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ABSORPTION AND METABOLISM OF VOLATILE FATTY ACIDS BY RUMEN AND OMASUM

Absorção e metabolismo de ácidos graxos voláteis pelo rúmen e omaso

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ABSTRACT

Volatile fatty acids (VFA) absorption and metabolic capacity of rumen and omasum were compared, *in vitro*. Fragments of rumen wall and omasum laminae were taken from eight adult crossbred bovines. An isolated fragment of the mucosa was fitted in a tissue diffusion chamber. Valeric acid and CrEDTA were added to ruminal fluid and placed on the mucosal side and buffer solution was placed on the serosal side. Fractional absorption rates were measured by exponential VFA:Cr ratio decay over time. Metabolism rate was determined as the difference between VFA absorbed and VFA which appeared on the serosal side over time. Mitotic index was higher in omasum (0.52%) than in rumen epithelium (0.28%). VFA fractional absorption rate was higher in omasum (4.6%/h.cm^2) than in rumen (0.4%/h.cm^2). Acetate, propionate, butyrate, and valerate showed similar fractional absorption rates in both fragments. Percentage of metabolized acetate and propionate was lower than butyrate and valerate in both stomach compartments. In the rumen, individual VFA metabolism rates were similar (mean of 7.7 µmol/h.cm^2), but in the omasum, valerate (90.0 µmol/h.cm^2) was more metabolized than butyrate (59.6 µmol/h.cm^2), propionate (69.8 µmol/h.cm^2) and acetate (51.7 µmol/h.cm^2). Correlation between VFA metabolism and mitotic index was positive in the rumen and in the omasum. In conclusion, VFA metabolism and absorption potential per surface of the omasum is higher than that of the rumen. Variations on rumen and omasum absorption capacities occur in the same way, and there are indications that factors capable of stimulating rumen wall proliferation are similarly capable of stimulating omasum walls.

Index terms: Morphology, physiology, bovine, ussing chamber, forestomach.

RESUMO

A capacidade de absorção e metabolismo de ácidos graxos voláteis (AGV) pelo rúmen e omaso foi comparada, *in vitro*. Fragmentos da parede do rúmen e das lâminas do omaso foram coletados de oito bovinos mestiços adultos. Um fragmento isolado da mucosa foi colocado em uma câmara de difusão tecidual. Ácido valérico e CrEDTA foram adicionados ao fluido ruminal e colocados no compartimento da câmara voltados para a mucosa e uma solução tampão foi colocada no compartimento voltado para a serosa. As taxas fracionais de absorção foram medidas pela queda exponencial da relação VFA:Cr ao longo do tempo. A taxa de metabolismo foi determinada pela diferença entre a quantidade de AGV absorvida e a detectada no compartimento serosal da câmara. O índice mitótico foi mais alto no epitélio do omaso (0.52%) do que no do rúmen (0.28%), bem como a taxa fracional de absorção, 4.6%/h.cm^2 e 0.4%/h.cm^2, respectivamente. Acetato, propionato, butirato e valerato tiveram taxas fracionais de absorções similares em ambos os compartimentos. As porcentagens do acetato e do propionato metabolizados foram mais baixas do que a do butirato e valerato em ambos os compartimentos. No rúmen, a taxa metabólica individual dos AGV foi similar (média de 7.7 µmol/h.cm^2), mas, no omaso, o valerato (90.0 µmol/h.cm^2) foi mais metabolizado do que o butirato (59.6 µmol/h.cm^2), propionato (69.8 µmol/h.cm^2) e acetato (51.7 µmol/h.cm^2). A correlação entre o metabolismo de AGV e o índice mitótico foi positiva no rúmen e no omaso. Concluiu-se que o potencial de metabolismo e de absorção de AGV por unidade de área do omaso é mais alto do que o do rúmen. A variação da capacidade de absorção do rúmen e do omaso ocorre na mesma direção e existem indícios de que os fatores capazes de estimular a proliferação da parede do rúmen são também capazes de estimular a parede do omaso.

Termos para indexação: Morfologia, fisiologia, bovino, câmara de ussing, proventrículo.

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INTRODUCTION

Absorptive surfaces of reticulorumen (DIRKSEN et al. 1984) and probably that of omasum (BALDWIN et al. 2004) are directly related to VFA absorption capacity. The understanding of omasal physiology seems to be as important in the control of current digestive disturbances as the understanding of ruminal physiology.

