PHYSIOLOGIC EVIDENCES OF GOOD TOLERANCE OF CONCURRENT RUMEN FISTULATION AND DUODENAL CANNULATION IN WEST AFRICAN DWARF SHEEP

[1]AKA, Lawrence Okonkwo, [1]OBIDIKE, Reginald Ikechukwu, [2]EZE, Chinedu Athenasius and [3]IGBOKWE, Casmire Onwuaso

[1]Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[2]Department of Veterinary Surgery, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[3]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

Corresponding Author: Aka L. O. Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** Lawrence_aka@yahoo.com **Phone:** +234 7033808840

ABSTRACT

Twelve adult West African Dwarf (WAD) sheep of mean age and body weight of 18 ± 1.19 months and 14.69 ± 2.56 kg body weight respectively were used to study the compatibility of concurrent rumen fistulation and duodenal cannulation with normal life. The compatibility with normal life was assessed by differences in body weight, voluntary feed intake, rectal temperature and some haematological and biochemical parameters for a period of eight weeks between test and control groups. During the study period, all the six animals survived the surgery. There was no significant (p < 0.05) difference in body weight between the test and control groups for the entire study period (week 1-8). The mean rectal temperature of the test group was significantly (p < 0.05) higher than the control group on week 1(44.6 ± 3.15^{0}C), week 2 (43.26 ± 4.14 0C) and week 3 (41.15 ± 6.62^{0}C) after implantation. Packed cell volume (PCV) and erythrocyte count (EC) were significantly reduced in the test group within the first three weeks (33.19 ± 1.41 to 24.45 ± 1.39% and 7.01 ± 1.34 to 4.37 ± 1.58 x 10^{6}μl) respectively. On the contrary, total leukocyte count (TLC), and neutrophils were significantly higher in the test group between weeks 1 to 3 (12.48 ± 4.35 and 31.09 ± 3.67 respectively) compared to the control (8.06 ± 0.95 and 23.41 ± 2.09 respectively. Lymphocytes were insignificantly higher in the test group while voluntary feed intake was insignificantly reduced in the test group throughout the eight weeks. Biochemical analyses revealed that serum creatinine was significantly higher in the test group at week 1 (3.62 ± 1.18) and week 2 (3.08 ± 1.44) after surgery. There was however, no significant difference in serum total proteins during the study period. Serum potassium concentrations were significantly decreased in the test group at week 1 (4.96 ± 1.03 to 1.34 ± 0.04). Sodium was also significantly reduced (146.08 ± 3.78 to 96.03 ± 6.21) but on week 3 post implantation (PI). On the other hand, serum globulin was significantly higher in the test group throughout the study period. Gross observations of incision sites showed adhesions characterized by coalesced tissue granulations. The study has shown that the use of polymetric materials in fabricating rumen fistulae and duodenal cannuale could pave way for increased routine nutritional studies in ruminants particularly in developing countries where the availability of these materials constitute great limitation to research involving rumen studies.

Keywords: Duodenal cannulation, Rumen fistulation, West African Dwarf sheep

INTRODUCTION

Rumenotomy is one of the important and commonest surgical procedures in ruminants (Remi-Adwunmi *et al.,* 2006). In Nigeria, the most significant indication of the procedure in small ruminant is in the relief of rumen impaction especially due to indigestible materials such as polythene bag, pieces of leather, bailing rope, rubber, cloth, metal and glass (Sanni *et al.,* 1998). Other indications include surgical treatment of toxic indigestion, primarily of rumen origin, relief of obstruction of the rumino-recticular and reticulo-omasal orifices, a prelude to the treatment of omasal and abomasal impactions and removal of neoplasm such as papillomas at the cardia of the rumen (Gyang, 1992). Another very important area where rumenotmy is indicated is in *in-vivo* nutritional studies where it is usually accompanied by implantation of rumen fistula alone or together with intestinal (duodenal) cannula.

In nutritional studies such as rumen degradation of forages, manipulation of fermentative activities, bioengineering of rumen functions and nutrient digestibility trials, rumen fistulation and duodenal cannulation are very essential. This is so because they afford the researcher opportunities to investigate digestive events under unaltered physiologic conditions (Leng, 1993).

Measuring feed fractions degradability within the rumen in usually a difficult task (Hungate, 1966). The difficulty usually arise from post surgical management of surgical complications often associated with rumenotomy, fistualtion and cannualtion, as well as physiologic variation likely to be introduced and which probably may lead to conflicting results. For this reasons *in vitro* studies involving cultures of rumen fluid are usually preferred.

Nevertheless, rumen fistulation and duodenal cannulation, adopted under well-managed conditions and minimal disruption of rumen environment, are becoming popular in digestibility studies in ruminants. However, the problem of unavailability of rumen fistulation and duodenal cannualtion materials in developing countries has limited this area of research in such countries including Nigeria. In other to make remarkable progress in rumen studies in these areas there is need to solve the problem of availability of fistulation and cannulation materials. For this reason, we fabricated rumen fistulae and duodenal cannulae using polymetric materials and consequently used them to perform concurrent rumen fistulation and duodenal cannulation so as to evaluate their compatibility with normal life using the West African Dwarf sheep.

