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Research Article

Blood Parameters and Rumen Wall Histology of Brahman Crossbred Steers in Response to the Administration of Rumen Mechanical Stimulating Brushes

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Abstract

Background and Objective: The effects of rumen mechanical stimulating (RMS) brushes on the health of Brahman crossbred steers were determined by measuring blood parameters and examining the rumen wall. **Materials and Methods:** This study used twenty Brahman crossbred steers divided into control and RMS groups. The steers were given low-forage diets (95.5% concentrate and 4.5% maize stover) based on 3% dry matter by body weight. The installation of RMS was conducted by a professional. Blood samples were collected at the end of the experiment from the coccygeal vein for blood profile and immunoglobulin G (IgG) measurements. The steers were slaughtered at the 93rd day of the experiment, samples of the rumen wall were collected and rumen histological observations were made. **Results:** The results showed that the RMS brush administration did not affect the hematocrit, hemoglobin (Hb), number of red blood cells (RBC), number of white blood cells (WBC) or IgG ($p>0.05$). However, there was a significant increase in neutrophils ($p<0.05$). The steers with RMS had a higher ratio of neutrophils to lymphocytes ($p<0.05$). Even though the treatment affected the neutrophil count and the ratio of neutrophils to lymphocytes, these values were in a normal range. The results of the rumen gross anatomy and histology observations showed that there was no tissue damage of the rumen, thus, it is in a normal state. **Conclusion:** The data indicate that the RMS brush utilization in steers does not result in adverse effects on the health of steers as measured by blood profiles, IgG concentrations and rumen histology.

Key words: Steers, rumen mechanical stimulation, blood parameters, rumen histology, IgG concentration

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rumen mechanical stimulations result in a series of processes where rumination is observed. This stimulation is induced by solid particles in the rumen that assist with processing a naturally fibrous diet[1]. Artificial fibers, such as rumen reticulators[2], plastic pot scrubbers[3] and rumen mechanical stimulators[4], have been used by researchers as a substitute for dietary fibers to provide mechanical stimulation in the rumen.

The utilization of rumen mechanical stimulating (RMS) brushes in dairy cattle and beef cattle has been explored by many researchers. It is introduced to improve the motility of the rumen wall when a high-concentrate diet is fed to cattle as reported by Matsuyama *et al.*[5]. A subsequent increase in the duration of rumination and saliva secretion is observed with RMS[6]. This is beneficial for coping with the acidic rumen environment created by the rapid production of short-chain fatty acids in the rumen. This research also found that RMS decreased the retention time as a result of a higher saliva supply to the rumen[5]. Moreover, Matsuyama *et al.*[7], reported that RMS increased dry matter digestibility.

However, the possible biological side effects of RMS in the rumen have not been investigated. The objective of the present study was to investigate the effects of RMS brush utilization on the health of steers as measured by blood profiles, serum IgG concentration and a rumen wall assessment.

MATERIALS AND METHODS

Experimental animals and diet: The Animal Care and Use Committee of Bogor Agricultural University reviewed and approved the animals used and the experimental procedures of this study (number 01/2015/IPB). This trial was conducted using twenty Brahman crossbred steers with an average initial body weight of 267.50±3.456 kg, divided into a control group (without RMS) and an RMS-administered group. The administration of RMS was carried out by a professional.

The diet was 3% dry matter of body weight with 18.7% crude fiber and 13.2% crude protein. It consisted of a low-forage diet (5.5% maize stover) and a high-concentrate diet (94.5%). Equal portions of the concentrate diet were given twice daily at 7 am and 12 pm, while the forage diet (maize stover) was given at 12 pm. Water was given to the Brahman cross steers *ad libitum*. Samples of the concentrate and corn stover were analyzed for dry matter content and OM total ash after drying at 135°C for 2 h, while CP was calculated as 6.25 times the total nitrogen content measured by the Kjeldahl method and EE according to AOAC[8]. The chemical analysis of feed ingredients used in this experiment is shown in Table 1. Cattle were given a week for adaptation before the experiment began.