Despite the absorptive surface of reticulorumen (7.7 m^2) being higher than that of the omasum (2.1 m^2) (DANIEL et al. 2006), and absorption and metabolism potential of the rumen being well documented, the potential of absorption and metabolism of the omasum and any comparison of these parameters among organs are poorly understood. The purpose of this work was to compare, *in vitro*, VFA absorption and the metabolism capacity of rumen and omasum.

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MATERIAL AND METHODS

Eight adult crossbred bovines, of different weights and ages, of both sexes, coming from a commercial slaughterhouse, were allocated to a completely randomized block design where each animal was considered one block. Animals were slaughtered by exsanguinations after stunning, and the forestomach was removed from abdominal cavity 5 to 10 minutes later. One portion of rumen wall was taken from the ventral sac (*Recessus ruminis*) and one portion of omasal laminae (*Laminae omasi*) were cut and placed in a Krebs-Ringer bicarbonate buffer solution (Sigma-Aldrich, Saint Louis, Missouri, USA) at 38°C with pH adjusted to 7.4, and immediately transported to the laboratory. In the laboratory, ruminal mucosa was isolated by removing serosa and muscular layers, and omasal mucosa was carefully separated. Mucosal sheets were cut into circles (3 cm diameter) and inserted between half-chambers of a tissue diffusion chamber with an inner aperture of 4.91 cm^2 (Indústria e Comércio Estanhof Ltda, Lavras/MG, Brazil). Bathing solutions on both sides of the chamber were kept circulating by a mini air compressor and maintained at 38° C in water-jacketed reservoirs. Absorption assays were started 30 to 40 minutes after slaughter.

Previously, about 320 mL of rumen fluid was collected from a fistulated cow, fed on tropical pasture and 2.5 kg d^{-1} of a ground corn and soybean meal based commercial concentrate. Fermentation was stopped by the addition of 6.5 mL of 50% sulfuric acid. Forty milliliter aliquots of rumen fluid were frozen at -20° C. For each animal one aliquot was thawed before incubation. Valeric acid (ALLEN et al., 2000) and CrEDTA solution (BINNERTS et al., 1968) were added to rumen fluid until reaching concentrations of 25 mM and 1 mM, respectively. PH was adjusted for 6.8 with addition of 50% NaOH solution and the final solutions of rumen fluid plus Valeric acid and CrEDTA were placed on mucosal side of the chamber fitted with rumen or omasum fragments. The Krebs-Ringer bicarbonate buffer solution with pH adjusted for 7.4 was used on the serosal sides of the chamber. All solutions were heated at 38° C before incubation and this temperature was maintained during incubation. Initial pH of mucosal fluid was the same for rumen and omasum because of their similarity in vivo (EDRISE et al. 1986).

Samples (300 µL) of mucosal and serosal fluids were obtained immediately after the assay was started (time zero) and at 25, 50, 75, 100, and 125 minutes thereafter. Mucosal pH was determined at each sampling time and serosal pH was determined at the start and end of the incubation. The samples were mixed with 100 µL of 10% sulfuric acid solution and immediately frozen at -20°C for subsequent analyses of VFA and chromium.

VFA and chromium concentrations were determined in supernatant obtained after centrifugation at 8,855 g for 15 minutes at room temperature. Samples were analyzed for VFA by Gas Liquid Chromatography (CP-3800 Gas Chromatograph Varian, with flame ionization detector, Varian Chromatography Systems, California, USA) using a capillary column with nitroterephthalic acid-modified phase, chemically bonded polyethylene glycol, 25 m x 0.25 mm I.D. and 0.2 µm of film thickness (CP-Wax 58 (FFAP) CB, Varian Analytical Instruments, California, USA), and N_2 as carrier gas. Oven temperature was kept at 65°C for 30 s, then increased to 125° C, at a rate of 20° C/minute, and then increased again to 170° C, at a rate of 50° C/minute. Total time of analysis was 4.9 minutes. Chromium concentration was determined by atomic absorption spectrophotometry (SpectrAA – 100 Varian, Varian Australia Pty LTD, Victoria, Australia).

Exponential VFA:Cr decay ratio over time on the mucosal side was used to estimate fractional absorption rate of VFA (RESENDE JÚNIOR et al. 2006). Since the major objective of this study was to compare compartments, only net absorption rates were determined. It was not accessed mucosal-serosal and serosal-mucosal unidirectional transport fluxes. VFA metabolism was estimated as the difference between VFA absorbed and that appearing in serosal fluid.