MATERIALS AND METHODS

Fabrication: Rumen fistula was fabricated using a car driving shaft cover made of polypropylene rubber. It was designed in such a way that the portion with larger internal diameter formed the flange, by cutting it to be together with the projected cylindrical neck (Figure 1).

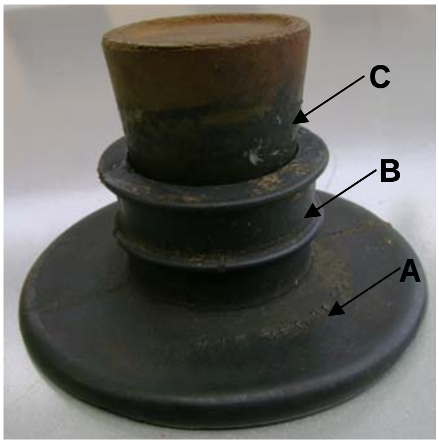

Figure 1: Showing the fabricated rumen fistula using a car driving shaft cover. The rubber bung is also shown. A, Fistula flange. B, projected end of the fistula. C, rubber bung stoppered on the fistula opening for firm closure

A suitable solid rubber bung was used as stopper (Figure 1). The internal diameter of the flange was 8 cm while the internal and external diameter of the cylindrical neck was 4cm and 4.8 cm respectively. A round fistula clamp made of synthetic polypropylene plastic; measuring 0.5 cm thick with a circular hole (about the size of the external diameter of the cylindrical neck of the fistula) in the centre (Figure 2) was used for firm anchorage of the fistula.

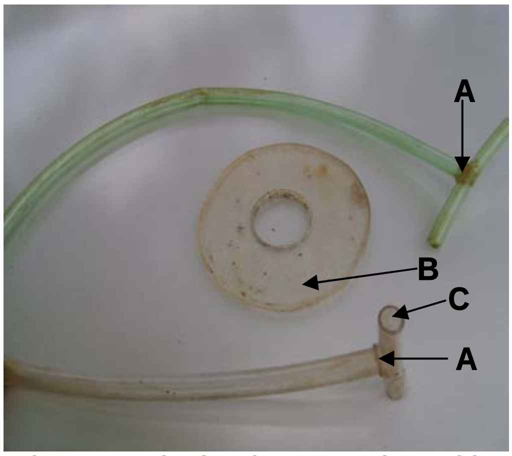

Figure 2: Fistula clamp together with duodenal cannula showing the T end. A, duodenal cannula. B, fistula clamp. C, internal diameter of the T end of the duodenal cannula

The duodenal T-canula (Figure 2) was fabricated using two polypropylene tubes-one measuring 6cm and the other 10 cm in length. A hole, about the size of the external diameter of the 10 cm tube was made at the middle of the 6cm tube (Figure 2). One end of the 10 cm tube was carefully inserted into the hole on the 6 cm tube, thus forming a T-junction (Figure 2).

The insertion was carefully done to avoid blockage of the lumen of the 6 cm tube. The junction was well sealed by heating. The choice of tube diameter was informed by predetermining the diameter of the small intestine of the WAD sheep few minutes after slaughter. All fabricated materials were alternately soaked in 70 % alcohol and Izol® germicide for 24 hours each before use.

Animal: Twelve West African Dwarf Sheep bought from Opi market in Nsukka area of Enugu state, Southeastern Nigeria were used for this study. They were kept at the animal house of the Department of Veterinary Physiology and Pharmacology where they were acclimatized for two months. During this period, they were fed with *Panicum maximum, Pennisetium purpureum* and *Centrosema pubescens.* Occasional supplementation with maize bran was performed. Water was provided *ad libitum* while salt was provided as a lick once (24 hours) a week. During the acclimatization period, ectoparasites and endoparasites were routinely controlled, together with the prescribed vaccination. At the end of the two months acclimatization period, blood samples were obtained via the jugular vein. The samples were examined for haemoparasite, which was found to be negative for all haemoparasites. Likewise, baseline haematological parameters were within normal ranges. Fecal samples were examined for worm eggs by the floatation technique and did not show worm eggs.

Experimental Design: The animals were then divided into two groups of six sheep each. The sheep in group A fitted with rumen fistulae and duodenal cannulae, served as the test group, while those in group B were neither fitted with rumen fistulae nor duodenal cannulae, and therefore served as the control group. The usual pre-surgical protocols were observed. Feed was withdrawn for 24 hours and water was withdrawn for 12 hours prior to surgery. The left Para lumbar area extending from the last rib to the tuber coxae transversely and from the vertebral column down to the ventral abdominal midline longitudinally was prepared for aseptic surgery. Xylazine® HCl administered intramuscularly at a dose rate of 0.01ml/kg was followed with an inverted L field infiltration of 2 % Lingnocain hydrochloride at the paralumbar region. Animals were restrained properly on right lateral recumbence.

