Comparative evaluation of incisional approaches to one stage rumen cannulation based on tumour necrosis factor-α expressions

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SUMMARY

Varieties of incisional approaches to the skin and rumen for one-stage rumen cannulation could ameliorate some of the challenges of the technique. Following comparisons, incisional approaches with minimal surgical stress were established. Nine (n = 9) Yankasa-Balami cross-bred rams aged 1-2 years and weighing 39 0.9 kg were grouped into A, B, and C, each composed of 3 animals. Group A had primary incisions on the skin-muscle and rumen; group B had primary-secondary skin-muscle incisions and only primary rumen incisions; and group C, primary-secondary skin-muscle incisions and primary-secondary rumen incisions. Blood sample were collected after the surgery, at 0 h, 4 h, 8 h, 12 h, 24 h, 48 h and 72 h, weeks 1, 2 and 3 postrumenostomy for sera that were stored at -20 °C until ELISA for TNF-α. The M±SE of TNF-α concentrations was calculated using GraphPad Prism version 9.0 (121) for Windows, and a One-way Repeated Measures ANOVA with Dunnett's multiple comparisons test was used to compare sampling periods to their respective groups' pre-values. A two-way repeated measures ANOVA with a Bonferroni post-hoc was used to compare the three groups (A, B, and C). Analysis was considered significant at $p < 0.05$. The pre-cannulation serum TNF-α concentrations were 36 ± 0.66, 38 ± 4.8 and 38 ± 1.7 pg/mL for groups A, B and C, respectively. Compared to respective Pre values, the concentrations became significantly ($p < 0.05$) higher in group A (19 ± 1.7) at 72 h and group C (73 ± 2.3 pg/mL) at 48 h. The TNF-α concentrations between-groups showed that at 0 h post-cannulation, the concentrations of TNF-α in groups B (72.35 ± 1.18) and C (85.47 ±11.78) were significantly ($p < 0.05$) higher than group A (26.4 ± 0.46 pg/mL). At 4 h, group C was significantly ($p < 0.05$) higher than group A while group B and C were significantly ($p < 0.05$) higher than group A, at 8 h. Group C had a significantly ($p < 0.05$) higher concentration than groups A and B at 12 h. The finding that group B had lower concentrations of the TNF-α than group C suggests that the numerous incisions were responsible for the TNF-α surge. The application of primary incisions on the skin and rumen alone influences the numerical concentration of serum TNF-α, and this effect is amplified when a number of secondary incisions are added to the skin and/or rumen. The application of primary incisions on the skin and rumen alone, as well as the length of incisions, influences the release and numerical concentrations of serum TNF-α, particularly when the number of primary incisions or secondary incisions are applied to the skin and/or rumen. Although the incisional patterns used did not preclude cannula drops, initial incisions on both the rumen and skin alone were a preferable choice in terms of the surgical welfare of the animals. The adoption of wider inner and outer rubber flanges avoided cannula drops, making primary incisions on both the rumen and skin alone a superior incisional technique due to the reduced surgical stress on the rams. Thus, above other adopted traditional procedures in the bovine species, the use of primary incisions on both the rumen and skin is by far the cheapest and least stressful incisional strategy for rumen cannulation in the ram. Overall, the use of locally improvised polyvinyl chloride plastisol for ram cannulation was successful.

KEY WORDS

Ovine species, Sheep, rumen cannulation, Tumour Necrosis Factor-α, Cytokines, Surgical stress.

INTRODUCTION

Rumen fistulation (RF), otherwise known as rumenostomy or rumen cannulation, is the fitting of an intra-ruminal cannula into a deliberate fistula created between the ruminal dorsal sac and the body wall in the left para-lumbar fossa of ruminants. The procedure is an indication for the treatment of chronic bloat and, most commonly, an integral part of the bovine, caprine, and ovine nutritional studies when the rumen ingesta must be collected and analysed[1,2]. Several methods for obtaining transfaunate have been reported; the use of a rumen liquor or rumen content donor through rumen cannulation for long-term access to the rumen fluid with minimal labour, time, and expense[3]. Rumen fistulation is preferred over stomach tube sampling[4], especially when repeated sampling is required, as it prevents the contamination of ruminal samples with saliva and gives a typical sample of ruminal fluid[5,6].

Three techniques of RF exist as the one-stage method, the two-stage method, and the Schalk and Amadon technique[1,2,7]. The one-stage method involves exteriorisation of the rumen through a circular[3,8] or vertical[9-12] skin incision through the left paralumbar fossa, and the cannula is then inserted into the ruminal opening on the same day of surgery. The two-stage tech-

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nique of RF is such that the rumen is exteriorized through a circular[13] or vertical[1] skin incision in the left paralumbar fossa. The third technique, called the Schalk and Amadon Technique, was first described by Shalk and Amadon[14] for cattle and modified for sheep by Hecker[15]. A fold of rumen wall is exteriorized and clamped between two metal bars. The fold necrotizes and sloughs, and a fistula between the lumen of the rumen and the outside is created.

Permanent cannula implantation has been associated with some complications[2] and may comprise peritonitis following leakage of rumen contents around the rumino-cutaneous fistula[16]. The leakage of large amounts of rumen content can subject the animal to dehydration and malnutrition, cause skin lesions, produce unpleasant odours and promote the emergence of myiasis[2, 7]. The leaks are primarily caused by an increase in the size of the fistula caused by necrosis of the tissue or a ruminal fluid leak[17]. It was also observed that cannulas fell at times, mainly weeks or months post cannulation, mostly in the morning, after sheep had spent the night lying with the rumen full, which increased the pressure on the cannulas. In sheep, due to the weakness of their abdominal wall muscles, the cannula attachment and maintenance of its stability can be considered a challenge for veterinary surgeons[1, 18]. Whether the number and length of incisions made on the skin and rumen at the time of making the fistulae could moderate the occurrence of these problems is yet to be fully investigated.