Table 1: Chemical composition and digestible nutrients

Chemical composition (%)	Concentrate mix.	Corn stover
DM	88.5	19.3
DM basis		
Organic matter	85.3	92.3
Crude fiber	18.2	27.5
Crude protein	13.6	6.4
Ether extract	4.1	1.4
Crude Ash	9.6	7.4
NFE	54.5	57.2
TDN	69.9	51.6
DE (MJ kg^{-1} DM)	12.89	9.52

NFE: Nitrogen free extract, TDN: Total digestible nutrient, DE: Digestible energy

Rumen mechanical stimulating brush administration: The RMS brush, commercially known as Rumen Fiber®, is an artificial fiber made from non-toxic materials used to stimulate motility of the interior wall of bovine rumen. There were three units of RMS administered/animal. The installation process was similar to the methods described by Golder *et al.*[4].

Blood collection and analysis: Blood samples were collected before feeding from the coccygeal vein and separated into bottles with and without anti-coagulant. Clear serum was stored at -20°C for further blood metabolite and IgG measurements, while heparinized blood samples were used for Hb, PCV, RBC, WBC and leukocytes differential counts.

Determination of packed cell volume (PCV) was performed using heparinized capillary tubes that were centrifuged at 2,500 rpm for 5 min. Hemoglobin was measured using a Sahli's hemoglobinometer, which converts hemoglobin to hematin. For the red blood cell count, blood samples were diluted into a Hayem solution and quantified using a Neubauer counting chamber with a dilution concentration of 10^6 cells μL^{-1}. White blood cells were counted by using similar chambers after dilution in Turk solution (1:50). Furthermore, white blood cells were smeared and fixed with methanol and Giemsa-wright solution. Then 100 white blood cells in a stained blood smear under a light microscope were examined to determine the proportion of each type of white blood cell. Clear serum was harvested and stored at -20°C for an immunoglobulin G measurement. The IgG serum concentrations were measured using ELISA according to the methods in Burgess[9].

Animal slaughter and post-mortem observation of the reticulorumen wall: The steers were slaughtered and post-mortem localization of the RMS brush was conducted in

ten steers at the abattoir. First, the ventral midline was opened and the forestomach was removed. Then, the ruminal contents were cleared and the exterior wall was photographed. The rumen wall was examined to find any gross lesion of the mucosa that could be related to the presence of the RMS brush.

Rumen papillae histology: Samples of the saccus ruminis ventralis and saccus ruminis dorsalis of each group were collected for histological evaluation. The ruminal tissues (1 cm^2) were fixed in 4% formalin solution. After rinsing with water, the samples were dehydrated in various dilutions (50, 60, 70, 80, 90 and 100) of absolute ethanol, then rinsed with benzene twice and, finally, embedded in paraffin. Sections with a 1 μm thickness (5 slices/sample) were stained with hematoxylin/eosin[10].

Statistical analysis: Data were presented as the mean values (SE). A t-test was completed using a GLM procedure in SAS. Data were considered significantly different if the p-value is less than 0.05.

RESULTS AND DISCUSSION

The chemical composition of the diet is displayed in Table 1. The diet provided was a low-forage diet consisting of 95.5% concentrate and 5.5% forage (maize stover).

Hematology attributes of RMS-administered Brahman steers: The RMS brush is an artificial fiber that provides a continuous physical stimulation on the rumen wall. The RMS brush is made of non-toxic plastic that is not digestible. The brush is neither to be expelled to esophagus nor to drop to the lower gastrointestinal tract because of its diameter. The RMS brush was removed after the animal was slaughtered.

The effects of RMS administration on the blood profile of steers are summarized in Table 2. Steers with RMS had a higher percentage of neutrophils ($p<0.05$) than those in the control group. Neutrophils in the RMS group reached 44.10% (4965 cells μL^{-1}), in contrast, steers without the RMS brush had 29.70% (3011 cells μL^{-1}). Together with an alteration in the percentage of neutrophils, the neutrophil-to-lymphocyte ratio increased with the administration of RMS (Table 2). There was no difference in lymphocyte number between the RMS brush and control groups ($p = 0.05$).

Neutrophils are known to be the first defensive response to microorganism infection, inflammation[11,12] and tissue injury[13,14]. The neutrophils directly communicate with macrophages, dendritic cells and lymphocytes to perform their functions[15]. There was no indication of inflammation in the rumen tissue of RMS-administered steers. Similarly, damaged cells were not found in the gross observation as well as in the histological evaluation of the rumen (Fig. 1a, b). Therefore, inflammation was not assessed in this study.