Following effective anesthesia, a longitudinal incision (about 8 cm) was made on the left para lumbar region. Upon exposure of the rumen wall, the stomach was retracted until the abomasum was revealed and using the abomasum as a landmark, the duodenum was located and a loop of the proximal duodenum (about 4 – 6 cm) with the shortest mesentery was isolated with sterilized gauze napkins. Then a longitudinal incision about (2 – 4 cm) was made on the isolated loop of the proximal duodenum after retraction of its content proximally and distally. The T end of the sterilized and paraffin treated cannula was carefully inserted into the duodenal lumen starting distally and then ending proximally (Lopukhin, 1976; McGillard, 1982). The incision was then sutured using absorbable chronic catgut (number 3.0). Correct alignment of the duodenal cannula in the duodenum was indicated by a free flow of intestinal content into the exteriorized end of the T-cannula. Following complete retraction of the duodenum and abomasum, stay sutures were then applied on the rumen wall and the abdominal muscle at 3, 6, 9 and 12 o'clock positions. The wall of the stomach was then cut open about the size of the internal diameter of the fistula. The protruding mucosa was griped with a haemostatic clamp at the middle of the incision and pulled upward. The cone thus formed was cut off, creating a circular opening on the stomach. The walls of the stomach (especially the two edges of the incision) were kept raised on hooks and forceps to prevent spillage of rumen content into the peritoneum. Thereafter, the flanges of the rumen fistula were folded and slipped carefully into the stomach through the opening created on the rumen. The ruminal incision was subsequently closed tightly around the neck of the fistula with medium chronic catgut (type C) using two rows of Cushing sutures. Soiled drapes were removed and fresh ones applied. Procaine penicillin (4,000.000 iu) diluted appropriately was sued to lavage the peritoneal cavity prior to closer of the laparotomy.

The peritoneum and abdominal muscles were closed using simple interrupted suture with chromic catgut (number 2). Subcuticular suture was used to appose the subcutaneous tissue using chronic catgut (number 2). The skin was closed using interrupted horizontal mattress suture with silk (number 2). All sutures were made to allow for exteriorization of the duodenal cannula. The fistula clam was used to hold the fistula firmly in place by passing the protruding neck through the central hole on the clamp. Postoperative medication with 5 % dextrose solution at 20ml/kg B.W (iv), procaine penicillin and streptomycin at recommended does were done. Fly repellants, Scabicur® together with Spray plus® (oxytetracycline + Gentian violet) were alternately applied on the surgical would. Skin sutures were removed 10 days post surgery.

Evaluation: After implantation of rumen fistulae and duodenal cannulae, the animals were evaluated weekly. Mean weekly rectal temperature, body weight, and voluntary feed intake, were recorded. Blood samples were collected weekly, from each sheep in a group, for determination of packed cell volume, erythrocyte count, total leukocyte count, lymphocyte and neutrophil count. Blood was sampled, using needle and syringe by jugular venipuncture into duplicate bijou bottles-one containing EDTA, an anticoagulating agent, and the other without EDTA for the hematological and biochemical analyses respectively. Erythrocyte count, packed cell volume, total leukocyte, lymphocyte and neutrophil count were determined using standard methods (Schalm *et al.*, 1975). Serum creatinine was analyzed by the Folin-Wu method (Coles, 1986). Serum total proteins were determined by the biuret method (Coles, 1986). Sodium and potassium levels were determined colorimetrically using Randox® test reagents based on standard protocol (Coles, 1986).

Data Analysis: The data obtained were analyzed statistically using student t-test (Steel and Torrier, 1980).

RESULTS

Figure 1 showed the fabricated rumen fistula. The rubber bung was also shown. Figure 2 showed fistula clamp together with duodenal cannula showing the T end. Figure 3 showed the duodenal cannula well fitted into the duodenal lumen.

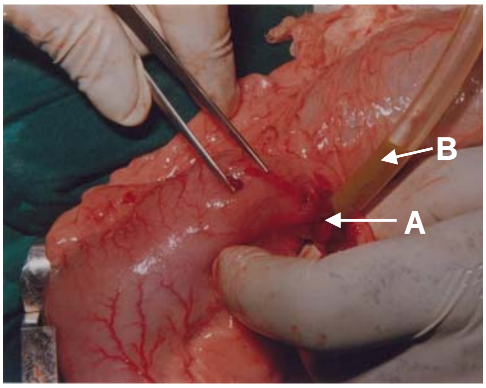

Figure 3: Shows duodenal cannula already implanted into the duodenum. A, point if insertion of the cannula. B, outflow of duodenal content into the cannula indicating correct alignment of the cannula within the duodenal lumen

Figure 4 showed a West African Dwarf sheep carrying the concurrent rumen fistula with clamp and exteriorized duodenal cannula 2 weeks after implantation.

