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Haematologic Tolerance of a Rumen Fistulation Protocol in UdaRamsata Tertiary Institution Livestock Teaching and Research farm, Kaduna State, Nigeria

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ABSTRACT This experiment was conducted at the Federal University Dutsin-Ma Livestock Teaching and Research Farm in Dutsin-Ma Local Government area of Katsina State, Nigeria. The study was aimed at studying haematological tolerance level of Uda rams to a rumen fistulation protocol involving incision of skin and muscles separation along the direction of muscle fibers by divulsion. Blood samples were collected and evaluated at the intervals of 0, 24 and 48hours to assess blood line response to the protocol. A total of 3 rams were used and assigned T1, T2 and T3 in a completely randomized technique. A significant ($P< 0.05$) continuum of decreased packed cell volume and haemoglobin concentration was observed from 0hrs through to 48hours connoting anaemia condition associated with the rumen fistulation protocol. However, significant ($P < 0.01$) tolerance levels were observed with the cell mediated immune response as evident in the increased lymphocyte counts (lymphocytosis) and an initial acute increase in neutrophils (neutrophilia). This technique was able to demonstrate good tolerance level to rumen fistulation by Uda rams making this breed a good candidate for nutritional and physiological studies requiring rumen fistulation.

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Ruminants have a stomach with four compartments or chambers—reticulum, rumen, omasum, and abomasum. The reticulum, rumen, and omasum are lined with non-glandular mucous membranes, while the abomasum is similar in function to the human stomach. The largest compartment is the rumen, which along with the reticulum serve as the sites of anaerobic fermentation. Ruminant nutritionists often refer to these compartments as the reticulo-rumen because together they function in the rumen cycle (coordinated contractions) to support the acts of eructation and rumination (DePeters *et al*., 2014). In a normally functioning rumen, fiber, starches, and sugars yield volatile fatty acids (VFAs), carbon dioxide (CO2), and methane. Protein sources are partially degraded to NH4, volatile fatty acids, and gases. Lipids are partly cleaved to glycerol and fatty acids, with unsaturated fatty acids being hydrogenated. Most organic compounds in the diet can be fermented by anaerobic microbes within the rumen. The extent to which feed components are degraded is limited either by the accessibility of various feed components to ruminal microbes, by the enzymatic activity of ruminal microbes (that will vary with ruminal conditions), or the amount of time available for fermentation (Owens *et al*., 2016). Methanogens metabolize the hydrogen produced during fermentation. This microbial population is found free in the rumen fluid, attached to particulate material and rumen protozoa, as well as to the rumen epithelium. The methanogens associated with these different fractions can be expected to have different growth rates since they are removed from the rumen at different rates. For this reason, the animal itself and the feed influence the rate of passage of digesta through the rumen system (Janssen *et al*., 2007). 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

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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).

Two surgical procedures for the ruminal fistulation and cannulation have been described. The first technique described by Schalk and Amadon (1928) is a one stage procedure and later a new technique in two stages was described by Jarret (1948). The ideal fistula is one that forms a seal around the cannula, preventing the leakage of the experimental period. In this study, two-stage technique was done in two different ways with the intention to compare and find out which one is better to reduce postoperative necrosis of the wound edges and consequent loosening of cannula and other complications (Malik *et al*., 2015).

At least three different ruminalfistulation techniques are used in ruminants: a one stage method, a two-stage method, and the Schalk and Amadon technique (Kristensen*et al*., 2010; Ghazy, 2017). The first technique is suitable for the implantation of small cannulae and is generally applied in small ruminants, whereas the second is preferred in larger ruminants. However, this criterion is not definitive since the use of one technique or the other will depend on the objectives pursued by the study (Resillez *et al*., 2009). Every year it is possible to read articles that make modifications to the previously mentioned techniques and that allude to the surgical approach, the type of cannula to be used, or even the strategies to be followed after surgery to maintain the anaerobic environment of the rumen. Regardless of the technique or location, the gap between the skin and rumen cannula can lead to fermentation gas leakage and atmospheric air ingress, which can negatively affect the anaerobic environment of the rumen; therefore, the choice of the appropriate material according to the species and/or the size of the animal is an aspect to be taken into account (Wang *et al*., 2019). From reading the available references, we would like to highlight two facts: (1) that a good surgical procedure allows for the long-term use of cannulated animals (Szakács *et al*., 2021), and (2) that this procedure is impractical for sampling a large number of animals (Song *et al*., 2018; Tapio *et al*, 2016). The first and probably the most critical aspect of experimental success is the selection of the animals. Their temperament is essential. Ideally, before surgery, the animals should be trained for the restraint and handling involved in the sampling. They will also have to get used to the space where they will go for cannulation, evaluating the dangers that may exist such as the walls of the facility—which must be smooth, the openings in the fences or doors—which may damage the animal or trap and pull out the cannula, the possibility of the cannula being ejected when the animal is moved or lifted, or even the free access of other animals that may pull out the cannula. Often, the lack of design forces surgery on animals that become excited and consequently damage themselves or the cannula after surgery (Harmon *et al*., 1997). The surgical site and the surface of the outer edge of the cannula next to the skin should be cleaned daily for 5–7 days with a diluted antiseptic solution. The wound should be protected from flies, and a broad-spectrum antibiotic must be administered after surgery for seven days. The use of postoperative analgesics if the animal appears to be uncomfortable is recommended (Laflin *et al.,* 2008).

