67	373.80 ± 18.04	374.30 ± 16.64	382.03 ± 12.82	388.66 ± 28.11b	329.64 ± 13.37	327.38 ± 4.54
	D	336.06 ± 14.53	398.30 ± 40.17	493.52 ± 54.56	419.19 ± 38.43	434.05 ± 40.44	402.32 ± 39.76	474.63 ± 57.09	469.10± 97.70	360.55 ± 14.87

Table 1. Mean ± SE serum creatine kinase activity (U/L) pre- and post-rumenotomy in the different experimental groups of the Kano-Brown Goats

Values with different superscripts in columns significantly (p < 0.05) differ Group A: Group that had undergone Rumen Skin Clamp Fixation (RSCF) technique of rumenotomy Croup B: Group that had undergone Stay Suture Rumenotomy (SSR) Group D: Group that had undergone standing position rumenotomy using Mobile Small Ruminant Surgical Chute (MSRSC) CK: creatine kinase

#### Table 2. Mean ± SE serum creatine kinase activity (CK, U/L) pre- and post-rumenotomy in the different experimental groups of female Kano-Brown Goats

Sampling Time	Group A	Group B	Group D	Group C (Control)
Pre	374.47 ± 14.99	254.96 ± 26.40	$354.12 \pm 5.56$	339.67 ± 18.08
0 h	492.31 ± 29.10	950.27 ± 298.67	379.83 ± 56.35	373.39 ± 33.72
5 h	417.70 ± 8.63	980.78 ± 418.17	432.85 ± 80.48	315.59 ± 5.56
24 h	529 38 + 57 03	1195 60 + 647 25	363 77 + 44 50	393 87 + 14 61
27 11	527.50 2 57.05	1175.00 1 047.25	303.77 1 44.30	575.07 ± 14.01
40.1	(11.00 + 140.10	7(2.50 + 114.74	202.02 - 0.25	207 (0 + 22 50
48 h	$611.09 \pm 140.12$	762.59 ± 114.74	383.03 ± 8.35	397.69 ± 23.58
72 h	921.18 ± 439.83	824.11 ± 179.96	355.73 ± 31.26	395.07 ± 1.39
Week 1	701.01 ± 285.66	577.22 ± 121.60	445.67 ± 15.49	416.77 ± 38.95
Week 2	799.61 ± 414.43	981.38 ± 495.68	315.59 ± 16.68	334.66 ± 21.02
Week 3	557.77 ± 234.47	485.74 ± 20.35	338.06 ± 15.32	$326.12 \pm 3.30$

All values within rows were not significantly (P > 0.05) different Group A: Group that had undergone Rumen Skin Clamp Fixation (RSCF) technique of rumenotomy Croup B: Group that had undergone Stay Suture Rumenotomy (SSR) Group D: Group that had undergone standing position rumenotomy using Mobile Small Ruminant Surgical Chute (MSRSC) CK: creatine kinase

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Table 3. Mean ± SE serum creatine kinase activity (CK, U/L) pre- and post-rumenotomy in the different experimental groups of male Kano-Brown Goats						
Sampling Time	Group A	Group B	Group D	Group C (Control)		
Pre	354.99 ± 3.75	316.62 ± 31.87	318.00 ± 26.42	355.32 ±0.70		
0 h	666.92 ± 198.87	359.59 ± 62.79	416.78 ± 67.48	342.08 ±7.74		
5 h	470.37 ± 179.79	417.12 ± 110.99	554.18 ± 68.73	368.57 ± 8.34		
24 h	886.85 ± 218.15	455.32 ± 43.34	474.60 ± 48.29	353.73 ± 31.80		
48 h	540.13 ± 121.31	426.33 ± 66.18	485.08 ± 74.19	350.91 ± 16.77		
72 h	569.77 ± 208.67	511.89 ± 89.91	448.91 ± 68.98	368.98 ± 25.49		
Week 1	**1148.09 ± 438.94 <sup>a</sup>	**459.56 ± 84.16 <sup>b</sup>	**503.60 ± 23.37 <sup>b,c</sup>	*360.56 ± 40.53 <sup>b,c,d</sup>		
Week 2	753.94 ± 249.00	391.62 ± 47.71	622.61 ± 154.56	324.62 ± 20.65		
Week 3	663.46 ± 154.51	324.23 ± 45.18	383.03 ± 19.12	328.63 ± 9.52		

Values with different superscripts within a row are significantly (p < 0.05) different (\* = p < 0.05, \* Group A: Group that had undergone Rumen Skin Clamp Fixation (RSCF) technique of rumenotomy Croup B: Group that had undergone Stay Suture Rumenotomy (SSR) = p < 0.01, \*\*\* = p < 0.001)

Group D: Group that had undergone standing position rumenotomy using Mobile Small Ruminant Surgical Chute (MSRSC)

CK: creatine kinas

#### Discussion

Findings in this study on evaluation of the effect of surgical stress on muscle cells consequent to differences in restraint positions for rumenotomy in KBGs based on serum CK activities were similar to the report by Kumbhare et al (2008) that investigated and ratified the validity of serum CK assessment as an index of skeletal muscle injury caused by lumbar decompression surgery (LDS). Serum creatine kinase as muscle injury biomarker post rumenotomy in recumbent and standing restraint positions was significantly higher in all the treated groups compared to their respective pre-rumenotomy CK activities. Comparison between the experimental groups revealed that KBGs in group A had significantly higher CK activities than the control group C, at week 1 is in tandem with the report of Lombao, Bagó et al (2014) that validates CK as a biomarker of muscle injury in the spinal injury of human surgical patients. Mbassa and Poulsen (1993), reported the influence of age and sex on CK profiles agrees with the incidences recorded in this experiment that creatine kinase activities were higher in the males than in the female KBGs. Similarly, the values of the CK activities at 0, 5, 24, 48, and 72 hours through weeks 1, 2 and 3, were higher in the goats restrained in lateral recumbencies (A and B) in both sexes compared to goats subjected to the standing restraint position for the rumenotomy in a mobile small ruminant surgical chute. These findings are suggestive that rumenotomy in the standing restraint position using MSRSC in KBGs is superior in the provision of relief (welfare) to the animals while they are subjected to surgical procedures than the recumbency restraint positions using both RSCF and SSR techniques. The

rumenotomy procedure in groups A and B KBGs subjected to lateral recumbent restraint positions were also associated with longer surgery time. These findings are in coherence with the findings of Lombao, Bagó et al (2014) who observed that severity and extent in time of surgery exhibit a correlation with postsurgical CK activities and that the occurrence of austere postsurgical pain is not significantly associated to CK levels or activity.

#### Conclusion

The fact that rumenotomy was successfully carried out on Kano-Brown goats (KBGs) restrained in standing position using a locally fabricated mobile small ruminant surgical chute (MSRSC) without significant alterations or rise in the creatine kinase activity as to the values of the conventional lateral restraint position, suggest that it is a technique that could be adopted in Small Ruminant Practice.

#### **Conflict of Interest**

The authors did not report any conflict of interest

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#### **Author Contributions**

Motivation / Concept: Abubakar Mshelia Saidu Design: Abubakar Mshelia Saidu Control/Supervision: Samuel Tanko Fadason, Sani Adamu and Gabriel Enenche Ochube

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#### **Ethical Approval**

This study was approved by the Ethics Committee of Veterinary Faculty on Experimental Animals (SUVDAMEK) (Decision Date & No: 22.11.2018, 2018/162).

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