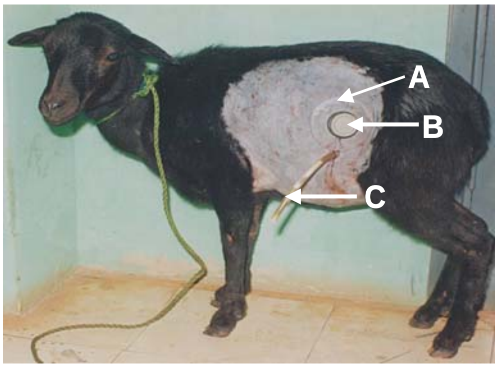

Figure 4: A West African Dwarf sheep carrying concurrent rumen fistula and duodenal cannula fabricated with polymetric materials. (A, rumen fistula with rubber bung for a firm covering, B, fistula clamp for firm anchorage, C, exteriorized duodenal cannula)

Changes in body weight and rectal temperatures in the test and control groups between week 1 and 8 is presented in table 1. There was no significant ($p < 0.05$) difference in body weight in both test and control groups between week 2 and week 8. However, on the first week after implantation, the body weight of the test group significantly decreased compared to the control. Rectal temperature in the test group increased significantly in week 1 and 2 than the control group. From week 3 onwards, no significant difference in rectal temperature was observed in both test and control groups.

Table 2 showed the packed cell volume (PCV) and erythrocyte count (EC) in both test and control groups for the study period. The packed cell volume was significantly decreased in the test group in week, 1, 2 and 3 compared to the control. Likewise, the erythrocyte count followed the same trend as the PCV, though it was significantly reduced in week 3 relative to other weeks including weeks 1 and 2 compared to the control.

Table 3 showed the total leukocyte, lymphocyte and neutrophil count in both test and control groups. Total leukocyte count increased significantly ($p < 0.5$) in the test group in week 1 through week 3. The increase persisted throughout the eight-week period but after week 3 it was not significantly different from the control. In addition, lymphocytes were insignificantly increased in the test group compared to the control throughout the study period. On the contrary, neutrophil in the test group was significantly higher than the control in week 2 and week 3.

Table 4 showed the voluntary feed intake in the test and the control groups. In the test group, voluntary feed intake decreased insignificantly from baseline value in week 0 and compared with the control group for the entire study period.

Table 1: Mean body weight and rectal temperatures in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	Body weight (kg) Group A	Body weight (kg) Group B	Rectal temperature (^{0}C) Group A	Rectal temperature (^{0}C) Group B
0	13.88 ± 2.19	14.96 ± 2.66	38.75 ± 1.50	39.94 ± 52.26
1	10.92 ± 1.46^a	14.53 ± 1.62^b	44.64 ± 3.15^a	39.83 ± 1.00^b
2	12.94 ± 1.93^a	14.08 ± 2.61^a	43.26 ± 4.14^a	38.93 ± 2.25^b
3	12.11 ± 0.88^a	13.63 ± 3.11^a	41.15 ± 4.62^b	38.66 ± 1.75^b
4	11.64 ± 2.43^a	13.84 ± 1.69^a	40.05 ± 6.11^b	39.15 ± 2.65^b
5	12.16 ± 1.56^a	14.02 ± 2.36^a	39.65 ± 2.93^b	38.50 ± 1.19^b
6	12.84 ± 3.14^a	14.17 ± 2.15^a	40.15 ± 2.15^b	39.50 ± 2.25^b
7	12.92 ± 1.92^a	14.24 ± 3.0^a	38.91 ± 1.58^b	38.39 ± 3.11^b
8	11.82 ± 1.27^a	13.62 ± 2.51^a	39.74 ± 2.67^b	39.75 ± 4.11^b

Means within row with different superscript are significantly (p<0.05) different

Table 2: Packed cell volume (%) and erythrocyte count (x10^6 µl) in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	PCV (%) Group A	PCV (%) Group B	Erythrocyte count (x10^6 µl) Group A	Erythrocyte count (x10^6 µl) Group B
0	33.19 ± 1.41	32.28 ±1.36	7.01 ± 1.34	6.68 ± 1.48
1	27.25 ± 2.62^a	33.46 ± 1.24^b	4.72 ± 0.44^a	6.99 ± 1.53^b
2	25.65 ± 2.87^a	32.61 ± 4.46^b	4.96 ± 1.46^a	7.26 ± 1.56^b
3	25.45 ± 1.39^a	33.65 ± 2.96^b	4.37 ± 1.58^a	7.86 ± 0.81^b
4	28.43 ± 5.56	30.29 ± 3.16	5.88 ± 1.18	6.23 ± 1.48
5	27.86 ± 4.11	31.90 ± 3.66	5.96 ± 0.13	6.84 ± 1.35
6	29.57 ± 1.62	30.45 ± 2.93	6.37 ± 1.77	7.95 ± 1.36
7	30.14 ± 2.63	37.34 ± 3.03	6.17 ± 2.51	6.82 ± 2.33
8	29.45 ± 2.71	34.95 ± 4.62	6.83 ± 3.16	7.69 ± 3.17

