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Original Research Article

Three incision patterns of one-stage rumen cannulation technique and associated haematological and glycaemic responses in Yankasa-Balami cross-bred rams

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ABSTRACT

Despite concerns of leaks, cannula drops, and expensive cannula costs, researches on the nutritional needs of ovine species had led to the use of bovine cannulation techniques to fistulate ovine species. Nine Yankasa-Balami crossbred rams were cannulated with locally improvised polyvinyl chloride plastisol after primary-secondary skin-muscle incisions but only primary rumen incisions; primary-secondary incisions on the skin-muscle of the left flank and the rumen; and a primary incision on the skin-muscle and the rumen, designated as groups A, B, and C, respectively. Glycaemic and haematological responses in the rams were measured at pre-anaesthesia (10 minutes), 0 h, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and weeks 1, 2, 3 and 4. At 10 minutes post-anaesthesia, blood glucose levels in groups A, B, and C were higher than Pre values: 108.33 ± 10.2, 118.33 ± 51.83 and 153.33 ± 46.31, respectively. Moderate dehydration was suggestive of PCV redue to fistula fluid loss while neutrophils, eosinophils, and monocytes were responsible for the increased leucocyte levels. As monocytes contribute to phagocytosis, neutrophils and eosinophils are indicative of surgical stress. Despite the fact that group B's blood glucose levels were much lower than group C's, the results were within the normal species range. Above all, the three incisional patterns are usable for rumen cannulation. Group B's rumen cannulation procedure was more traumatic than groups A and C's, and group A's was ranked second among the most stressful procedures.

Keywords: Cannulation, fistulation, polyvinyl chloride plastisol, sheep, surgery

INTRODUCTION

Rumen cannula is a porthole-like device that allows veterinarians to gain access to a rumen in order to undertake research and analyse contents of the digestive system as well as provides a long-term, readily available source of rumen material, which can be used to transfaunate herd mates that have experienced digestive problems. Rumen cannulation as a fistulation operation between the rumen's dorsal sac and the body surface on the left para-lumbar fossa, is an indication of ruminal content research or treatment of persistent bloating (Saeed *et al.,* 2007). Rumen cannulation entails inserting a flanged rubber cylinder behind the 13th rib in the left para-lumbar fossa of ruminants. To retain the rumen anaerobic digestion, the cylinder is usually fitted with a plastic, rubber, or metal lid (Laflin & Gnad, 2008). The practice of rumen cannulation was originally documented in 1928 by North Dakota Agricultural College's Arthur Fredric Schalk and R S. Amadon. Cannulation in small ruminants is currently in high demand, whether for investigating digestion or collecting ruminal fluids, and this can be accomplished using a variety of cannulae and procedures (Azizi *et al, 2007*). The materials employed could be conventional factory made or locally improvised for sheep (Saidu, 2021; Saidu *et al.*, 2022). The procedure for its implantation could consist of varied techniques in stylized incisional patterns for one stage cannulation (Saidu, 2021). To maintain the ruminal environment clean, facilitate nursing of operated animals, and retain a normal ruminal environment, the device is

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modified to allow sampling of the entire ruminal contents using cannula of identical diameters that were tightly sealed within the ruminal fistula (Brown *et al.,* 1968). Accordingly, researchers will be more inclined to conduct long-term investigations of the ruminal environment in small ruminants.

Rumen cannulation is performed either by one or two stage rumen cannulation methods. The one stage rumen cannulation involves implantation of a silicon cannula in a fistulated procedure between the dorsal sac of the rumen and the body surface of the left paralumbar fossa, for experimental purposes or to treat persistent bloat, is known as one stage cannulation (Saeed *et al.,* 2007). Cannulation is required to keep the fistula open and prevent it from interfering with regular digestion (Komarek, 1981). Cannulas are utilized in a variety of species and parts of the digestive tract to avoid gas and rumen contents leaking during sampling intervals (Stedile *et al.,* 2008). The two-stage rumen cannulation procedure involves using a wooden clamp to exteriorize the rumen segment and 6 mattress sutures to secure the clamp to the skin. The necrotic rumen portion is then removed after one week, leaving a rumen fistula into which a 7.5 cm catheter could be placed. After another week, the cannula can be replaced with a 10 cm cannula. The procedure can take up to 30 minutes on average and the technique necessitates at least one assistant surgeon (Saeed *et al.*, 2007).

Infection, anaemia, inflammation, haemophilia, leukaemia, and the body's response to foreign bodies and chemotherapeutic treatments among others are best alerted in the profiles of haematology (Prasse's, 2011). At the time of trauma and haemorrhage, the body's immune system starts to fight back with the wound as a bed of capillaries and blood elements, stimulate and activate macrophages that release pro-inflammatory mediators (Harris & Gelfand, 1995). Acute and chronic inflammations are the two types of inflammation. Although acute inflammation is characterized by pain due to the release of chemicals, it is a protective attempt by the immune system to remove injurious stimuli and to initiate healing process (Ferrero-Milliani *et al.,* 2007) and a phase characterized by increased flow of plasma leukocytes, particularly granulocytes (i.e. neutrophils and eosinophils) from the blood to the injured tissue (Parakrama *et al.,* 2005). Chronic inflammation is characterised by progressive shift in the type of cells present at the site of the inflammation with a rise in simultaneous destruction and healing of the tissue from the inflammatory process (Parakrama *et al.,* 2005). In a study that explored haematologic tolerance of a rumen fistulation regimen in Uda Rams, Jamilu *et al.* (2022) revealed good physiologic tolerance to rumenotomy and fistulation procedures.