Stress is defined as external and internal disruptive events (stressors) applied to animals or other biological systems, leading to measurable alterations of homeostasis that may comprise metabolic, immunologic, and neuroendocrine changes following injury or trauma[19, 20]. Oxidative stress is a common accompaniment of nearly all forms of surgery, especially major surgical procedures. The severity of the inflammatory response is connected to different physiological events and correlates with the concentration of the cytokine produced[21]. The pro-inflammatory cytokines are the most necessary mediators to set up an anti-infectious response. Cytokines have been associated with poor outcomes in human pathologies such as sepsis[22]. While anti-inflammatory cytokines are a prerequisite to controlling the cascade of pro-inflammatory mediators, their excessive production is associated with severe immune depression[23].

Tumour necrosis factor (TNF-α) also known as cachectin, is a pro-inflammatory cytokine produced mainly by monocytes, macrophages, and T-lymphocytes and is abundant in the peritoneum and splanchnic tissues. It is also present in neurons and glial cells, with functions that are important both in inflammatory and neuropathic hyperalgesia. TNF-α is one of the earliest and most potent mediators of the inflammatory response after a surgical procedure, trauma, or infection, causing significant metabolic and hemodynamic changes as well as activating other cytokines distally. As a potent inducer of muscular metabolism and cachexia, TNF-α stimulates lipolysis and inhibits lipoprotein lipase. It also plays the role of activating coagulation, stimulating the expression or release of adhesion molecules, platelet activating factor, glucocorticoids, and eicosanoids, and influences apoptosis[24]. The amount of other cytokines in the experimental model influenced some of the effects of tumor necrosis factor (TNF-α). Low doses were found to induce angiogenesis, whereas high concentrations were associated with an inhibition of angiogenesis[25].

The specific objective of the study was to evaluate serum concentrations of TNF-α as a biomarker of surgical stress in two newly innovated and one conventional incisional patterns of one-stage rumen cannulation using a locally improvised polyvinyl chloride plastisol. The incisional patterns are composed of primary incisions on the skin of the left flank and rumen, primary and secondary skin incisions but primary incision on the rumen and the two primary and secondary incisions on both the skin of the left flank and the rumen in Yankasa-Balami cross-bred rams. This was to identify which could be minimally stressful while maintaining leak proof for cannulation in ovine species. To the best of our knowledge, this is the first study comparing these stylized incisional patterns for rumen cannulation in sheep.

MATERIALS AND METHODS

Ethical Clearance

Animal utilization protocol with approval letter dated 26th February 2020 as formal Ethical Clearance was obtained from the University of Maiduguri Animal Use and Ethics Committee with reference number FVM/UNIMAID/AUEC/2020/002.

The Research Station

The study was conducted at the Surgery Theatre, Department of Veterinary Surgery and Radiology, University of Maiduguri while the ELISA was performed at the North-East Zonal Biotechnology Centre of Excellence, University of Maiduguri.

Research Animals

Nine apparently healthy Yankasa-Balami cross-bred rams aged 1-2 years were procured from Maiduguri Livestock Market, Borno, State, Nigeria. The rams weighed 39 ± 0.9 kg and designated for rumen nutritional studies, hence, required to be cannulated to aid collection of samples from the rumen. As pre-surgical evaluations for acclimatization, the rams underwent physical examination followed by collection of blood and faecal samples for haematology and parasitological evaluations, respectively.

The animals were dewormed with ivermectin (Bremamectin®, Brema pharma GmBH, 34414 Warburg, Germany) at 200 µg/kg S/C. A 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 was administered to each ram against bacterial infections. The animals were adequately fed groundnut husk, mixtures of beans, sorghum shafts and maize offal, three times daily. Clean drinking water were provided *ad libitum*, except where specified, and allowed to acclimatize through confinement at the stated facility for three weeks before the commencement of the study.

Grouping of Research Animals and the Treatments Allocated to the Respective Groups

A random number generator was employed and allocated the nine experimental rams into three groups A, B and C such that each group had 3 animals.

Group A (n = 3): primary incision on the skin-muscle and rumen

Group B (n = 3): primary and secondary skin incisions but only primary rumen incision

Group C (n = 3): primary and secondary incisions on the skin of the left flank and the rumen

Pre-operative Preparation

The animals were fasted 12 and 6 hours for feed and water, respectively. The rams in all the groups were sedated with Xylazine hydrochloride (XYL-M2® VMD nv/sa-Hoge Mauw 900-B-2370 Arendok-Belgium) at 0.1mg/kg administered intravenously. The rams were then placed on right lateral recumbency and the left paralumbar fossa were shaved using a razor blade. The shaved left paralumber fossa of each ram 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 in an inverted-L block fashion with 2% lidocaine hydrochloride (NCL Lidocaine®, Syncom Formulations, NCL Pharm Chem Ind. Ltd., India) at 4 mg/kg.

The male Adaptor Polyvinyl Chloride Plastisol

The polyvinyl chloride plastisol material used for the rumnen cannulation in this study was a T09 Male Adaptor plumbing device, Conexiones®, S.Tigre Otros Paises, China, www.tigre.com.br (Figure 1).