** Period of most significant reduction. Means within row different superscript are significantly (p<0.05) different*

Table 3: Total leucocyte, lymphocyte and neutrophil count in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	Total Leukocyte (x10^3 µl) Group A	Total Leukocyte (x10^3 µl) Group B	Lymphocyte (%) Group A	Lymphocyte (%) Group B	Neutrophils (%) Group A	Neutrophils (%) Group B
0	8.36 ± 2.71	7.85 ± 1.38	60.33 ± 4.15	58.93 ± 4.19	23.96 ± 4.11	20.46 ± 2.83
1	11.89 ± 1.94^a	7.64 ± 2.16^b	63.81 ± 3.91	59.84 ± 3.34	28.42 ± 3.28^a	21.35 ± 2.75^b
2	12.17 ± 1.89^a	7.86 ± 1.05^b	62.11 ± 4.11	58.74 ± 9.14	30.94 ± 2.98^a	20.38 ± 3.11^b
3	12.48 ± 1.35^a	8.06 ± 0.95^b	58.44 ± 3.47	59.73 ± 2.81	31.09 ± 4.23^a	23.41 ± 2.09^b
4	11.36 ± 1.41^a	7.58 ± 0.42^a	61.17 ± 4.19	60.03 ± 4.71	26.65 ± 5.11	22.95 ± 2.88
5	10.08 ± 2.56^a	8.01 ± 0.94^a	62.27 ± 2.81	59.73 ± 6.48	24.06 ± 3.56	23.16 ± 2.71
6	9.63 ± 1.42^a	8.44 ± 1.33^a	61.95 ± 5.13	57.44 ± 4.18	25.45 ± 4.31	22.75 ± 3.17
7	8.58 ± 1.56	7.41 ±1.7	59.12 ± 3.67	43.11 ± 2.68	26.82 ± 3.71	24.65 ± 2.81
8	8.93 ± 2.82	6.48 ± 0.92	63.14 ± 2.86	51.78 ± 3.41	29.01 ± 2.43	21.03 ± 3.04

Means within row with different superscript are significantly (p<0.05) different

Table 5 showed the serum creatinine and total protein profile in the test and control groups. There was significant ($p < 0.05$) increased in serum creatinine in the test group from week 1 to week 3 compared to the control. However, beyond week 3, the increase persisted but was insignificant up until week 5. There was no significant difference in total protein between the test and control groups fro the entire study period.

Table 6 showed the mean serum globulin, potassium and sodium concentrations in both experimental groups. Serum globulin was significantly higher in the test group throughout the eight weeks. On the other hand, potassium was significantly decreased in week 1. This decrease was restored to near normal almost after this period (i.e. week 2). Serum sodium concentration was normal in week 1 and 2 and only significantly decreased in week three after which an insignificant increase was maintained up till week 5.

DISCUSSION

In the study, we observed that concurrent rumen fistulation and duodenal cannulation is well tolerated by West African dwarf sheep as evidenced by remarkable positive progressive physiological changes within eight weeks after implantation. These observed changes characterized good healing processes, effective defense mechanism against infectious organisms and adequate restoration of electrolyte balance in interstitial fluid.

Table 4: Voluntary feed intake in WAD sheep fitted with concurrent rumen fistula and duodenal cannula

Expt. Period (Wks)	Feed intake kg/day	
	Group A	Group B
0	3.86 ± 0.96[a]	3.94 ± 1.71[a]
1	2.93 ± 0.73[a]	3.96 ± 1.86[a]
2	3.16 ± 0.46[a]	3.72 ± 0.66[a]
3	3.46 ± 0.62[a]	3.83 ± 1.17[a]
4	3.53 ± 0.48[a]	3.76 ± 1.31[a]
5	3.66 ± 0.93[a]	3.84 ± 0.81[a]
6	3.71 ± 0.76[a]	3.93 ± 0.73[a]
7	3.16 ± 1.12[a]	3.97 ± 1.02[a]
8	4.11 ± 1.51[a]	4.94 ± 0.49[a]

Means within row with different superscript are significantly (p<0.05) different

Table 5: Serum creatinine and total proteins in WAD sheep fitted with concurrent rumen fistulae and duodenal cannula

Expt. Period (Wks)	Biochemical parameters			
	Serum creatinine (mg/100ml)		Total proteins (mg/100ml)	
	Group A	Group B	Group A	Group B
0	1.31 ± 0.04	0.98 ± 0.09	9.01 ± 0.89	7.18 ± 1.44
1	3.62 ± 1.18[a]	1.03 ± 0.06[b]	6.69 ± 1.16[a]	7.21 ± 2.02[a]
2	3.08 ± 1.44[a]	1.14 ± 0.09[b]	6.34 ± 2.04[a]	6.73 ± 1.82[a]
3	2.94 ± 0.33[a]	0.95 ± 0.04[b]	7.16 ± 2.05[a]	6.73 ± 1.84[a]
4	2.09 ± 0.0[a]	0.86 ± 0.07[a]	6.84 ± 1.63[a]	6.28 ± 2.01[a]
5	1.36 ± 0.03[a]	0.91 ± 0.03[a]	7.04 ± 0.99[a]	6.19 ± 1.02[a]
6	1.33 ± 0.04[a]	0.94 ± 0.06[a]	6.96 ± 1.38[a]	7.47 ± 1.92[a]
7	1.55 ± 0.11	0.96 ± 0.43	7.32 ± 1.77	7.19 ± 0.83
8	1.19 ± 0.06	0.82 ± 0.21	6.49 ± 0.09	6.39 ± 0.76