Surgery induces stress responses that include a wide range of hormonal and metabolic changes, with two major systemic consequences to consider: neuroendocrine and haemato-immunological effects (Desborough, 2000). Although the stress response to surgery is a protective mechanism, it has also been proposed that in today's surgery, such reactions may be unnecessary if the stress response is prolonged as disease resistance decreases, morbidity increases, and hospitalization periods lengthen (Kehlet, 1999). The neuroendocrine responses to surgery is characterised by the glycaemic changes during the postoperative periods (Jokela *et al.,* 2007). The typical range of blood-sugar readings in fed sheep is regarded to be 25-50 mg/dL at any time of day. Generally speaking, normal fasting blood sugar levels are less than 100 mg/dL and 5.6 mmol/L. Pre-diabetes is defined as a fasting blood sugar level of 100 to 125 mg/dL (5.6 to 7.0 mmol/L). Impaired fasting glucose is a term used to describe this result (Zhuang *et al.,* 2014).

Castillo and Hernández (2021) observed that rumen fistulation and cannulation are critical tools for the advancement of ruminant research in the investigation of new food sources, notably in the evaluation of productivity, health status, and the higher or lesser potential for greenhouse gas generation. It must be done with a small number of animals and subjected to strong clinical and managerial controls to ensure their welfare at all times. The use of in vitro fermenters does not replace the data provided by live animals, but it can provide extra information regarding the changes that occur in the rumen environment under typical settings, regardless of the species. The most recent advancements are aimed at creating a robust and continuous artificial rumen system that will allow for a better knowledge of rumen dynamics with as few animals as feasible. Despite this, there are still segments of the population who are hesitant to perform fistulation and cannulation in ruminants, partly due to a lack of understanding of the technique, which must be performed with a minimum number of animals while taking into account their welfare and health status, according to legislative requirements. This study aims at revealing the neuroendocrine responses to rumen cannulation with the specific objective of evaluating the haematological and glycemic changes that could be associated with three incisional approaches of one-stage rumen cannulation in Yankasa-Balami cross-bred rams in order to find a less stressful technique. The improvised cannula is cheap, applicable, readily available as polyvinyl chloride plastisol (PVC) that withstands mechanical damage as well as ruminal degradation while maintaining normal ruminal environment. The purpose of the study was to evaluate the effect of a locally improvised polyvinyl chloride plastisol (PVC) material in performing rumen cannulation in different

primary and secondary incisional approaches on the skin and rumen that may be employed in the provision of leak-proof rumen fistulae based on periodic haematologicalogical and glycaemic indices in Yankasa-Balami cross-bred rams.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

Nine Yankasa and Balami crossbred adult sheep were purchased from the Maiduguri Livestock Market, Borno State. The rams weighed between 35 - 45 kg and were designated for rumen nutritional studies.

The animals were acclimatized for three weeks prior to surgery in a fold at the Department of Veterinary Surgery and Radiology, University of Maiduguri. Blood samples were collected for haematology and parasitology purposes.The animals were dewormed with ivermectin (Bremamectin®, Brema pharma GmBH, 34414 Warburg, Germany) at a dose of 200 g/kg S/C. Each ram received a preventive dose of oxytetracycline (Kepro Oxytet® 20 percent LA inj, Kepro B.V. Magdenburgtraat 17, 7421 ZA Deventer-Holland) at a rate of 20 mg/kg IMagainst bacterial infections and was administered only once. Groundnut husk, beans shaft, and maize offal were provided as feed, along with access to clean water on an *ad libitum* basis.

ETHICAL CLEARANCE

The Animal Utilization Protocol approval letter for the study was acquired from Faculty of Veterinary Medicine Animal Use and Ethics Committee, University of Maiduguri dated February 26th, 2020, with a reference number FVM/UNIMAID/AUEC/2020/002.

EXPERIMENTAL DESIGN

The nine animals were grouped into groups A, B and C. Each group had 3 animals, and were numbered A1, A2, A3; B1, B2, B3 and C1, C2, C3 for groups A, B and C respectively, with the different rumen incisional patterns for the cannulation as below: Group A; n = 3: primary-secondary skin-muscle incisions but only primary rumen incision

Group B; n = 3: primary-secondary incisions on the skin of the left flank and the rumen

Group C; n = 3: primary incision on the skin-muscle and rumen

ONE STAGE RUMEN CANNULATION TECHNIQUES AND SAMPLING PERIODS

Prior to cannulation, the animals were fasted 6 and 12 hours of water and feed, respectively. Blood samples were taken in an EDTA bottle for haematology and an immediate glucose assay to determine pre-anaesthesia values. Following anaesthesia, samples were obtained as to establish post-anaesthesia values. Following the respective stylized rumen incisional patterns and cannulation, sample times were 0 h, 4 h, 8 h, 12 h, 16h, 20h, 24 h, 48 h and 72 h, 96 h, 120 h, 144 h, and also at weeks 1, 2, 3 and 4.

PREOPERATIVE PREPARATION

The experimental rams in all the groups were sedated by receiving 0.1 mg/kg of Xylazine hydrochloride (XYL-M2® VMD nv/sa-Hoge Mauw 900-B-2370 Arendok-Belgium) intravenously. The rams were then positioned in right lateral recumbency, and the left paralumbar fossa was clipped and aseptically prepared by scrubbing with 0.2 percent Chlorhexidine gluconate (Savlon®, Johnson and Johnson Ltd, London) and smearing with povidone iodine (Sawke-10 percent®, Jawa International Limited, 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.

GROUP A: PRIMARY-SECONDARY SKIN-MUSCLE INCISIONS AND PRIMARY RUMEN INCISION

A 7 cm skin incision was made through the abdominal muscles and into the abdominal cavity in this group of animals. The rumen was located, and a 7 cm primary incision was made on its dorsal sac. To exit the plastic hose of the cannula after insertion into the rumen, it was brought out through a 3 cm secondary incision (Fig I) performed parallel to the initial incision in a 4 cm away from the primary rumen incision. The internal hose diameter was screwed out of the smaller 3 cm incision and secured with a purse string suture pattern using nylon size 2 USP. The primary incision was closed with polyglycolic acid suture material (PGA) size 2 USP in a combination of Lembert and Cushing suture patterns, while the secondary incision was fitted with the cannula and left to heal after the edges were apposed with the suture (Fig. I).