A seven-centimeter dorsoventral incision were made on the skin of the left flank within the Para lumbar-fossa (Figure 2, A). The incisions were approximately five centimeters ventral to the transverse process of the lumbar vertebrae and eight centimetres caudal to the last rib. This was followed by blunt undermining of the subcutaneous tissue while haemostasis was achieved as may be desired. The muscle layers were incised with a scalpel blade such that a finger was inserted into the opening separating the muscles along the direction of their fibres. The exposed rumen was exteriorised and the dorsal sac was incised up to 7 cm

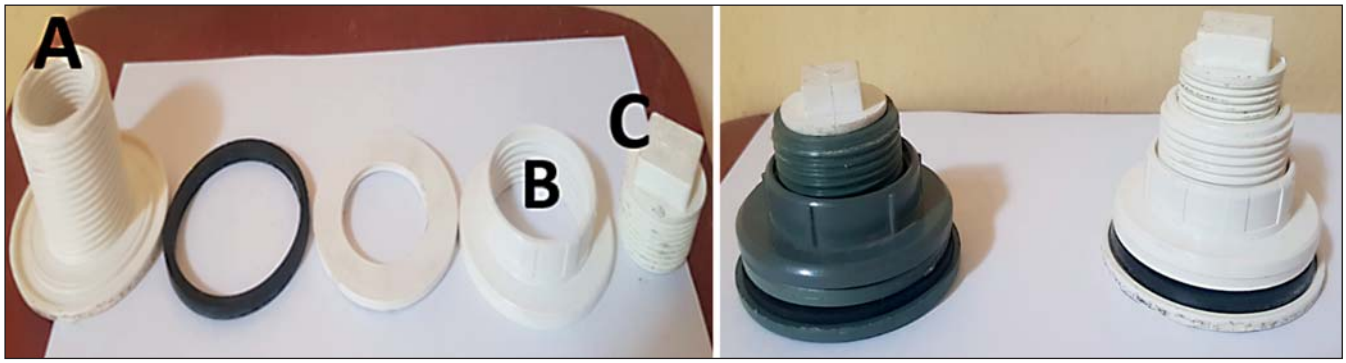

Figure 1 - A polyvinyl chloride plastisol main T09 Male Adaptor plumbing device modified into a rumen cannula and demonstrating its various components for rumen cannulation. **A**: Plastic hose with inner flange. **B**: Plastic outer flange and screw. **C**: Stopper.

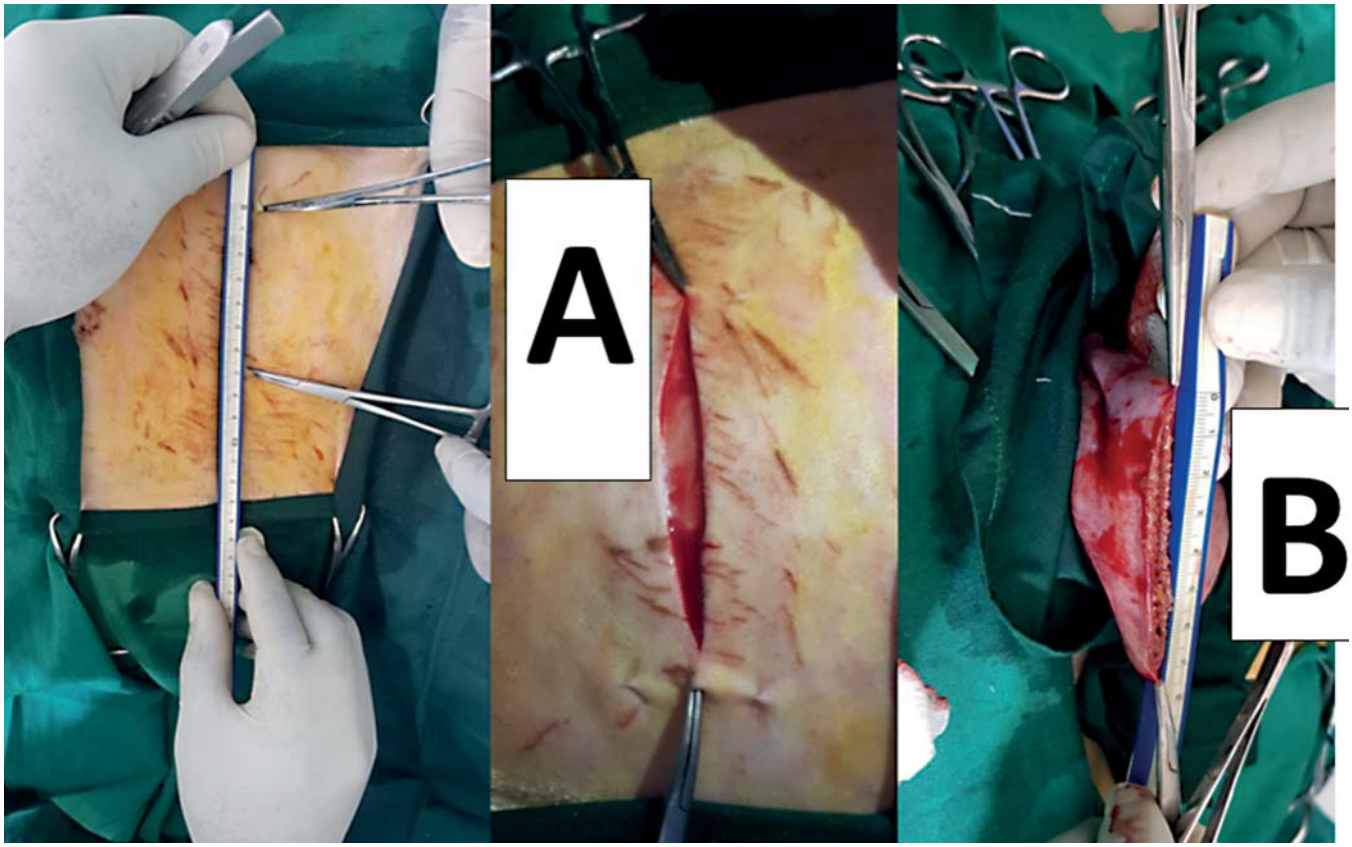

Figure 2 - **A** 7cm primary incision of the skin (left flank) A and the dorsal sac of the rumen **B** shown in stylized incisional approaches for rumen cannulation.