Table 2: Hematology of steers with RMS

Parameters	Control	RMS	p-value
Haemoglobin (g dL^{-1})	11.27±0.21	11.28±0.21	0.98
Haematocrit (%)	36.45±1.00	37.27±1.00	0.57
Red blood cells (x10^6 μL^{-1})	9.83±0.41	10.20±0.41	0.53
White blood cells (x10^3 μL^{-1})	10.14±0.71	11.26±0.71	0.28
Lymphocyte (%)	62.70±4.51	49.50±4.51	0.05
Neutrophil (%)	29.70±4.03	44.10±4.03	0.02
Monocyte (%)	2.30±0.47	2.20±0.47	0.59
Eosinophil (%)	5.30±1.03	4.20±1.03	0.36
N/L ratio	0.56 ±0.13	1.01±0.17	0.04
IgG mg μL^{-1}	8.42±0.28	8.83±0.28	0.31

Values with different letters in a row differ significantly ($p<0.05$)

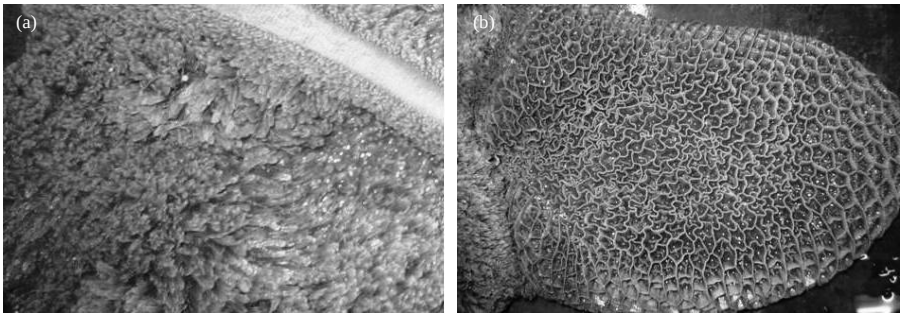

Fig. 1(a-b): (a) Rumen opened at the abattoir after removal of RMS brush, (b) Shows reticulum of RMS administered steer

In this study, changes in the leukocyte profile of the RMS brush-administered steers, associated with a rise in the population of neutrophils, might be a stress response resulting from continuous mechanical stimulation within the rumen. Davis *et al.*[16] mentioned that an increase in leukocytes induces an increase in glucocorticoids. Changes in the leukocyte profile, particularly of neutrophils and the concentration of glucocorticoids, have been used to assess stress level in animals[16].

A possible reason for the changes in neutrophils and the neutrophil-to-lymphocyte ratio found in present research may be stress, as reported by previous studies[17,18]. The stress condition was probably developed in association with exposure to a novelty that might lead to mild stress.

Salak-Johnson and McGlone[19] stated that an activation of stress hormones led to a reduction in lymphocyte proliferation which was not observed in current study. The RMS brush administration did not affect the percentage of lymphocytes in the blood profiles. Thus, the steers with the RMS brush may not be stressed due to the treatment. In agreement with the results of present study, Golder *et al.*[4] mentioned that the RMS brush did not affect the plasma oxidative stress of lactating Holstein cattle. Even though there were alterations in white blood cell parameters, the proportion of neutrophils and the neutrophil-to-lymphocyte ratio examined in present research were considered normal as described by Jones and Allison[20]. Neutrophils act as key components in the activation and regulation of the innate and adaptive immune system[19,11]. Thus, the RMS brush in the rumen might have an effect on the innate immunity of steers characterized by an increase in neutrophil percentage. The proportion of monocytes, eosinophils and the number of white blood cells was similar in both groups ($p>0.05$). The reason for the observed changes in the neutrophil count and the neutrophil-to-lymphocyte ratio in the RMS brush group is unknown.

Insignificant results were also found for hemoglobin, hematocrit and the number of red blood cells ($p>0.05$). Radostits *et al.*[21] observed that the hematocrit percentage of normal beef cattle ranged from 24-46%, we observed a hemoglobin concentration of 8.5-15 g dL^{-1} and red blood cells varied from 5×10^{6}-10×10^{6} μL^{-1}. In observations of both groups, no sample was beyond the normal range of hematology values.