GROUP B: PRIMARY-SECONDARY SKIN-MUSCLE INCISIONS AND PRIMARY-SECONDARY RUMEN INCISIONS

This group received a primary incision through the skin and abdominal muscles, as well as a primary incision on the rumen, to insert the cannula. To exit the cannula, a secondary incision (3cm) was made lateral to the first (primary) incision (7cm) on the rumen. The internal hose diameter was exited by screwing it out through the smaller secondary incision. A purse string suture was used to secure the cannula in the secondary incision, which was adjusted to fit the cannula. To close the primary incision on the rumen, a double layer of Lembert and Cushing suture patterns with a number 2 USP PGA suture was deployed while a Ford interlocking suture pattern with nylon suture material were utilized to close the primary incision on the skin (Fig. II).

GROUP C: PRIMARY INCISION ON THE SKIN-MUSCLE AND RUMEN

This group had a single straight 7 cm incision into both skin and rumen, termed primary incision. Muscles beneath the skin into the abdominal cavity were included in the incisions. The cannula and its inner flange were entered and exited through the incision. The cannula hose was exited through the rumen and a nylon purse string sutures were used to stabilize the cannula in the ruminal neck. The cannula tube was then exited through the only primary incision on the skin and the outer flange was attached.

In all the surgical groups, primary or secondary incisions on the skin as the case may be, were closed with nylon suture size 2 USP using a blanket or Ford interlocking suture pattern.

POST-OPERATIVE CARE

All the rams were each given intramuscular injection of flunixin meglumine (Bremafluxin® Bremapharma Warburg, Germany), an analgesic, at 2.2 mg/kg and a course of antibacterial treatment with amoxicillin trihydrate (Amoxinject LA®) 17.22 mg/kg i.m x 3/7. Daily, the wounds were dressed by cleaning them with 0.2% chlorhexidine gluconate (Savlon® Johnson and Johnson Ltd, London) and followed by topical application of 10% povidone-iodine gel (Sawke® Jawa International limited, Lagos, Nigeria) and a wound healing ointment, Charmil ointment, (Charmil Plus®, AYURVET LIMITED, Kaushambi, Ghaziabad- 201010 (UP). The rams returned to feeding troughs almost immediately after surgery (Fig. III).

HAEMATOLOGY

Microhematocrit method as described by Coles (1986) was used to determine the packed cell volume. Red blood cell and white blood cell counts were determined using haemocytometer. Spectrophotometer was used to estimate the haemoglobin concentrations. Blood smear were made and stain with Giemsa staining method (Schalm *et al.,* 1975), viewed under oil emersion objective and leukocyte were counted using the battlement method.

BLOOD GLUCOSE TEST

A glucometer (OneTouch® Ultra® 2 meter, Johnson & Johnson, USA) and glucometer strip were used to determine the blood glucose levels of the rams at the experimental design's sampling periods.

DATA ANALYSIS

The M±SE periodic haematological and glycaemic profiles 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 Two-Way Repeated Measures ANOVA followed by Bonferroni post-test (Multiple comparisons post-test) was employed to compare the respective variable values sampled at different periods between the three groups. Statistical significance was defined at $P < 0.05$.

RESULTS

The haematological profiles of Yankasa-Balami rams subjected to different incisional technique of rumen cannulation showed that the period before anaesthesia and surgery termed Pre, the values were 28.67 ± 2.91, 29.67 ± 1.45 and 27.67 ± 1.33 for PCV in groups A, B and C respectively. These values did not significantly ($p > 0.05$) differ from one another (Table I). The values for other haematological variables at this same time points were total red blood cell counts (TRBC), total white blood cell counts (TWBC), and the differential leucocyte variables that include monocytes, lymphocytes, neutrophils, eosinophils and basophils, were all within the species normal range.

The Packed cell volume [PCV (%)] levels 10 minutes' post anaesthesia and just before the rumen cannulation termed post had group C (29 ± 0.23) significantly ($P < 0.05$) lower than the values in group A (33.5 ± 0.29). This phenomenon turned out that group A (25.50 ± 0.29) became lower afterwards than group C (28 ± 0.58) at 24 h. Similarly, at week 3, the PCV value of group A (24.4 ± 0.06) was significantly lower than group B (26.5 ± 0.29).

The haemoglobin concentration [HGB (g/dl)] was observed at 16 h, 24 h and 120 h to have significantly dropped in concentration in group A (8 ± 0.76, 8.67 ± 0.17 and 7.2 ± 0.76) than that of group C (11.55 ± 0.84,10.50 ± 0 and 9 ± 0.58), respectively. Similarly, group B (6.25 ± 0.38) had lower HGB (g/dl) concentration than group C at 120 h. The group A had lower concentration than group B at Wk 1.

The total red blood cell counts [TRBC X 10^{12}/L] at 4h and 16 h showed that group B (11.06 ± 1.19 and 8.96 ± 0.73) had significantly ($p < 0.05$) lower levels than group C (12.39 ± 1.2 and 12.1 ± 0.9). At the periods of 20 h and Wk 2 the TRBC X 10^{12}/L were lower than group C while at 96 h, group A was significantly ($p < 0.05$) lower than group B.

The total white blood cell counts [TWBC X 10^{9}/L] showed group B values (7.67 ± 0.67) significantly ($p < 0.05$) lower than group C (9 ± 0.5) at 72 h while group A was significantly ($p < 0.05$) lower than that of B at 96 h.

The neutrophil [NEUTRO X 10^{9}/L] values of group C (344.67 ± 30.69) was significantly ($p < 0.05$) lower than the values of group A (505.67 ± 15.88) at 72 h while group B had significantly ($p < 0.05$) lower values than group A at 96 h.