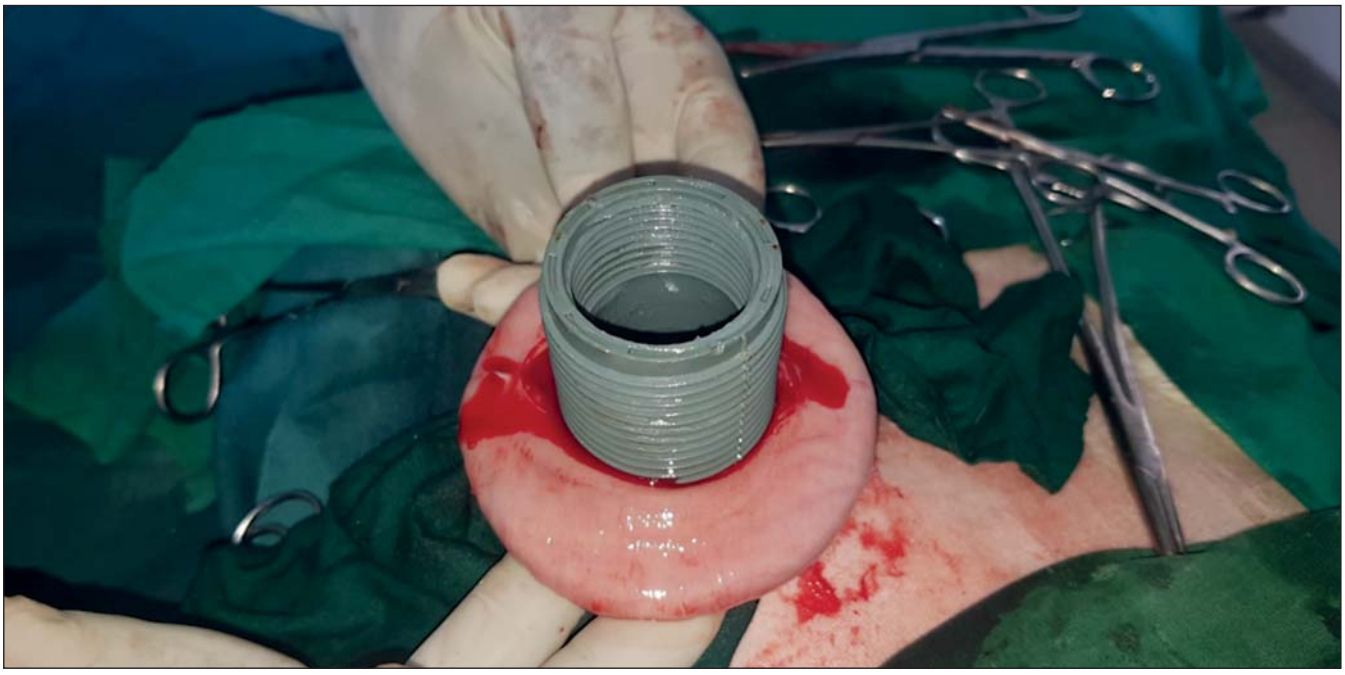

Figure 3 - A rumen cannula and its inner flange were placed in the rumen and the cannula hose exited through a primary rumen incision just before it was held in place with a purse string suture pattern.

over less vascular portion (Figure 2, B). The polyvinyl chloride plastisol rumen cannula along with its rubber inner flange was inserted into the rumen via the incision.

Group A: Primary Incision on the Skin-Muscle and Rumen

This group had only a 7 cm incision known as primary incision on both the skin and the rumen (Figure 2, A and B). For the skin incisions, it included muscles that are beneath the skin into the abdominal cavity. Similarly, an incision on the rumen (primary incision) such that the cannula and its inner flange were inserted and the hose of the cannula was exited through the same primary incision (Figure 3). A combination of Lambert and Cushing suture patterns with polyglycolic acid (PGA) sutures were applied until a fit around the exited cannula was achieved. The cannula was further secured around the rumen with polyamide suture material in a purse string suture pattern (Figure 5, P). A Ford interlocking suture pattern was employed with a polyamide suture material to reduce the 7 cm primary skin incision to a fit around the cannula.

Group B: Primary and Secondary Skin Incisions and Primary Rumen Incision

The animals in this group were subjected to 7 cm primary and secondary incisions on the skin passing through the abdominal muscles into the abdominal cavity (Figure 2, A). However, the rumen dorsal sac had only 7 cm primary incision (Figure 2, B). The cannula was inserted and exited through the same 7cm primary rumen incision such that a fit was achieved following a combination of Lambert and Cushing suture patterns with polyglycolic acid (PGA) sutures were applied. A purse string suture was further placed around the exited cannula through the rumen primary incision to advance securing it. Ford interlocking suture pattern was employed using a number 2 polyamide suture material to close the skin primary incision.

Group C: Primary-Secondary Skin-Muscle Incisions and Primary-Secondary Rumen Incisions

This group had both primary and secondary incisions on both the skin and rumen. The cannula was inserted through a 7 cm primary incision and the plastic hose of the cannula was exited via a 3 cm secondary incision (Figure 4, ʊ) was made parallel to the primary incision in a space of 4 cm (Figure 4, β). Similarly, the secondary incisions on the skin was 3 cm, done

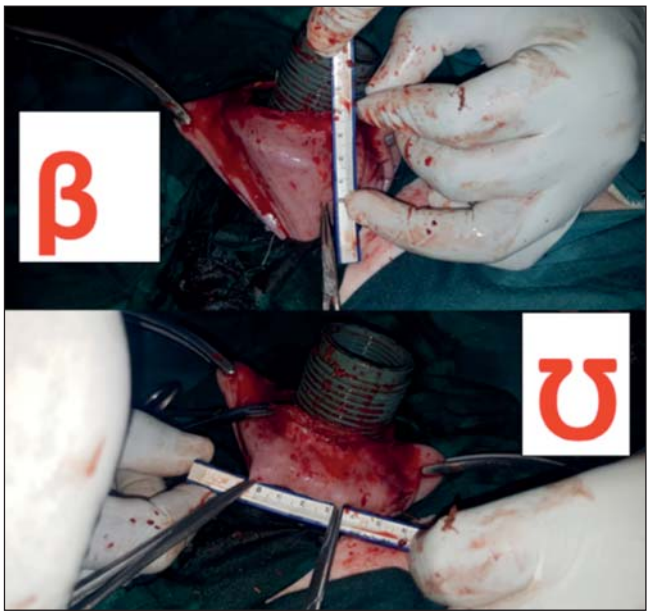

Figure 4 - The creation of a secondary rumen incision demonstrates the 4 cm distance between primary and secondary rumen incisions (as measured with the ruler in **β**) and the 3 cm length of the secondary rumen incision (as measured with the ruler in **ʊ**) on the dorsal sac of the rumen for the various rumen cannulation incisional approaches.