Means within row with different superscript are significantly (p<0.05) different

The decrease in body weight which was insignificant during the entire observation period (Table 1) was probably due to reduction in fed intake which was also insignificantly decreased (Table 4) in the test group for the same entire study period . This reduction was probably due to surgical stress that gave rise to inappetance. Stress in general has been shown to reduce appetite and thus feed intake in both humans and animals (William, 2004). Under this experimental condition, the surgical stress in addition to the relative increase in rectal temperature (Table 1) (thermal stress) within the first three weeks post implantation probably gave rise to sustained anorexia which lasted for the entire observation period, thus probably responsible for the decreased body weight. Another factor, which probably was responsible for the suppression of appetite, was the stretching of the rumen and intestine. Stretching of the rumen and the intestine causes anorexia (William, 2004). The rumen fistulae and duodenal cannula (especially considering the materials used) probably produced stretching effect on the rumen and intestine such that intragastric and intra-intestinal pressures were so increased as to send satiation signal via stretch receptors to the feeding centre in the brain, which in turn regulated feeding negatively. These stretching effects probably contributed to anorexia especially few days after implantation. Whatever was the mechanism (s) that resulted in reduced feed intake and body weight, we had the opinion that the reductions were not inimical to health since there variations in the test and control groups were insignificant for the entire observation period.

The decrease in mean packed cell volume and erythrocyte count within the first three weeks suggested anaemia. Post surgical anaemia has been demonstrated few days after surgery (Dougherty, 1981) and is usually associated with the extent of blood loss during surgery (Gyang, 1992; Venugopalan 1997), post surgical complication (Santra and Karim, 2002), post surgical feeding behaviour and quality of feed offered (Ragab, 1989; Appleby and Hughes, 1997). In this study, the surgery that lasted for 56 minutes was characterized by relative high blood loss. This level of blood loss together with the suppressed appetite and decreased feed intake probably accounted for the decrease in PCV and EC at least for the first 3 weeks. The fact that beyond the third week, PCV and EC began to improve led us to conclude that their decrease were surgery related particularly due to degree of blood loss and post-surgical inappetance, which waned with time. Thus, three weeks and beyond was enough for improvement in PCV and EC especially as intake of protein rich legumes, *Centrosema pubescens* and *Stylosanthese gracilis* during the first one week after implantation was assured.

The significant increase in total leukocyte and neutrophil within the first three weeks suggest physiologic response to inflammation and enhanced immune response. Various factors such as time of day, a meal, exercise, epinephrine (endogenous), anesthesia and stress conditions are known to contribute to physiological leukocytosis (William and Melvin, 2004). With some anesthesia, animals may undergo an excitement period that causes epinephrine release, which contributes to a leukocytosis. However, in this study, most of the sheep showed some excitement immediately after administration of anesthetic agent (xylazine). The excitements were very short-lived however. Therefore, it was difficult to relate the observed leucocytosis to epinephrine influence, weeks after surgery. Increase in total leucocytes few weeks after surgery has been earlier reported (Leifer *et al.*, 1983; Buckner, 1995; Abdel-Fattah, 1999). In localized traumatic conditions such as rumenotomy, leucocytosis and neutrophilia has been reported (Weisis, 1984; Hassanein *et al*; 1988; Aka *et al.*,

Table 6: Serum globulin, potassium and sodium levels in WAD sheep fitted with concurrent fistula and duodenal cannula

Expt. Period (Wks)	Serum biochemical parameters					
	Globulin (g/dl)		Potassium (mEq/L)		Sodium (mEq/L)	
	Group A	Group B	Group A	Group B	Group A	Group B
0	2.83 ± 0.36	3.41 ± 0.63	4.96 ± 0.98	5.18 ± 0.98	146.08 ± 4.87	55.14 ± 6.11
1	4.939 ± 0.71	3.08 ± 0.14	1.34 ± 0.06[a]	4.12 ± 0.54[b]	158.46 ± 4.33	63.44 ± 3.83
2	6.86 ± 1.08	3.14 ± 0.61	3.89 ± 0.93	4.62 ± 0.65	67.93 ± 3.78	153.43 ± 2.96
3	6.36 ± 1.42	2.94 ± 0.48	3.66 ± 0.87	4.17 ± 0.84	96.03 ± 4.11[a]	116.37 ± 4.2[b]
4	5.16 ± 1.33	2.88 ± 0.55	4.28 ± 10.06	4.86 ± 0.76	143.78 ± 3.98	156.18 ± 3.22
5	5.98 ± 0.96	3.16 ± 0.43	4.06 ± 1.05	4.82 ± 0.42	157.63 ± 4.81	167.46 ± 3.71
6	5.48 ± 1.17	3.66 ± 0.37	4.71 ± 1.13	4.56 ± 1.11	141.67 ± 3.77	163.83 ± 3.23
7	4.93 ± 0.88	3.61 ± 0.44	4.45 ± 0.54	4.34 ± 0.32	144.23 ± 3.21	171.55 ± 3.88
8	5.33 ± 0.23	3.12 ± 0.43	3.97 ± 1.02	4.63 ± 0.54	146.98 ± 3.65	166.98 ± 4.62

Means within row with different superscript are significantly ($p<0.05$) different

2006). Tissue destruction, irrespective of its cause will produce an increase in the number of circulating neutrophils. Increase in neutrophils occurs in prolonged surgical procedures where there has been considerable tissue damage (Buckner, 1995). These observations, which have been represented in this study, made it apt for us to conclude that the increase in total leucocytes in this study was more of a physiologic relative leucocytosis that resulted from neutrophilia.

