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## Characterization of the absorptive surface area of the reticulorumen and omasum of buffaloes

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**Abstract.** The absorption of volatile fatty acids by the reticulorumen and omasum in buffaloes is poorly understood. In order to evaluate the absorption capacity of the proventriculus in buffaloes, five adult animals had their stomachs removed after slaughter, the compartments of the stomachs were dissected, and the rumen, reticulum, and omasum were separated; the rumen was separated into its several bags. Thereafter, these were digitized in a scanner and the images processed using software (UTHSCSA software Image Tool version 3.00) to determine their surface areas. In addition, a fragment of the various rumen sacs was removed to determine the area and the correlation with the total area of the compartments. The ventral sac of the rumen exhibited the largest absorption surface area, not differing from the omasum. There was no difference between the ventral, cranial, blind caudoventral, caudodorsal, and dorsal cavities of the rumen. Thus, our results confirm that the absorptive surface area of the compartments of the proventriculus can be estimated via a biopsy of the cranial sac of the rumen.

**Keywords:** Acidosis, morphology, physiology, short chain fatty acids

## *Caracterização da superfície de absorção do ruminoretículo e omaso de bubalinos*

**Resumo.** A absorção de ácidos graxos voláteis pelo ruminoretículo e omaso em búfalos é mal compreendida. Para avaliar a capacidade de absorção do proventriculo de búfalos, cinco animais adultos tiveram o estômago removido após o abate, os compartimentos dos estômagos foram dissecados e o rúmen, retículo e omasum foram separados. O rúmen foi separado em seus vários sacos. Posteriormente, estes foram digitalizados em um scanner e as imagens processadas usando um software (UTHSCSA software Image Tool versão 3.00) para determinar suas áreas de superfície. Além disso, um fragmento dos vários sacos do rúmen foi removido para determinar a área e a correlação com a área total dos compartimentos. O saco ventral do rúmen exibiu a maior área de superfície de absorção, não diferindo do omasum. Não houve diferença entre os sacos ventral, cranial, caudoventral, caudodorsal e dorsal do rúmen. Assim, nossos resultados confirmam que a superfície absorptiva dos compartimentos do proventriculus pode ser estimada através de uma biópsia do saco cranial do rúmen.

**Palavras-Chave:** Ácido graxo de cadeia curta, acidose, fisiologia, morfologia

## Introduction

The absorption of short chain fatty acids (SCFA) in ruminants is directly related to the available absorptive surface of the reticulo-rumen, omasum ([Daniel and Resende Júnior, 2012](#)), abomasum, and cecum-colon ([Cardoso et al., 2013](#)), and it can be manipulated by diet ([Lima et al., 2015](#); [Santoro et al., 2015](#)).

[Daniel et al. \(2006\)](#) evaluated the absorption surface of the reticulo-rumen and omasum of bovines and observed that although the absorptive surface area of the reticulo-rumen is greater than that of the omasum, the surface/digest ratio was greater for the omasum. The authors found a regression model that can estimate the absorptive surface area of the reticulo-rumen through a biopsy of the ventral sac of the rumen. Thus, it is possible to evaluate the manipulation of the absorptive surface area of the epithelium of the reticulo-rumen in cannulated animals without having to kill them and to measure the absorptive surface area of slaughtered experimental animals without having to evaluate the area of all the compartments.

There are several studies characterizing the SCFA absorptive surface area of cattle, but only a few in the case of buffaloes. In addition, the reticulo-rumens of buffaloes reportedly have a higher absorptive capacity than those of cattle of the same metabolic weight ([Alves and Sales, 2000](#); [Leão et al., 1985](#)); thus the results of studies conducted with cattle cannot be considered representative of buffaloes. Thus, the objective of this study was to characterize the SCFA absorptive surface area of the reticulo-rumen and omasum of buffaloes.

## Material and methods

All procedures were approved by the Bioethical Committee for Animal Utilization at the Federal University of Amazon, Brazil (Draft protocol 048 / 2017- CEUA/UFAM).

Five, adult half-breed buffaloes, weighing  $349 \pm 79$  kg from a commercial slaughterhouse, were fed exclusively on pasture. The buffaloes had their stomach removed immediately after slaughter and the reticulo-rumen and omasum were immediately transported in isothermal boxes to an animal anatomy laboratory where they were washed in running water. After washing, dissection was performed to remove excess adipose tissue and aponeuroses with the separation of anatomical regions.

The anatomical regions of the reticulo-rumen were separated by isolating the reticulum, cranial sac, dorsal sac, caudo-dorsal blind sac, ventral sac, and caudo-ventral blind sac of the rumen. Post separation, samples ( $\sim 1$  cm<sup>2</sup>) were taken of the various anatomical regions. The fragments were immersed in a physiological solution (0.9% NaCl; pH 6) to preserve their biological characteristics.

All methodologies used were as described by [Daniel et al. \(2006\)](#). Briefly, each anatomical region was fragmented to enable scans of its images through a scanner (HP Deskjet 2050, EUA). Morphological measurements of the fragments previously preserved in physiological solution were performed by counting the number of ruminal papillae present in each fragment. Ruminal papillae were sectioned in the base using scissors and scanned later. After being scanned, the organ fragments were measured using the UTHSCSA software Image Tool version 3.00 (Health Sciences Center of San Antonio, University of Texas). The rumen area was estimated by scanning the entire surface of a fragment with given a number of papillae. Thus, after determining the surface area of the fragment, it was correlated with the rest of the anatomical region. The reticulum area was determined in a manner similar to that of the rumen. The area of omasum absorption was determined by dissecting all slides followed by scanning and analysis using the UTHSCSA software Image Tool.