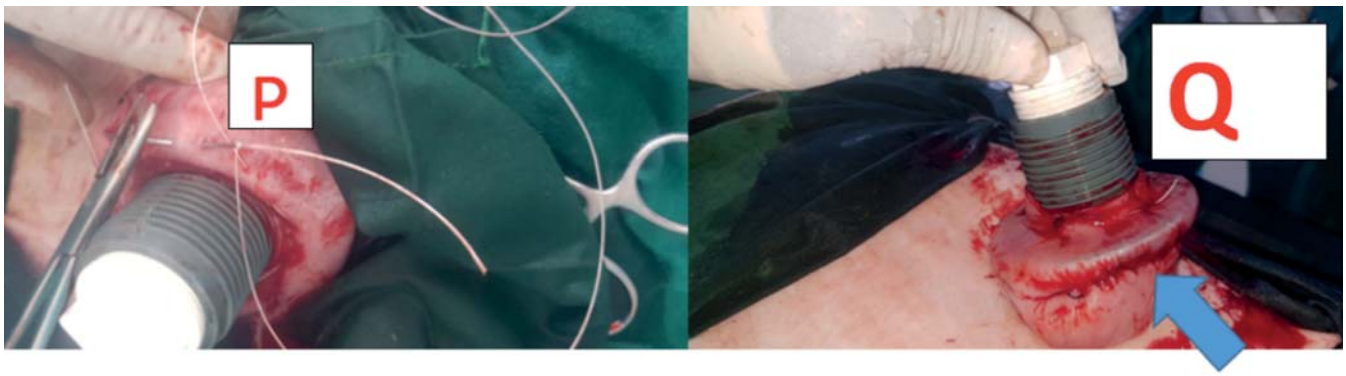

Figure 5 - The purse string suture pattern (**P**) secures the rumen cannula after it was exited through a secondary incision (**Q**) following closure of the primary rumen incision (Blue arrow) in Lambert and Cushing's double suture pattern.

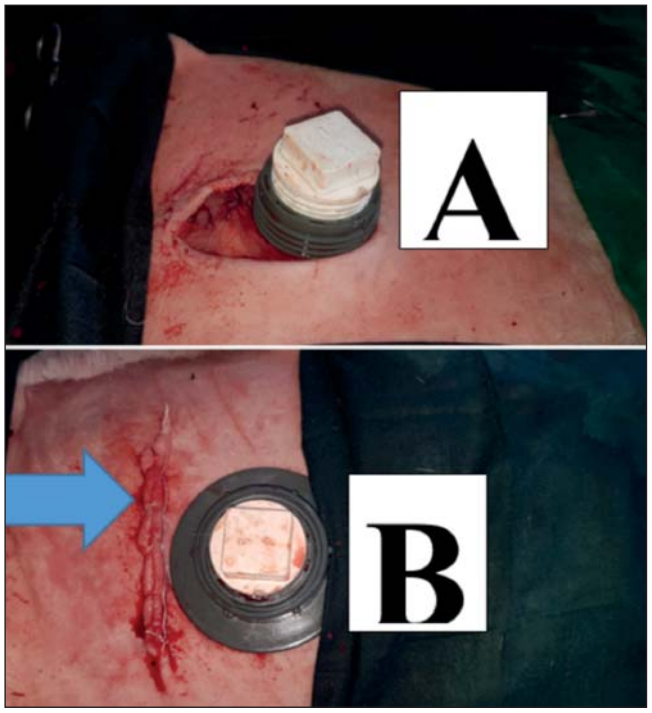

Figure 6 - Rumen cannula hose and stopper (white cap) exited through primary incision (**A**) on the skin just before apposition of the skin with Ford-interlocking suture pattern in a primary-skin-rumen and an exited cannula hose with fastened outer plastic flange through secondary skin incision post Ford-interlocking suture pattern closure (**B**, Blue arrow) following primary and secondary skin incisions in stylized incisional approaches for rumen fistulation.

in the same fashion as that on the rumen (Figure 6, B). The primary incision on the rumen was closed by double layer combinations of Lambert and Cushing suture pattern with a number 2 PGA suture (Figure 5Q, Blue arrow) while a purse string suture pattern was applied on the rumen around the exited cannula hose (Figure 5, P). The secondary incision was a tight fit to the cannula and the wound was allowed to heal through second intension healing (Figure 7, B). The stopper (Figure 6, white cap) is the point that is screwed open when rumen samples are to be collected.

Collection of Blood Samples for Serum Tumour Necrosis Factor-α (TNF-α) Analysis

On the day each rumen cannulation for the groups A, B and C to be carried out and just about to perform the surgery, blood sample (5 ml) were collected via the jugular vein of each ram and established preliminary (Pre) inflammatory biomarker TNF-α profile. Blood sample were collected after the surgery, at 0 h, 4 h, 8 h, 12 h, 24 h, 48 h and 72 h, weeks 1, 2 and 3 post-rumenostomy.