The insignificant increase in lymphocytes in this study points to mild proliferative response by the immune cells. This probably was to boost immunity to combat infection by microorganisms. This was particularly corroborated by significant increase in serum globulin level for the same period after surgery (Table 6). Increased serum globulin level with concomitant leucocytosis highlights an improved immune status (Obidike *et al*; 2009).

At first, second and third week after implantation there was almost double increased serum creatinine level in the test group compared to the control. This observation agrees with the report of other workers. Swanson and Wilkinson (1972) observed that trauma associated with surgery and accidents with high degree of muscle damage caused significant rise in serum ceratinine. In this study, serum creatinine level was high in test group at the earlier stages after implantation (week 1 to 3) and was decreased as wound healing progressed as observed from week 4 onwards. Surgical trauma to muscle, or any form of trauma, bruises, fracture and even severe exercise may cause a marked elevation of blood creatinine and serum kinase activity, which may persist for a week or longer (Tietz, 1978). The increase in serum creatinine in this study seems to be a physiologic response to surgical trauma and thus was probably not inimical to the health status of the animals as it waned with time.

Relative changes in electrolyte balance in interstitial fluid have been reported to be characteristic of most surgeries. The significant reduction in serum potassium and sodium concentrations in week 1 and week 3 respectively lays credence to this assertion. Potassium is one of the major cations of fluids of the cell (cytosol) while sodium occurs in small amounts within the cell. In surgical condition, the cells that are usually lost in greater numbers are the erythrocytes and consequently potassium is lost much more than sodium. This probably explains its (potassium) significant reduction (Table 6) within the first week as against sodium that required up to 3 weeks recording a significant fall (Table 6). A change in electrolyte balance affects the osmotic pressure of cells and body fluids. Changes in electrolytes and osmotic pressure of cells and body fluids probably did not develop in this study. This could be due to the sharp fall in potassium level, which was restored in week 2. Restoration of potassium deficiency via diet is usually faster than sodium (Rosa *et al*., 1992) as most foodstuffs contain considerable potassium, and the kidneys are more capable of excreting this excess than of conserving it. Ruminants have a good ability to vary their urinary excretion rate of potassium to meet wide and rapid changes in intake (William, 2004). Thus, the time taken for the physiologic adjustments of these electrolytes after depletion was not long enough to produce any adverse effects on the health of the animals.

Furthermore, the study showed no significant difference in the total plasma proteins for the entire observation period. This shows that in all groups (test and control), the normal profiles and functions of serum proteins were maintained. Since plasma proteins are chiefly formed in the hepatic cells, it could be said that the surgical technique had no obvious clinical effect on the liver. Again, since there was no obvious change in total protein we also speculate that cellular proteins were not depleted in the prevailing circumstance. This could be so because in situation(s) where cellular proteins are depleted reduction in plasma protein occurs due to their breakdown into amino acids by the mononuclear phagocytic system (MPS) cells. These amino acids are then used for the formation of cellular protein. Plasma proteins are known to maintain the colloidal osmotic pressure, contribute to viscosity of blood, influence the suspension stability of erythrocytes, and help regulate acid-base balance, transport substances (vitamins, hormones, nutrients etc) and affect solubility of carbohydrates, lipids and other substances held in solution in the plasma. It then follows that in both groups these functions probably were not affected by the surgical technique since no significant difference in total protein was observed – a situation that maintains homeostatic balance between blood and cellular fluids.

Conclusion: In conclusion, we have observed that some physiologic changes do occur in WAD sheep following concurrent rumen fistulation and duodenal cannulation. Most of these changes were thought to be normal (physiologic) consequences of most surgical techniques and were usually restored with 3 to 4 weeks post implantation. On the other hand, those changes, which could compromise normal health if prolonged, did not persist beyond three weeks after implantation. Therefore, it was concluded that the WAD sheep tolerates concurrent rumen fistulation and duodenal cannulation without obvious clinical effects for at least the observation period (eight weeks). In small ruminants, particularly the WAD sheep, the use of local polymetric materials to fabricate rumen fistulae and duodenal cannula could provide more opportunities for *in vivo* nutritional studies.

REFERENCES

ABDEL-FATTAH, M. (1999). *The goat as a model for experimental surgery*. PhD thesis, surgery, anesthesiology and radiology. Faculty of Veterinary Medicine Cairo University, Beni-Suef. Brach, Egypt.