Table I: Mean ± SEM Haematological profiles at pre, post-anaesthesia and different periods following three incisional Patterns of one stage rumen cannulation in Yankasa-Balami cross-breed Rams

Sampling Time	Haematology Variables	GROUP A	GROUP B	GROUP C
Pre	PCV (%)	28.67 ± 2.91	29.67 ± 1.45	27.67 ± 1.33
	HGB (g/dl)	9.5 ± 1.04[a]	11.83 ± 0.73[b]	10.8 ± 0.99
	TRBC X 10^{12}/L	10.33 ± 2.18	11.83 ± 0.82	12.07 ± 0.89
	TWBC X 10^9/L	9.5 ± 1.04	10.83 ± 0.17	9.33 ± 0.73
	NEUTRO X 10^9/L	318.67 ± 80.67	320.67 ± 19.92	262.33 ± 47.03
	LYMPHO X 10^9/L	581.67 ±170.79	535.67 ± 31.18	714.33 ± 52.4
	MONO X 10^9/L	25.67 ± 8.69	32.67 ± 2.03	38.33 ± 6.89
	EOSINO X 10^9/L	33 ± 10.79	42 ± 3.46	70.33 ± 15.45
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
Post-Anaesthesia (10 Mins)	PCV (%)	33.5 ± 0.29[c]	27.67 ± 2.03	29 ± 0.23[d]
	HGB (g/dl)	10.47 ± 0.03	10 ± 1.15	12 ± 0.29
	TRBC X 10^{12}/L	12.8 ± 0.06	10.9 ± 1.24	13.25 ± 0.16
	TWBC X 10^9/L	10.77 ± 0.15	9.83 ± 0.44	9.5 ± 0.29
	NEUTRO X 10^9/L	218.33 ± 10.4	320.33 ± 30.05	251 ± 28.88
	LYMPHO X 10^9/L	647.33 ± 22.23	638 ± 78.89	697.67 ± 45.63
	MONO X 10^9/L	18.67 ± 4.91	25 ± 7.81	37 ± 4.04
	EOSINO X 10^9/L	46.67 ± 6.06	46.67 ± 5.21	58.33 ± 4.91
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
0 h post-cannulation	PCV (%)	27.67 ±1.45	28.67 ± 1.45	27.5 ± 1.44
	HGB (g/dl)	9.83 ± 0.93	11 ± 0.58	10.25 ± 0.72
	TRBC X 10^{12}/L	10.08 ± 1.67	11.68 ± 0.87	11.74 ± 0.99
	TWBC X 10^9/L	9.33 ± 0.67	9 ± 1.15	9 ± 0.29
	NEUTRO X 10^9/L	296 ± 72.45	384 ± 24.43	322.33 ± 3.76
	LYMPHO X 10^9/L	723.33 ± 14.53	680.33 ± 119.04	684 ± 12.42
	MONO X 10^9/L	18.33 ±4.41[e]	39.33 ± 2.03	28 ± 4.04
	EOSINO X 10^9/L	42.67 ± 3.71	45.67 ± 6.12	79 ± 15.31
	BASO X 10^9/L	0 ±0	0 ± 0	0 ± 0
4 h	PCV (%)	28.33 ± 1.45	26.33 ± 2.03	27.33 ± 1.76
	HGB (g/dl)	10 ± 0.58	9.83 ± 1.17	10.83 ± 1.01
	TRBC X 10^{12}/L	9.71 ± 1.91	11.06 ± 1.19[g]	12.39 ± 1.2[h]
	TWBC X 10^9/L	9.67 ±	9.5 ± 0.29	9.33 ± 0.44
	NEUTRO X 10^9/L	329.67 ± 34.35	351.67 ± 48.07	311.67 ± 19.78
	LYMPHO X 10^9/L	615.67 ± 26.31	665 ± 60.38	667.33 ± 31.35
	MONO X 10^9/L	30.33 ± 9.67	18 ± 3.51	25 ± 2.65
	EOSINO X 10^9/L	61.33 ± 14.44	54.67 ± 12.72	72.67 ± 16.59
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
8 h	PCV (%)	26.33 ± 0.88	25 ± 0.58	27.33 ± 0.88
	HGB (g/dl)	9 ± 0.58	8.75 ± 0.14	11.2 ± 0.76
	TRBC X 10^{12}/L	7.39 ± 1.41	8.66 ± 0.03	12.07 ± 0.99
	TWBC X 10^9/L	8.67 ± 0.83	7.75 ± 0.72	9.5 ± 0.29
	NEUTRO X 10^9/L	395.33 ± 12.99	379.33 ± 62.04	303 ± 26.85
	LYMPHO X 10^9/L	695.33 ± 95.54	835 ± 48.42	663 ± 16.86
	MONO X 10^9/L	33 ± 12	26.33 ± 2.6	25 ± 10.02
	EOSINO X 10^9/L	54.33 ± 23.62	73 ± 10.44	70.33 ± 4.33
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
12 h	PCV (%)	26.67 ± 2.6 7	25.67 ± 2.6	31.67 ± 3.67
	HGB (g/dl)	8.67 ±1.67	9 ± 0.58	12.17 ± 1.48
	TRBC X 10^{12}/L	7.55 ±2.86	7.18 ± 1.49	12.87 ± 1.19
	TWBC X 10^9/L	8.33 ± 1.45	8.83 ± 0.44	9.83 ± 0.44
	NEUTRO X 10^9/L	428.33 ± 94.78	441 ± 68.51	302.67 ± 27.72
	LYMPHO X 10^9/L	751.67 ± 136.95	650 ± 14.15	632.67 ± 26.3
	MONO X 10^9/L	36.67 ± 6.89	23 ± 1.15	16.67 ± 2.85
	EOSINO X 10^9/L	67 ± 23.86	28.67 ± 2.03	69 ± 15.72
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
16 h	PCV (%)	25.67 ± 1.2	26.67 ± 0.88	29 ± 0.58
	HGB (g/dl)	8 ± 0.76[i]	9.17 ± 0.22	11.55 ± 0.84[j]
	TRBC X 10^{12}/L	6.36 ± 1.69	8.96 ± 0.73[k]	12.1 ± 0.9[l]
	TWBC X 10^9/L	7.83 ± 0.88	8 ± 0.66	10 ± 0.29
	NEUTRO X 10^9/L	434.67 ± 51.72	413 ± 30.44	281.33 ± 25.41
	LYMPHO X 10^9/L	789 ± 81.91	780 ± 93.52	630.33 ± 6.64
	MONO X 10^9/L	31.67 ± 12.13	29 ± 1.73	25 ± 2.31
	EOSINO X 10^9/L	53.67 ± 10.93	59 ± 6.43	65 ± 1.15
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
20 h	PCV (%)	31 ± 0.58	26 ± 2	29.25 ± 0.14
	HGB (g/dl)	11.17 ± 0.44	8.83 ± 1.3	11.93 ± 0.2
	TRBC X 10^{12}/L	10.75 ±0.26	7.09 ± 1.69	12.5 ± 0.23[n]
	TWBC X 10^9/L	10.5 ± 0.29	9 ± 0.29	10.12 ± 0.07
	NEUTRO X 10^9/L	292 ± 32.92	363.33 ± 26.86	269.33 ± 6.06
	LYMPHO X 10^9/L	594.67 ± 8.37	664 ± 16.04	627 ± 1.73
	MONO X 10^9/L	33.33 ± 3.76	33.33 ± 6.69	26 ± 0.58
	EOSINO X 10^9/L	33.00 ± 1.73[o]	52.67 ± 16.76	65.33 ± 0.33[p]
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
24 h	PCV (%)	25.50 ± 0.29[q]	27 ± 2.89	28 ± 0.58[r]
	HGB (g/dl)	8.67 ± 0.17[s]	9.33 ± 1.45	10.50 ± 0[t]
	TRBC X 10^{12}/L	6.73 ± 0.42	8.18 ± 1.77	11.9 ± 0.78
	TWBC X 10^9/L	9.25 ± 0.43	9.17 ± 0.33	9.83 ± 0.17
	NEUTRO X 10^9/L	448.67 ± 0.88	400.33 ± 52	343 ± 23.07
	LYMPHO X 10^9/L	577.33 ± 45.95	601.67 ± 38.08	589.33 ± 10.67
	MONO X 10^9/L	27 ± 1.73	33.33 ± 7.54	20.33 ± 0.33