The 5 ml blood samples were dispensed into a plain vacutainer tube and was allowed to clot for two hours at room temperature before centrifugation for 20 minutes at approximately 1000×g. The harvested serum samples from all the animals were emptied into micro-vials and stored at -20 °C until the ELISA, with ELISA kits obtained from Abbkine Scientific® (Abbkine, Inc, China, www.abbkine.com).

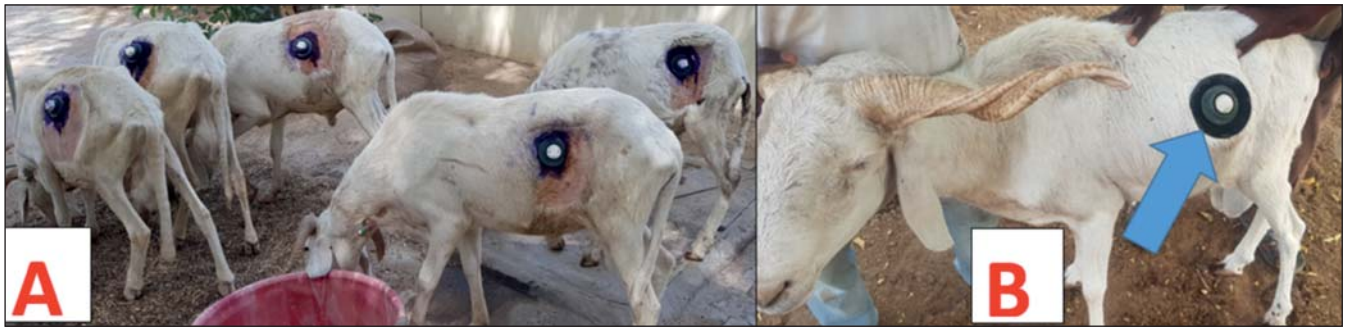

Figure 7 - Post-surgical and post-healing conditions of some of the experimental animals following stylized incisional approaches for rumen cannulation in Yankasa-Balami cross-bred rams.

Post-operative Care

The rams in all the groups were provided analgesia with intramuscular injection of flunixin meglumine (Bremafluxin® Brema pharma GmBH, 34414 Warburg, Germany) 2.2 mg/kg for 3 days and antibiosis with Amoxicillin trihydrate (Amoxinject LA®) 172.2 mg/10kg intramuscularly for 5 days. The wounds were aseptically dressed daily with 0.2% Chlorhexidine gluconate (Savlon®, Vervaading deur, Johnson and Johnson (Pty) Ltd., London) and smeared with povidone-iodine gel (Sawke-10%®, Jawa International Limited, Jawa House Compound, Plot 6, Abimbola Estate, Isolo, Lagos, Nigeria) on the first day and skin gel, blend of herbal oils possessing anti-bacterial, anti-fungal, anti-inflammatory, anti-pruritic, vulnerary & miticidal properties were topically applied daily for ten days serving as flies repellant, (Charmil® Ayurvet Limited Unit No. 101, 103 1st Floor, KM Trade Tower, Plot No-H3, Sector-14, Kaushambi, Ghaziabad- 201010 (UP) Landmark: Radisson Blu Hotel).

Assay Principle

Sheep Tumor necrosis factor (TNF/TNFA/TNFSF2) ELISA employs a two-site sandwich ELISA to quantitate TNF/TNFA/TNFSF2 in samples. An antibody specific for Sheep TNF/TNFA/TNFSF2 was pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF/TNFA/TNFSF2 present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated TNF/TNFA/TNFSF2 detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and colour develops in proportion to the amount of TNF/TNFA/TNFSF2 bound in the initial step. The colour development is stopped and the intensity of the colour is read and measured.

Statistical Analysis

The M±SE periodic serum concentrations of TNF-α for all the groups were established through column statistics using GraphPad Prism version 9.0.0 (121) for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. A One-way Repeated Measures ANOVA followed by Dunnett's multiple comparisons test was performed to compare the respective sampling periods in a group to its respective Pre values. Two Way Repeated Measures ANOVA with Bonferroni posttest was employed in comparing the three groups A, B and C along the different sampling periods. Analyses was considered as significant at $P < 0.05$.

RESULTS

All the animals subjected to the stylized incisional patterns in one stage technique of the rumen cannulation recovered uneventfully. The animals in group A were seen to have returned to feeding practically immediately after the operations were conducted as compared to groups B and C that returned to feeding 12 h following surgery. The quality of feeding was best in group A than that of groups B and C up to 72 h after cannulation. With good hydration status, the animals in all the groups voided urine and defecated soon after cannulation.

Ambulation of the animals within their enclosures was better in group A, which had mild lameness, compared to animals in groups B and C, which had typically demonstrated some degrees of lameness after cannulation. Within the first 24 hours after cannulation, all groups had equivalent reflexes and/or responses to touch.

There was no rumen cannula drop within three weeks of post-cannulation sampling, but signs of infection, such as pus at the cannula site, were observed on the left flank in groups A and B. There was considerable drop at some occasions outside of the sampling periods in groups B and C. The cannula drop was managed using a wider rubber inner and outer flanges, (Figure 7, blue arrow).

The pre-cannulation serum TNF-α concentrations within the respective groups were 36 ± 0.66 for group A, 38 ± 4.8 for B and 38 ± 1.7 pg/mL for group C (Table 1). Generally, the serum TNF-α concentrations post rumen cannulation in groups B and C were above their respective pre rumen cannulation values but that of group A were below the Pre rumen cannulation TNF-α values (Table 1).

The concentrations of the TNF-α in group A was significantly ($p < 0.05$) higher at 72 h compared to the preliminary levels (Pre) of the group. The levels of the serum TNF-α did not significantly ($p > 0.05$) differ at any of the sampling periods in group B but that of group C was significantly ($p < 0.05$) higher (73 ± 2.3 pg/mL) at 48 h compared to the group's Pre value (Table 1).