The statistical analyses were performed following the GLM procedure of the SAS statistical package, accounting for random effects according to the following model:  $Y_i = \mu + C_i + e_i$ , where  $Y$  = area of the anatomical regions of the provector,  $\mu$  = general mean,  $C_i$  = Compartment or region of the proventricle, and  $e_i$  = independent error and identically distributed in assumed normal distribution with mean 0 and variance  $\sigma^2$ . The averages were compared by one using the 5% Tukey test. A Pearson Correlation test was performed between the areas of the fragments of the various compartments of the rumen and real total areas of the rumen, reticulum, reticulo-rumen, omasum and total proventriculus area.

## Results and discussion

The mean absorptive surface area of each region the reticulo-rumen and omasum and the percentage of surface area that each region accounts for in relation to the total absorptive surface area of the proventriculus are presented in [table 1](#). The ventral sac of the rumen was had the largest absorptive surface area surface of absorption; however, the difference in surface areas between the ventral sac of the rumen and omasum was not significant despite the large numerical difference. The rumen had an absorptive surface area of 10.23 m<sup>2</sup>, representing 78.9% of the total proventriculus area. Although the omasum represents only 17.0% of the total proventriculus area, similar to the 21.5% found in cattle by [Daniel et al. \(2006\)](#). The absorptive surface area of the reticulo-rumen was approximately five times greater than that of the omasum. Using a similar approach [Daniel et al. \(2006\)](#) observed that the absorptive surface area of the reticulo-rumen in cattle was four times greater than that of the omasum, with the animals having an average weight greater than that identified in this study (479 ± 185 kg of live weight). Although it was not compared in this study, the data suggest that the higher feed efficiency of buffaloes in relation to cattle (as cited in some studies) can be explained in part by the greater absorption capacity of reticulo-rumen, which would provide a greater absorptive capacity of SCFA ([Resende Júnior et al., 2006](#)).

**Table 1.** Average absorptive surface area of the different regions of the rumen, reticulum, and omasum, and the percentages that each region represents in relation to the total area of the proventriculus of half-breed buffaloes - Parintins/Amazonas, Brazil - 2017

Regions	Area (m <sup>2</sup> )	% of the total proventriculus area
Ventral Sac	3.72a	28.5%
Cranial Sac	2.10ab	16.1%
Blind caudoventral sac	1.21b	9.3%
Blind caudodorsal sac	1.55b	11.9%
Dorsal sac	1.65b	12.6%
Reticulum	0.60c	4.6%
Omasum	2.22ab	17.0%
SD	0.389	
P	<0.01	

a, b, c Means followed by different letters in the column are not equal (Tukey  $P < 0.05$ ). SD = standard error.

The development of ruminal papillae and consequently the ruminal absorption surface is correlated with the type of diet. Animals that consume diets with higher non-fibrous carbohydrate content, produce more volatile fatty acids (VFAs) and need a larger absorption surface area ([Melo et al., 2013](#)). In addition, VFAs stimulate the multiplication of cells in the ruminal and omasal epithelium in similar proportions ([Costa et al., 2008](#)).

The ruminal content is stratified: gaseous in the cranial region, fibrous in the intermediate region, and liquid in the ventral region of the rumen ([Melo et al., 2013](#)). This stratification may explain the greater absorptive surface area of ventral region of the rumen, since it is the liquid phase that has the highest concentration of VFAs, than another phase stimulating organ development ([Costa et al., 2008](#)).

The correlation of the area (in square meters) of a fragment of the cranial sac of the buffalo rumen with the total area of the rumen, reticulum, reticulo-rumen, omasum and total proventriculus are presented in [table 2](#). There was no correlation between the areas of fragments ventral sac, dorsal sac, caudorsal blind sac, and caudoventral blind sac. [Daniel et al. \(2006\)](#) found a positive correlation between the fragment area of the ventral sac of the rumen and the total area of the rumen and reticulorumen. However, here we found that it is more suitable to use the cranial sac of the buffalo rumen in the estimation of the absorptive surface area of the rumen, reticulorumen, and total proventriculus.

**Table 2.** Pearson correlation between the area of a fragment of the cranial sac of the rumen in buffaloes and the reticulum, reticulo-rumen, omasum, and total area of the proventriculus - Parintins/Amazonas, Brazil - 2017

Organs	Equations	r	P
Rumen, m <sup>2</sup>	(2781,6* fragment +62834,0) /10000	0,83	0,08
Reticulum, m <sup>2</sup>	(297,14* fragment +993,8) /10000	0,47	0,43
Reticulorumen, m <sup>2</sup>	(3078,8* fragment +63828,0) /10000	0,91	0,03
Omasum, m <sup>2</sup>	(940,0* fragment +6236,3) /10000	0,69	0,20
Total proventriculus, m <sup>2</sup>	(4018,7* fragment +70064) /10000	0,86	0,05

Fragment = Area of the fragment (cm<sup>2</sup>) / area of the fragment base (cm<sup>2</sup>); the area of the fragment corresponds to the area of the base plus the area of each papilla times the number of papillae of the fragment.

## Conclusion

The absorption surface of the buffalo rumen is five times larger than that of the omasum, and the ventral sac showed the highest absorptive surface area. The absorptive surface area of the total proventriculus, rumen and buffalo reticulo-rumen can be estimated by using a fragment of the dorsal rumen sac, facilitating the evaluation of the effects of diet on the development of buffalo stomachs

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