	EOSINO X 10^9/L	33 ± 7.81	58.67 ± 19.2	71.33 ± 10.73
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
48 h	PCV (%)	28 ± 1.15	24 ± 2.31	27.25 ± 0.14
	HGB (g/dl)	9.83 ± 0.44	8.25 ± 0.72	10.5 ± 0
	TRBC X 10^{12}/L	8.89 ± 1.06	7.61 ± 1.58	12.12 ± 0.23
	TWBC X 10^9/L	10.17 ± 0.73	9.25 ± 0.14	10 ± 0
	NEUTRO X 10^9/L	423.67 ± 14.17	401.67 ± 56.3	312.67 ± 7.22
	LYMPHO X 10^9/L	501.33 ± 39.93	594.33 ± 21.94	605 ± 2.89
	MONO X 10^9/L	22 ± 2.89	21.67 ± 6.06	20 ± 0
	EOSINO X 10^9/L	24.33 ± 4.33^{u}	64.33 ± 11.55	67.67 ± 1.45^{v}
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
72 h	PCV (%)	22.5 ± 1.44	22.33 ± 1.2	21.67 ± 0.88
	HGB (g/dl)	7.5 ± 0.29	7.23 ± 0.96	7.57 ± 0.74
	TRBC X 10^{12}/L	5.58 ± 0.33	4.21 ± 1.01	7.08 ± 0.9
	TWBC X 10^9/L	9 ± 0	7.67 ± 0.67^{w}	9 ± 0.5^{x}
	NEUTRO X 10^9/L	505.67 ± 15.88^{y}	481.33 ± 71.74	344.67 ± 30.69^{z}
	LYMPHO X 10^9/L	544.33 ± 25.69	743.33 ± 57.45	687 ± 51.4
	MONO X 10^9/L	22 ± 0	39.67 ± 3.33	26.33 ± 4.48
	EOSINO X 10^9/L	39 ± 9.81	82 ± 12.9	59.67 ± 11.2
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
96 h	PCV (%)	22 ± 0.58	23 ± 1.15	21.67 ± 0.88
	HGB (g/dl)	7.25 ± 0.14	6.5 ± 0.29	7.9 ± 0.67
	TRBC X 10^{12}/L	5.18 ± 0.3$^{\alpha}$	6.7 ± 0.4$^{\beta}$	6.81 ± 0.89
	TWBC X 10^9/L	7.25 ± 0.43$^{\mu}$	8.75 0.43$^{\upsilon}$	9 ± 0.58
	NEUTRO X 10^9/L	476 ± 0.58$^{\eta}$	400.67 ± 6.94$^{\Sigma}$	363 ± 38.42
	LYMPHO X 10^9/L	796.67 ± 87.92	633.33 ± 44.8	676.33 ± 46.62
	MONO X 10^9/L	34.33 ± 2.03	23 ± 1.15	25.67 ± 9.39
	EOSINO X 10^9/L	82.67 ± 3.18	91.67 ± 4.63	55.67 ± 5.67
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
120 h	PCV (%)	22.67 ± 1.76	23 ± 0.58	23.33 ± 1.33
	HGB (g/dl)	7.2 ± 0.76^{ab}	6.25 ± 0.38^{ab}	9 ± 0.58^{c}
	TRBC X 10^{12}/L	5.75 ± 1.21	5.64 ± 0.89	8.23 ± 0.14
	TWBC X 10^9/L	7.67 ± 1.01	8.04 ± 0.53	9.67 ± 0.33
	NEUTRO X 10^9/L	436.33 ± 41.51	416.33 ± 13.45	336.33 ± 24.36
	LYMPHO X 10^9/L	827.33 ± 121.44	732.67 ± 84.46	620.67 ± 53.55
	MONO X 10^9/L	22.33 ± 5.81	25.33 ± 1.86	24 ± 3.06
	EOSINO X 10^9/L	65 ± 24.85	95.67 ± 2.6	56 ± 14
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
144 h	PCV (%)	20.5 ± 1.44	22.67 ± 0.44	23.67 ± 0.33
	HGB (g/dl)	6.4 ± 0.92	6.87 ± 0.59	8.33 ± 0.88
	TRBC X 10^{12}/L	5.87 ± 1.63	6.54 ± 0.74	7.92 ± 0.27
	TWBC X 10^9/L	8 ± 0.58	8.83 ± 0.62	9.67 ± 0.17
	NEUTRO X 10^9/L	343.33 ± 39.35	400.67 ± 11.32	344.33 ± 31.31
	LYMPHO X 10^9/L	844.67 ± 46.88	632.33 ± 72.31	594 ± 40.81
	MONO X 10^9/L	12.67 ± 0.88	29.33 ± 5.36	21 ± 6.08
	EOSINO X 10^9/L	63.33 ± 4.33^{de}	89.33 ± 4.7^{f}	75.67 ± 6.44^{de}
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
WK 1	PCV (%)	22 ± 1.73	23.73 ± 1.4	21.67 ± 1.2
	HGB (g/dl)	5.75 ± 1.01$^{\alpha}$	8.07 ± 0.69$^{\beta}$	7 ± 0.58
	TRBC X 10^{12}/L	6.23 ± 2.58	8.4 ± 1.02	5.9 ± 0.97
	TWBC X 10^9/L	7.25 ± 1.01	8.87 ± 0.35	8.5 ± 1.04
	NEUTRO X 10^9/L	490.67 ± 29.41	475.33 ± 75.83	406.33 ± 27.29
	LYMPHO X 10^9/L	844.67 ± 162.05	641.33 ± 42.68	674 ± 93.79
	MONO X 10^9/L	34.33 ± 0 .88	47.33 ± 24.67	32 ± 3
	EOSINO X 10^9/L	65.67 ± 13.69	73 ± 8.96	99.33 ± 22.66
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
WK 2	PCV (%)	24.27 ± 0.43	22.63 ± 0.95	22.67 ± 0.33
	HGB (g/dl)	7.43 ± 0.32	8.33 ± 0.2	8.73 ± 0.64
	TRBC X 10^{12}/L	9.31± 0.59$^{\mu}$	7.4 ± 1.15	6.86 ± 0.77$^{\pounds}$
	TWBC X 10^9/L	8.57 ± 0.26	8.2 ± 0.4	9 ± 0.29
	NEUTRO X 10^9/L	453 ± 5.2	487 ± 11.27	338.33 ± 28.11
	LYMPHO X 10^9/L	635.33 ± 27.14	759.67 ± 67.63	666.67 ± 4.33
	MONO X 10^9/L	33.67 ± 0.33	33.67 ± 11.57	23 ± 12
	EOSINO X 10^9/L	48 ± 2.31	59 ± 3.46	86 ± 17.93
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
WK 3	PCV (%)	24.4 ± 0.06$^{\sigma}$	26.5 ± 0.29$^{\epsilon}$	26 ± 1
	HGB (g/dl)	7.97 ± 0.32	9.3 ± 0.17	10.33 ± 0.17
	TRBC X 10^{12}/L	8.91 ± 0.22	8.7 ± 0.95	7.9 ± 0.98
	TWBC X 10^9/L	8.92 ± 0.19	9.5 ± 0.29	9.5 ± 0.29
	NEUTRO X 10^9/L	434.33 ± 10.68	397.67 ± 36.68	305 ± 17.21
	LYMPHO X 10^9/L	619.33 ± 8.37	582 ± 48.23	653 ± 7.37
	MONO X 10^9/L	25 ± 5.2	16 ± 3.46	21.33 ± 6.64
	EOSINO X 10^9/L	45.67 ± 1.45	59 ± 17.04	75.33 ± 29.08
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0
WK 4	PCV (%)	23.67 ± 0.88	24.33 ± 1.45	24.67 ± 1.2
	HGB (g/dl)	8 ± 0.5	7.83 ± 0.88	9.67 ± 0.6
	TRBC X 10^{12}/L	7.37 ± 1.2	6.1 ± 1.03	7.09 ± 0.94
	TWBC X 10^9/L	8.83 ± 0.44	9 ± 0.58	9.33 ± 0.17
	NEUTRO X 10^9/L	402.33 ± 13.97	336 ± 21.7	318 ± 13
	LYMPHO X 10^9/L	654.67 ± 58.92	676 ± 43.31	661 ± 15.31
	MONO X 10^9/L	15 ± 3.06	14.67 ± 2.73	25.33 ± 7.17
	EOSINO X 10^9/L	66 ± 26.31	94 ± 9.85	85.67 ± 25.98
	BASO X 10^9/L	0 ± 0	0 ± 0	0 ± 0