Following group comparisons, the pre-rumenostomy TNF-α concentrations did not significantly ($p > 0.05$) differ between groups A (36.45 ± 0.66), B (38.1± 4.80) and C (37.97 ± 1.68 pg/mL). Subsequent to rumenostomy at 0h, the concentrations of TNF-α in groups B (72.35 ± 1.18) and C (85.47 ±11.78) were significantly ($p < 0.05$) higher than that of group A (26.4 ± 0.46 pg/mL) (Table 2). This significantly ($p < 0.05$) higher concentration was maintained in group C as against the values of group A at 4 h. Similarly, it was observed that group B and C recorded a significantly ($p < 0.05$) higher concentrations than that of group A, at 8 h.

It was observed that group C recorded significantly ($p < 0.05$) higher concentration than groups A and B, at12 h post rumenostomy. The TNF-α expressions at 24 h post rumenostomy, showed that group C maintained significantly ($p < 0.05$) higher values than groups A and B, which similarly maintained its pace at 48 h in its comparison to group A.

Table 1 - Mean ± SE Serum concentrations of TNF-α pre and post skin-rumen stylized incisions for rumen cannulation with locally improvised polyvinyl chloride plastisol.

GROUPS	PRE	0 h	4 h	8 h	12 h	24 h	48 h	72 h	Week 1	Week 2	Week 3
A	36 ± 0.66[a]	26 ± 0.46	34 ± 6.1	22 ± 6.2	25 ± 1.3	25 ± 0.43	23 ± 5.1	19 ± 1.7[b]	20 ± 2.0	26 ± 2.3	25 ± 1.2
B	38 ± 4.8	72 ± 1.2	58 ± 15	60 ± 14	51 ± 20	62 ± 5.0	52 ± 9.3	25 ± 9.3	46 ± 24	35 ± 5.8	40 ± 8.4
C	38 ± 1.7[a]	85 ± 12	89 ± 11	112 ± 16	90 ± 15	77 ± 7.2	73 ± 2.3[b]	64 ± 4.6	72 ± 7.7	77 ± 11	59 ± 8.8

Values with different superscripts within a row are significantly ($p < 0.05$) different.

This was similarly observed at 72 h and through weeks 1 and 2. There was no significant ($p > 0.05$) difference in the concentrations of serum TNF-α between the three groups at week 3 (Table 2).

The pre-rumenostomy serum TNF-α concentrations within the respective groups were 36 ± 0.66 for group A, 38 ± 4.8 for B and 38 ± 1.7 pg/mL for group C (Table 1). Generally, the serum TNF-α concentrations post rumen cannulation in groups B and C were above their respective pre rumen cannulation values but that of group A were below the Pre rumen cannulation TNF-α concentrations (Table 1). The concentrations of the TNF-α in group A was significantly ($p < 0.05$) higher at 72 h compared to the preliminary levels (Pre) of the group. The levels of the serum TNF-α did not significantly ($p > 0.05$) differ at any of the sampling periods in group B but that of group C was significantly ($p < 0.05$) higher (73 ± 2.3 pg/mL) at 48 h compared to the group's Pre value (Table 1). Following group comparisons, the pre-cannulation TNF-α concentrations did not significantly ($p > 0.05$) differ between groups A (36.45 ± 0.66), B (38.1± 4.80) and C (37.97 ± 1.68 pg/mL). Subsequent to rumen cannulation, at 0h, the concentrations of TNF-α in groups B (72.35 ± 1.18) and C (85.47 ±11.78) were significantly ($p < 0.05$) higher than that of group A (26.4 ± 0.46 pg/mL) (Table 2).

This significantly ($p < 0.05$) higher concentration was maintained in group C as against the values of group A at 4 h. Similarly, it was observed that group B and C recorded a significantly ($p < 0.05$) higher concentrations than that of group A, at 8 h. It was observed that group C recorded significantly ($p < 0.05$) higher concentration than groups A and B, at12 h post cannulation. The TNF-α expressions at 24 h post cannulation, showed that group C maintained significantly ($p < 0.05$) higher values than groups A and B, which similarly maintained its pace at 48 h in its comparison to group A. This was similarly observed at 72 h and through weeks 1 and 2.

There was no significant ($p > 0.05$) difference in the concentrations of serum TNF-α between the three groups at week 3 (Table 2).

Table 2 - Mean ± SE Serum TNF-α concentrations (pg/mL) in the contrast of three stylized skin-rumen incisional techniques for rumen cannulation using locally improvised polyvinyl chloride plastisol.

Sampling Periods	Group A	Group B	Group C
PRE	36.45 ± 0.66	38.1 ± 4.80	37.97 ± 1.68
0h	26.4 ± 0.46^{a}	72.35 ± 1.18^{b}	85.47 ±11.78^{b}
4h	34.3 ± 6.06^{a}	58.43 ± 15.22^{a}	88.53 ± 11.32^{b}
8h	22.27 ± 6.24^{a}	60.4 ± 13.68^{b}	111.83 ± 16.33^{c}
12h	25.15 ± 1.30^{a}	51 ± 19.68^{a}	90.17 ± 14.88^{b}
24h	25.17 ± 0.43^{a}	62.07 ± 5.01^{a}	76.9 ± 7.22^{b}
48h	22.73 ± 5.13^{a}	52.2 ± 9.30	73.17 ± 2.33^{b}
72h	18.8 ± 1.73^{a}	25.17 ±9.27^{a}	64 ± 4.63^{b}
Week 1	20.4 ± 1.96^{a}	46.07 ± 23.58	71.63 ± 7.67^{b}
Week 2	25.8 ± 2.25^{a}	34.97 ± 5.80^{a}	77.43 ± 10.84^{b}
Week 3	25.48 ± 1.17	39.5 ± 8.43	59.45 ± 8.82

Values with different superscripts within a row are significantly ($p < 0.05$) different.