AKA, L. O., EZE, C. A. and ABIADE-PAUL, C. U. (2006). *Body weight, haematological and serum creatinine profile following rumen fistulation in West African dwarf X UDA sheep.* Pages 75 – 79. *In:* Proceeding of 43rd Annual Conference of NVMA, Niger State, Nigeria,

APPLEBY, M. C. and HUGHES, B. O. (1997). *Animal Welfare*. CAB International. New York.

BUCKNER, R. (1995). Surgical correction of left displaced abomasums in cattle. *Veterinary Records,* 136: 265 – 267.

COLES, E. H. (1986). *Veterinary clinical pathology.* 4th edition, W. B. Saunders Company, Philadelphia.

DOUGHERTY, R. W. (1981). *Experimental surgery in farm animals*. 1st edition, The State University Press, Ames, Iowa.

GYANG, E. O. (1992). *The digestive system: Introduction to large Animal Surgery*. Agitab Publishers, Zaria, Nigeria.

HASSANEIN, A., SOLIMAN, A. S. and EIFAT, J. F. (1988). Rumen cannulation in sheep. *Alexandria Journal of Veterinary Science,* 4: 429 – 435.

HUNGATE, R. E. (1966). *The rumen and its microbes*. Academic Press, New York.

LEIFER, C. E., MATUS, R. E., PATNARK, A. K. and MACEWEN, E. G. (1983). Chronic myelogenous leukaemia in the dog. *Journal of Americans Veterinary Medical Association,* 183: 686 – 689.

LENG, R. A. (1993). Quantitative ruminant nutrition. A green science. *Australian Journal of Agricultural Research,* 44: 363 – 380.

LOPUKHIN, Y. M. (1976). *Experimental surgery*. Mir Publishers, Moscow.

MCGILLARD, A. D. (1982). Surgical techniques-advances and caution. Pages 31 – 36. *In:* Owens, F. N. (Ed.) *Protein requirements for cattle.* Symposium Miscellaneous Publication Number 109, Oklahoma State University.

OBIDIKE, R. I., AKA, L. O. and OKAFOR, C. I. (2009). Time-dependant peri-partum haematology-cal, biochemical and rectal temperature changes in West Africa dwarf ewes. *Small Ruminant Research,* 82; 53 – 57.

RAGAB, G. A. (1989). *Some studies on the surgery of the urogenital system in small ruminants.* PhD Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

REMI-ADEWUNMI, B. D., FAGBAMILA, O. I., IBRAHIM, N. D. G., OGUNDLE, J. A. (2006). Evaluation of chromic catgut and polyalycolic acid sutures for closure of rumen incision in Savannah brown goats. Pages 12 – 15. *In: Proceedings of 43rd Annual Conference of. NVMA, Niger State, Nigeria.*

ROSA, R. M., WILLIAMS, M. E. and EPSTEIN, F. H. (1992). Extra renal potassium metabolism. Pages 213 – 133. *In:* SELDIN, D. W. and GILBISCH, G. (Eds.). The *kidney: physiology and pathophysiology.* Raven Press, New York.

SANNI, B. D., GYANG, E. O. and OSINOWO, O. A. (1998). Polythene-bag-induced rumen impaction in small ruminants. An environmental hazard. Pages 97 – 98. *In: Proceedings of the Silver Anniversary Conference of the Nigerian Society for Animal Production and the inaugural conference of the West African Society for Animal Production,* Abeokuta, Nigeria.

SANTRA, A. and KARIM, S. A. (2002). Rumen cannulation in sheep and goats. Fabrication of cannula and surgical procedure for its implantation. *Indian Journal of Animal Science,* 72: 978 – 980.

SCHALM, O. W., JAIN, N. C. and CARROL, E. J. (1975). *Veterinary Haematology*. 3rd edition, Lea and Febiger, Philadelphia.

STEEL, R. G. D. and TORIRIER, J. G. (1980). *Principles and procedures of statistics. A biometric approach.* 2nd edition. McGraw-Hill New York.

SWANSON. J. R. and WILKINSON, J. H. (1972). Measurement of creatinine kinase activity in serum. Pages 33 – 42. *In:* Copper, G.R. (Ed).*Standard methods of clinical chemistry*, Volume 7, Academic Press, New York.

TIETZ, N. (1976*). Fundamentals of clinical chemistry.* W.B. Saunders Company. Philadelphia.

VENUGOPULAN, A. (1997). Suture materials and suture patterns. Pages 11 – 15. *In: Essentials of veterinary surgery*. I.B.H. Publishing Company, New Delhi,

WEISIS, D. J. (1984). Disorders of voluntary muscle: Uniform evaluation and semi-quantitative reporting of haematological data in Veterinary Laboratories. *Veterinary Clinical Pathology,* XIV: 27.

WILLIAM, O. R. and MELVIN, J. S. (2004). The composition and function of blood. Pages 26 – 49. *In:* REECE, W. O. (Ed.) *Duke's physiology of domestic animals,* 12th edition, Cornel University Press, London.

WILLIAM, O. R. (2004). Pages 26 – 49. *In:* Reece, W. O. (Ed.) *Duke's physiology of domestic animals,* 12th edition, Cornel University Press, London.