Table II: Mean ± SE Blood Glycaemic levels at Pre, Post-anaesthesia and different periods following Three Incisional Patterns of One Stage Rumen Cannulation in Yankasa-Balami Cross-Bred Rams

	GROUP A	GROUP B	GROUP C
PRE	48.17 ± 5.57	54.5 ± 25.29	72.67 ± 25.86
Post anaesthesia (10 Mins)	108.33 ± 10.2	118.33 ± 51.83	153.33 ± 46.31
0 h	92 ± 16.65	121 ± 55.05	160 ± 56.86
4 h	66 ± 13.23	103 ± 50.5	51.67 ± 6.17
8 h	39.33 ± 4.2 ±6	49.33 ± 6.98	51 ± 6.93
12 h	41.67 ±1.2	48.67 ± 2.67	53.67 ± 2.96
16 h	44.67 ± 4.81	42.67 ± 0.88	54.67 ± 2.85
20 h	37.67 ± 6.74	41.33 ± 1.33[a]	55.33 ± 2.03[b]
24 h	55.67 ± 14.17	40.33 ± 1.2	53.33 ± 4.41
48 h	55.67 ± 14.24	43.67 ± 4.18	54.67 ± 4.63
72 h	42.67 ± 4.67	46.33 ± 6.94	47 ± 2.08
96 h	44 ± 3.79	49 ± 6.66	48 ± 2.89
120 h	46.67 ± 1.67	52 ± 1.15	49.33 ± 3.38
144 h	47.33 ± 3.38	47.67 ± 1.45	48.67 ± 3.53
Wk 1	44.67 ± 1.2	53.67 ± 3.76	51 ± 3.79
Wk 2	49.67 ± 5.17	53.33 ± 3.33	54.33 ± 2.96
Wk 3	54 ± 5.86	59 ± 10.5	58.33 ± 1.67
Wk 4	60 ± 6.56	59.33 ± 8.35	57 ± 3.79