DISCUSSION

The uneventful recovery of all animals treated with the stylized incisional patterns of the one-stage rumen cannulation approach implies that all stylistic incisions were successful for rams that could be intended for rumen cannulation. This discovery will help with high quality rumen content digestibility or nutritional research, alleviation of recurrent bloat, and cannulating rams to serve as transfaunate on farms that may be too far away from abattoir sources of transfaunate or when disease transmission may be avoided[10].

The early return to feeding by the rams in group A over those in groups B and C might be linked to the minimal surgical trauma associated with the technique in group A, where only primary incisions on both the skin and rumen were made. Thus, this was possible because the group B rams were subjected to primary-secondary incisions on the skin and only primary incisions on the rumen, while those in group C were subjected to primary-secondary incisions on both the skin and the rumen. This finding agrees with reports that show minimally invasive procedures are associated with marginal changes in the biomarkers of surgical stress[26, 27]. The shorter duration of surgery in group A could have contributed to the fact that analgesia from the local anaesthesia and the administered NSAID might have greatly contributed in this regard[27].

Although there is a paucity of baseline data for TNF-α values in Yankasa rams in Nigeria, the pre-cannulation values in this study suggest further research to establish the cytokine's baseline. The following comparison within groups showed that group A values were lower than the corresponding pre-cannulation levels but were not significantly ($p > 0.05$) different. Flunixin meglumine's analgesia may be responsible for the reduction in serum TNF-α concentrations. Flunixine meglumine, just like other NSAIDs, is frequently used for the relief of pain and inflammation in large animals. It could modulate the host's response to traumatic injuries, including surgical incisions and/or infections[28] NSAIDs work by inhibiting the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) that mediate the production of prostaglandins (PGs), which play a variety of biological roles in homeostasis and inflammatory responses.The inhibition of PG production by NSAIDs could affect the pathogenesis of the surgical injury response in many ways, such as altering susceptibility to infection by modifying expression of angiotensin-converting enzyme 2 (ACE2) and modulating the immune response to injuries[28]. The findings of this study also agree with the report of Donalisio and Barbero[29] who observed in the dairy cows, as it was in the horse, that ketoprofen and flunixine meglumine, at therapeutic doses, have a similar anti-inflammatory action and also observed that they are able to inhibit the production of CXCL8, IFN-γ, and TNF-α.

The significantly higher serum TNF-α concentrations in group C at 48 h compared to the group's Pre value could be attributed to the fact that the number of incisions in group C's technique was associated with eliciting a greater or enormous inflammatory process much earlier than was observed in the responses of groups A and B during the study's periods. The fact that the TNF-α concentrations of the Yankasa-Balami-cross bred rams in groups B and C were observed to be at their peaks at 0 h and other periods within the first 24 h suggests that rumen cannulation in the ovine species is more tolerated when they are subjected to only primary incisions on the skin and

rumen for both entry and exit of the rumen cannula in a one-stage technique. This finding agrees with the observations by Ahmed and Paraskeva[27] that the single-incision technique is a less invasive alternative to conventional laparoscopic surgery, requiring only one incision concealed within the umbilical folds as opposed to three to five incisions in conventional laparoscopic surgery. The successful use of single-incision laparoscopic surgery for a variety of surgical procedures in minimally invasive surgeries (MIS) has been reported, and it offers several potential benefits over conventional laparoscopic surgery, including reduced pain, shorter recovery time, and improved cosmetics[27]. These desired advantages were similarly observed in the conventional contrast surgery of rumen cannulation, involving only the primary incisions on both the skin and the rumen in bovine species.

Depending on what the outcome may be, the desire of the surgeon or an investigator who may wish to have the rams cannulated to serve as sources of transfaunate or for the relief of recurrent bloat as well as for research purposes, the welfare of the animals must be the principal guide in the choice among the stylized incisional patterns in one-stage cannulation. Because animals in all of the groups were cannulated with the same material, polyvinyl chloride plastisol, the inflammatory reactions may be attributed to the stylistic incisional patterns rather than the material utilized in this study. The fact that group B had lower TNF-α concentrations after primary and secondary skin incisions but only primary rumen incisions than group C, which had primary and secondary rumen incisions, suggests that the multiple incisions could have accounted for the higher serum TNF-α concentrations. This finding is unswerving with the findings of Van der Wal and Jeekel[30], who reported that the degree of injury determines the extent of the inflammatory response to that injury, which in turn determines the extent of adhesion formation and must be taken into account when performing intra-abdominal surgeries. As a result, incisions in the rumen alone might impact greater TNF-α production, and much more so with several incisions on the skin passing through skeletal muscles. This also explains the increase in serum TNF-α concentrations in group C compared to group B 72 h after cannulation[30]. The significantly higher TNF-α surge values at weeks 1 and 2 post-cannulation can be attributed to the fact that these incisional patterns were associated with ruminal fluid leakages, which, in addition to the numerous incisions of the primary and secondary styles on the rumen, skin passing through skeletal muscles in groups B and C, may have added to the burden of infection as previously reported[9, 11]. As the cause of the pus development found in groups B and C during these times, these leakages might have exposed the animal to infection at the surgical sites[23].

CONCLUSION

Overall, the use of locally improvised polyvinyl chloride plastisol for ram cannulation was successful. The application of primary incisions on the skin and rumen alone, as well as the length of incisions, influences the release and numerical concentrations of serum TNF-, particularly when the number of primary incisions or secondary incisions are applied to the skin and/or rumen. Although the incisional patterns used did not preclude cannula drops, initial incisions on both the rumen and skin alone were a preferable choice in terms of the surgical welfare of the animals. The adoption of wider inner and outer rubber flanges avoided cannula drops, making primary incisions on both the rumen and skin alone a superior incisional technique due to the reduced surgical stress on the rams. Thus, above other adopted traditional procedures in the bovine species, the use of primary incisions on both the rumen and skin is by far the cheapest and least stressful incisional strategy for rumen cannulation in the ram.

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