Clear and concise description of procedures and level of care given to animal, in our opinion, constitute a valuable tool that adds value to the experiment from an ethical point of view since it demonstrates that the researchers subscribe to an ethical position on animal experimentation (EU, 2010). On numerous occasions, and contrary to popular belief, thecannulated animals are subjected to reconstructive surgery at the end of the study. This allows for the removal of the cannula and the closure of the layers of tissue by planes; animals are then incorporated into productive and reproductive life within their herd of origin, thus avoiding their sacrifice (Resillez *et al*., 2009).

MATERIALS AND METHODS

The experimental animals were 9Uda rams aged between 9 to 12 months. The average live weight of the rams was 15kg. The animals were rested for 10days before the conduct of the rumenotomy protocol. The animals were prepared for surgery under local anaesthesia. The protocol was based on the methods of Malik *et al.,* 2015. However, a modification was made through the use of an improvised cannula in the form of a sterilized empty top gum rubber bottles. The skin was marked with an indelible marker along the circumference of the improvised cannula to be used. Cruciate incision was made through the skin and the pieces of skin were undermined and removed along the marking. The muscles were separated along the muscle fibre direction by divulsion. The abdominal wall muscles were sutured with skin using silk of varying sizes for each treatment: T1 (size 1), T2 (size 2) and T3 (size 3) to retract the muscles thereby giving a regular circular shape to the hole for ease at the time insertion of improvised cannula. Sufficient portion of the rumen wall was seized and pulled through the abdominal incision, exposing part of the rumen wall. The exposed portion of the rumen was sutured to the peritoneum around the surgical opening; using chromic catgut No. 2 in simple continuous pattern. Post-surgery, gauze was fixed to cover the rumen wall and dressed regularly.Animal welfare includes not only adequate conditions in the experimental pen, but also the enrichment of the environment to improve their emotional state, prevention of post-surgical infection through the administration ofKeproPenstrep® (procaine penicillin and streptomycin combination) for 4 days.

Blood samples were collected using commercial sample bottles pre-coated with EDTA as anticoagulants. Time intervals for blood sample collection were 0, 24 and 48hrs and at morning hours.The data obtained were analyzed statistically using ANOVA (Steel and Torrier, 1980).

RESULTS AND DISCUSSIONS

Hematological results of this study revealed remarkable positive progressive physiological changes within 48 hours of the study. Packed Cell Volume (PCV) and Haemoglobin concentration decreased (P<0.05) from 0 hour through to 48 hours (Table 1.0). Invasive surgical procedures are associated with blood loss with resultant slight to moderate anaemia usually within the range the body homeostasis can address.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, they were relative low blood loss. This level of blood loss together with the suppressed appetite and decreased feed intake post-surgery could be attributed to the decrease in PCV and haemoglobin concentration.

Table 1: Packed Cell Volume, Haemoglobin Concentration and Total Leucocytes Count in Uda Rams with Rumen Fistula

Expt. Period (Hours)	Packed Cell Volume (%)			Haemoglobin concentration (g/L)			Total leucocyte count (x 10^3 μl)		
	T1 N = 3	T2 N = 3	T3 N = 3	T1 N = 3	T2 N = 3	T3 N = 3	T1 N = 3	T2 N = 3	T3 N = 3
0	28±1.41[a]	30±1.36[b]	34±1.35[b]	9.3±1.34	10.1±1.48	11.3±1.36	11.4±2.71[a]	15.6±1.38[b]	10.4±1.45[a]
24	27±2.62[a]	29±1.24[a]	33±1.30[b]	9.6±0.44	10.2±1.53	11±1.45	10.8±1.33[a]	9.9±1.58[b]	14.1±4.33[c]
48	27±2.71[a]	28±1.38[a]	31±2.16[b]	8±2.31	9.8±1.24	10.3±1.55	11.9±1.35[a]	10.2±0.95[a]	13.8±2.54[b]

Means within row with different superscript are significantly (p<0.05) different, N = number of animals per treatment

Table 2: Lymphocytes and Neutrophils Counts in Uda Rams with Rumen Fistula

Expt. Period (Hours)	Lymphocytes (%)			Neutrophils (%)		
	T1 N = 3	T2 N = 3	T3 N = 3	T1 N = 3	T2 N = 3	T3 N = 3
0	40±4.15[a]	48±12.70[b]	28±12.70[c]	58±4.11[a]	85±12.71[b]	80±12.71[c]
24	44±3.91[a]	59±11.71[b]	36±14.20[c]	46±3.28[a]	83±18.88[b]	71±6.45[c]
48	54±2.82[a]	59±0.95[b]	42±2.86[c]	46±3.41[a]	79±2.43[b]	58±3.04[c]

Means within row with different superscript are significantly different, N = number of animals per treatment

Acute neutrophilia and subsequent lymphocytosis as shown in Table 2.0 suggest physiologic response to inflammation and enhanced immune response (cell mediated immunity). In localized traumatic conditions such as rumenotomy, leucocytosis due to lymphocytosis and neutrophilia has been reported (Weisis, 1984; Hassanein *et al*; 1988; Aka *et al*.,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 cell mediated immune response seen as leucocytic lymphocytosis that was triggered by an initial neutrophilia.

*Conclusion:*In conclusion, the study was able to demonstrate haematologically that Uda rams have a good physiologic tolerance to rumenotomy and fistulation protocols. Going by the findings of this study, Uda rams could be considered as suitable candidates for carrying out nutritional and physiological studies requiring rumen fistulation.

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