There was lower monocyte infiltration in group A (18.33 ±4.41) than group B (39.33 ± 2.03) immediately after rumen fistulation termed 0 h.

Eosinophil [EOSINO X 10^9/L] values at 20 h and 48 h were significantly ($p < 0.05$) lower in group A (33.00 ± 1.73 and 24.33 ± 4.33) than group C (65.33 ± 0.33 and 67.67 ± 1.45). At the period of 144 h, groups B and C were significantly ($p < 0.05$) lower than A and B, respectively, (Table I).

The glycaemic profile of the animals at Pre, revealed values that were within the species normal range of 48.17 ± 5.57, 54.5 ± 25.29 and 72.67 ± 25.86 for groups A, B and C, respectively. Following premedication, the values for each of the groups doubled as 108.33 ± 10.2, 118.33 ± 51.83 and 153.33 ± 46.31 for A, B and C respectively, but were not significantly ($p > 0.05$) different (Table II). Although, not significantly ($p > 0.05$) different, there was decreased glycaemic concentration in group A at 0 h, as against observed increased concentrations in groups B and C at 0 h. These values dropped below 100mg/dl in group A (66 ± 13.23) and C (51.67 ± 6.17) while that of group B (103 ± 50.5) remained above the stated values at 4 h, until at 8 h post rumen fistulation that it dropped below 100mg/dl similar to the values in groups A and C. At 20 h post rumen cannulation, the blood glucose levels were significantly ($p < 0.05$) lower in group B (41.33 ± 1.33) than group C (55.33 ± 2.03), (Table II).

DISCUSSION

The nine rams subjected to three incisional methods of rumen cannulation recovered from the procedure and all surgical wounds healed within one-month post-surgery. The fact that the packed cell volume (PCV) 10 minutes post anaesthesia and just before the rumen cannulation is termed post, had group C values significantly lower than the values in group A could be due to mild dehydration. This finding agrees with the report of Bani Ismail *et al.* (2009) in their study that evaluated the effects of xylazine–ketamine–diazepam anaesthesia on blood cell counts and plasma biochemical values in sheep and goats. The group A (25.50 ± 0.29) became lower afterwards than group C (28 ± 0.58) at 24 h. This could be connected to the fact that group A had primary and secondary incisions on the skin with only primary incision on the rumen, thereby increasing its chances of blood loss during the surgery than group C that underwent only primary incision on both the skin and the rumen. Similarly, the significant differences at week 3 that the PCV value of group A was lower than group B could be related to the same phenomenon. The haematocrit or PCV were increased significantly ($p < 0.05$) following blood reinfusion is in tandem with the work of Najarnezhad *et al.* (2016) who observed decrease at 48 h thereafter. The Significant drop in the haemoglobin concentration observed at 16 h, 24 h and 120 h in group A than that of group C could be linked to more blood loss associated with the technique of group A than in C. The group B lower HGB (g/dl) concentration than that of group C at 120 h and the findings of group A with lower concentration than group B at week 1 could be best described by the same principle. The total red blood cell counts [TRBC X 1012/L] at 4h and 16 h that revealed group B significantly ($p < 0.05$) lower than group C could be.

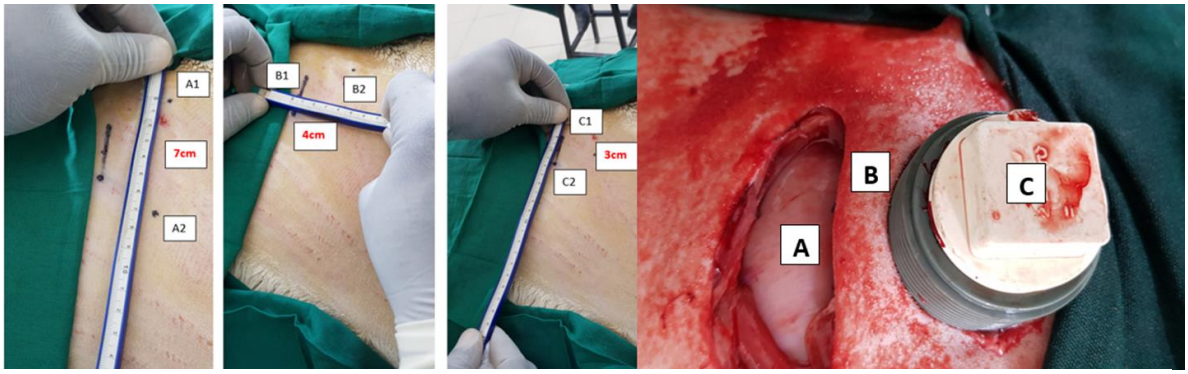

Figure I: A marked 7cm (A1-A2) and 3cm (C1-C2) primary and secondary incision points with an in-between 4cm gap (B1-B2) for cannulation on the skin of the left paralumbar fossa of Yankasa-Balami cross-bred rams