An effect of RMS brush administration on IgG was not observed in this study ($p>0.05$). Thus, there was no increase in immune activity. The total leukocyte count was also unaffected by the treatment ($p>0.05$). In general, continuous mechanical stimulations in the rumen by an RMS brush did not result in undesirable health effects.

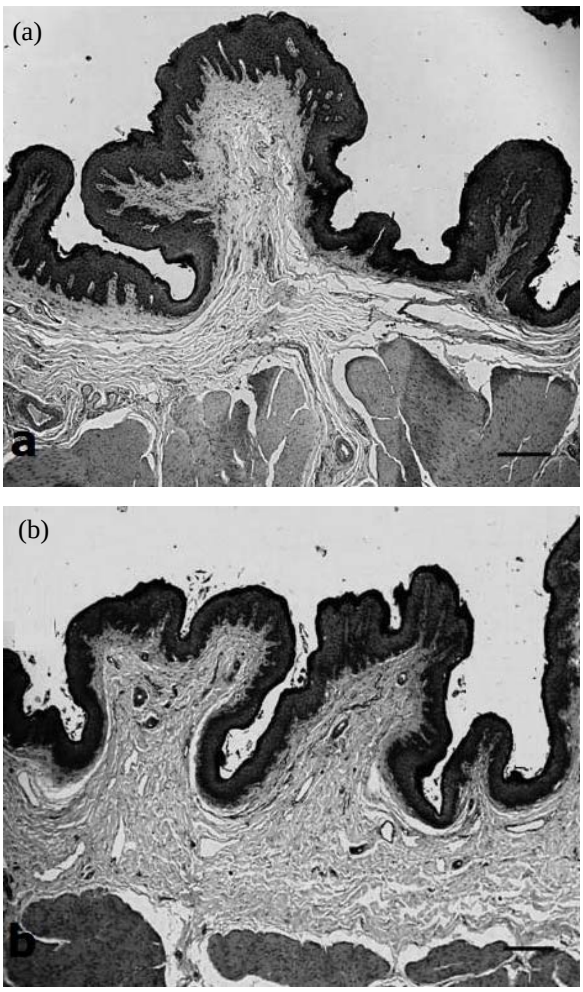

Fig. 2(a-b): Comparison of rumen papillae of ventral sac, (a) control steers (scale bar 50 µm), (b) RMS brush steers (scale bar 50 µm)

Post-mortem examination and histological findings: Immediately after being removed from the body cavity, the rumen was opened and the RMS units were displaced. A gross visual scrutiny showed healthy and normal rumen papillae (Fig. 1a, b). Furthermore, a histological examination revealed no abnormalities in the mucosa rumen (Fig. 2, 3).

The gross description revealed that the papillae of both groups were a dark-brown color. This is expected because both groups were given similar diets. Variations in the shape and size of the papillae of the RMS brush group and the non-RMS brush group were observed in this study. RMS brush is an indigestible material that did not undergo changes in shape or materials. This characteristic made it possible to continuously provide stimuli to certain spots in the gut wall. Reid *et al.*[22] stated that the digesta surface consistency, the origin of tactile stimulation and the reticulorumen wall are the