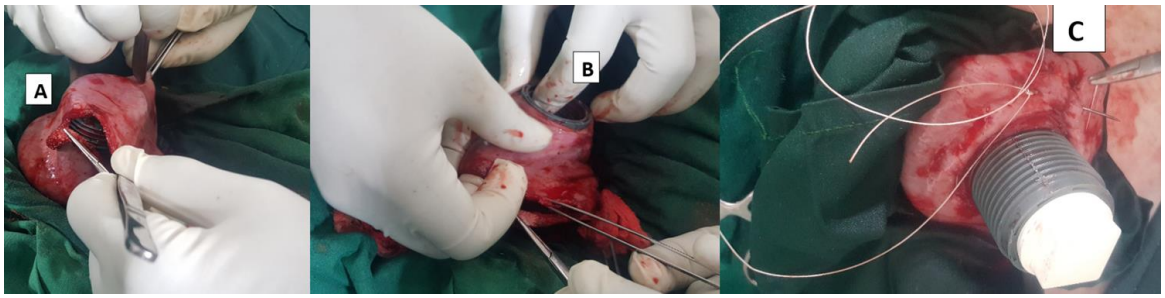

Figure II: A 7 cm primary rumen incision (A) and a 3cm secondary rumen incision (B) with purse string suture pattern (C) after closure of the secondary rumen incision in a stylized incisional approaches for rumen cannulation in Yankasa-Balami cross bred rams

Figure III: Post-cannulation condition of the animal drinking water immediately after the procedure and exhibition of the exited cannula hose with fastened outer plastic flange through a secondary skin incision in Yankasa-Balami cross-bred ram

associated to the more blood loss associated with the technique in group A The fact that the total white blood cell counts showed group B values significantly ($p < 0.05$) lower than group C at 72 h while group A was significantly lower than that of B at 96 h could be linked to the healing process, being a part of the whole regenerative reaction (Schwartz *et al.*, 2002). The high leucocyte levels were generated by the neutrophils, eosinophils and monocytes. These variables play the fundamental roles of defence mechanisms, locally and generally; the monocytes contributed in phagocytosis while neutrophils and eosinophils were indicative of surgical stress. This agrees with the report of Lucas *et al.* (2002) on wound healing in cell studies and animal model experiments. The stress leucogram by endogenous steroid or epinephrine release, anaesthetics or surgery may influence changes in the stress leucogram.

Chronic stress occurs when animals are unable to deal with a persistent stressor with species-typical responses, or when several stressors are present concurrently (Polizopoulou, 2010). Chronic stress is most frequently considered in intensive systems, but it may also be a welfare concern for extensively managed species, such as the sheep. The haematological responses of sheep to experimentally induced chronic stressors to determine relevant indicators of the chronic stress have been a system of concern among researchers, but, greatly rely on the haemogram especially those of the leucogram for insights. Neuroendocrine responses to chronic stress are difficult to interpret because initial responses are followed by an apparent normalisation. Chronic stress can also affect reproductive function, impair body and wool growth and meat quality, reduce immune function, and is associated with greater parasite burdens in sheep making it a subject of concern when subjecting these animals to surgery which is a stressor. Chronic stress induces alterations in behaviour patterns, particularly activity and feeding, and circadian rhythms of behaviour. The existence of many sources of chronic stress in the management of sheep suggests that the welfare of this species requires more attention than it has currently received (Dwyer & Bornett, 2004). In contrast to the findings in this study, which found significant differences in haematologic and glycemic parameters, Kim *et al.* (2018) evaluated the health risks associated with rumen cannulation over a 1-month period and found no significant differences in red blood cell counts or morphologies between pre- and postoperative time points using only one method of cannulation. Furthermore, no inflammation or infection was discovered, which contradicted the findings of this study. Despite the lack of obvious clinical manifestations following surgery in their investigation, serum chemistry results revealed changes in blood urea nitrogen levels and the activities of liver enzymes such as aspartate transaminase, lactate dehydrogenase, and creatinine kinase from postoperative days 1 to 14.

Castillo and Hernández (2021) observed that rumen fistulation and cannulation are critical tools for the advancement of ruminant research in the investigation of new food sources, notably in the evaluation of productivity, health status, and the higher or lesser potential for greenhouse gas generation. It must be done with a small number of animals and subjected to strong clinical and managerial controls to ensure their welfare at all times. The use of in vitro fermenters does not replace the data provided by live animals, but it can provide extra information regarding the changes that occur in the rumen environment under typical settings, regardless of the species. The fact that the blood glucose levels were significantly lower in group B than group C in this study could then mean that since all the values were within the normal species range, over and above all the report by Kim *et al.* (2018), the three incisional patterns experimented in this study could be employed for rumen cannulation in the sheep. This is even for the fact that exercise during a short period of stress may modify or prevent the normal hyperglycaemic response and adrenaline release (Desborough, 2000). The most recent advancements are aimed at creating a robust and continuous artificial rumen system that will allow for a better knowledge of rumen dynamics with as few animals as feasible. Despite this, there are still segments of the population who are hesitant to perform fistulation and cannulation in ruminants, partly due to a lack of understanding of the technique, which must be performed with a minimum number of animals while taking into account their welfare and health status, according to legislative requirements (Castillo & Hernández, 2021).

CONCLUSION

Changes in the haematological parameters and blood glucose levels of rams observed in the "stress syndrome" of three incisional techniques of one stage rumen cannulation are primarily a response to physical, chemical or emotional stress which reflects that rumen cannulation by the technique in group B: primary-secondary incisions on the skin and the rumen; was more stressful, than that of A: primary-secondary skin- but only primary rumen incisions; and C: Only primary incision on both the skin and rumen. In the same vein, group A was second in the ranking of higher production of surgical stress. In view of these findings, **r**umen cannulation by way of using the locally improvised polyvinyl chloride plastisol is recommended for use based on the haematologic and glycaemic profiles of the animals following fistulation and for the fact that as it is cheap, safe and readily available. The rumen cannulation by the technique in group C and A are preferred for adoption based on their superiority of being associated with minimal surgical stress by their glycaemic profiles and the responsive leucogram status of the Yankasa-Balami cross-bred rams at different periods post rumen cannulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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