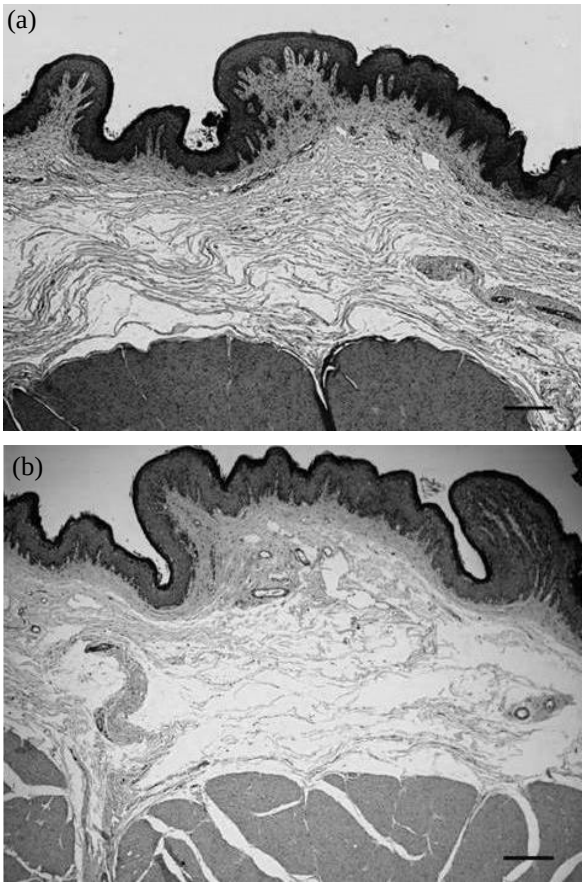

Fig. 3(a-b): Comparison of rumen papillae of dorsal sac (a) Control steers (scale bar 50 µm), (b) RMS brush steers (scale bar 50 µm)

three important factors employed in producing physical stimulation in the rumen. Thus, the brush's role resembles the function of the digesta surface texture. In addition, its physical characteristics affected its retention and localization in cattle[23]. The RMS brush might have been situated in the rumen mat and performed as floating fiber. It provided mechanical stimulation to the rumen wall as fiber does, but it did not attach to the rumen wall. Matsuyama *et al.*[5] reported that an increased rumination time in RMS brush-administered steers was associated with an increase in rumen contraction. Therefore, the RMS brush application might contribute to a modification in rumen motility without negative effects on the rumen wall. Similar to the RMS brush utilization, the insertion of a ceramic transponder bolus (e-bolus) was reported to change rumen motility and thus alter rumination pattern[23,24]. However, there was a clear mark in the ventral sac of the rumen where the e-bolus was attached. Atrophied mucosa was reported as a side effect of e-bolus use[23]. An *in vitro* study of e-bolus utilization showed an increase in mucosal cell proliferation and changes in the gene expression of *Escherichia coli*[25].

The rumen plays an important role both in digestion and immune function, thus allowing for a homeostatic condition[26]. The luminal environment should accommodate a symbiotic relationship to support a homeostatic condition in the rumen[27]. In this research, the presence of RMS did not result in negative effects on the rumen presentation as seen in our gross rumen evaluation.

Similarly, observations of the histology, morphology and integrity of the rumen epithelium showed that both groups were healthy. Hollmann *et al.*[28] explained that the function of the rumen epithelium is to protect the rumen wall from the luminal environment, such as acidic rumen liquor. It is also responsible for securing the rumen from high osmotic pressure and the secondary metabolites produced by rumen microbes. Another protective function is to restrict the rumen wall from dietary material abrasion to maintain ideal metabolic and absorption function. Steele *et al.*[29] reported that the high concentrate diet created a greater risk of changes to the epithelium of the rumen. In the present study, the RMS brush administration provided tactile stimulation by mildly scrubbing the reticulorumen. This triggers regurgitation, rumination and saliva secretion. Reid *et al.*[22] reported that tactile stimulation resulted from the relative motion of digesta texture and reticulorumen mucosa. A continuous tactile stimulation produced by an RMS brush during a 3 month fattening period did not result in detrimental effects to the rumen as observed in gross evaluation and microscopic examinations.

This study assessed the health status and rumen wall condition of Brahman crossbred steers after 93 days of RMS brush administration. In general, the blood profiles and IgG levels were in normal ranges; thus, the application of an RMS brush does not result in adverse effects to the health of steers. Therefore, the utilization of an RMS brush is safe to use as artificial fiber. The RMS brush administration is useful for steers fed with a high-concentrate diet and a low-forage diet. It is recommended that the installation of the RMS brush is conducted by a professional. Future research should focus on the adaptation of the steers to the presence of RMS by conducting periodic health assessments. It is also recommended that the experimental animal should be conditioned to regular blood collection to minimize stress.

CONCLUSION

The RMS brush characteristics induced motility by resembling the natural physical stimuli of the digesta surface texture rubbing the rumen wall. The application of the RMS brush may lead to changes in leukocyte profiles. Increases of neutrophils and the neutrophil-to-lymphocyte ratio were observed; however, the values are within normal ranges. Therefore, the alteration will not affect the health condition of the RMS brush-administered steers. It is clear from this gross examination and microscopic testing of the ruminal reticulum that there were no lesions or tissue abnormalities. It can be concluded that the RMS brush-administered steers are in good health as measured by their blood parameters and rumen wall conditions.

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