Rumen surface area was measured with the aid of a scanner (Scanjet 4C, Hewlett Packard). The number of papillae was counted and twelve papillae were randomly sectioned and digitalized for surface determination through the image analysis software UTHSCSA Image Tool, version 3.00 (Free Software, The University of Texas Health Science Center in San Antonio). Total fragment surface area was the sum of papillae surface plus wall surface minus surface of the base of papillae, assumed as 0.002 cm^2 per papilla (DANIEL et al. 2006). Omasum absorptive surface area was considered as constant (4.91 cm^2 – chamber orifice), since omasum papillae were not take into account, because of their very small size.

To determine mitotic index, 5 µm sections of histologically prepared fragment were stained with hematoxylin and eosin, and to measure thickness of epithelium layers, 5 µm sections were stained with Masson's Tricromic. Mitotic index of cells of epithelium basal layer, epithelium thickness, keratin layer thickness,

and non-keratin layer thickness were determined with an optic microscope (Ernst Leitz Wetzlar Nr. 438895, Germany) at 400 magnification. Mitotic index was determined by counting all mitotic nucleus, which were expressed as percentage of total visible nucleus. Percentage of cells undergoing mitosis was the average of five independent evaluators.

Morphological variables were compared using the MIXED procedure (LITTELL et al., 1998) of SAS (SAS INSTITUTE 1999), according to the following model: $y_{ij}=\mu+\alpha_i+\beta_j+\varepsilon_{ij}$; where μ: overall mean; α_i: random effect of animal (i = 1 to 8); β_j: fixed compartment effect (j = rumen or omasum); and ε_{ij}: residual error assumed independently and identically distributed in a normal distribution with mean zero and variance σ^2.

Fractional absorption and metabolism rates in both compartments (rumen and omasum) were analyzed as a split plot arrangement, using the MIXED procedure of SAS, according to the following model: $y_{ijk}=\mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\gamma_k+\beta\gamma_{jk}+\varepsilon_{ijk}$; where μ: overall mean; α_i: random animal effect (i = 1 to 8); β_j: fixed compartment effect (j = rumen or omasum); $\alpha\beta_{ij}$: interaction between animal and compartment effect (error term used for testing compartment effect); γ_k: fixed VFA effect (k = acetate, propionate, butyrate or valerate); $\beta\gamma_{jk}$: interaction between compartment and acid effect; and ε_{ijk}: residual. For pH analysis the time collection effect was added to the model.

Pearson correlation coefficients among variables within compartments were performed with CORR procedure of SAS. Linear regressions among measurements of both forestomach compartments were performed with the REG procedure of SAS.

RESULTS AND DISCUSSION

Mitotic index (Table 1) indicates a faster cell proliferation in omasum than in rumen epithelium. Rumen absorptive surface area, however, was higher than that of the omasum, mostly due to ruminal papillae. Despite systemic regulation on cell proliferation (SAKATA et al. 1980; SHEN et al. 2004), the local stimulus affects epithelial cells dynamics (SAKATA; TAMATE 1976; GÁLIFI et al. 1986). Thus, higher surface:digesta mass ratio in omasum (DANIEL et al. 2006) could maximize VFA local stimulation on cell proliferation, since large amounts of VFA pass from reticulorumen to omasum incorporated to the ruminal fluid phase (RESENDE JÚNIOR et al. 2006). Omasum mitotic index was highly correlated with rumen mitotic index (Figure 1), indicating that stimulating factors for rumen wall proliferation may be the same for omasum wall.

Total thickness, thickness of keratin layer, and thickness of non-keratin layers of the rumen and omasum epithelium were similar (Table 1) and highly correlated with each other (Figure 2). Given that the epithelium dimension is the result of cellular synthesis and deletion (TAMATE; FELL 1977), higher omasum epithelium cell proliferation might have been compensated by higher cell loss, since larger surface:digesta mass ratio and dehydration of content in the omasum could increase abrasive effects on the epithelium.

Table 1 – Absorptive surface area, mitotic index, epithelium layers thickness and mucosal fluid VFA concentration in rumen and omasum.

	Compartment			
	Rumen	Omasum	SEM[1]	*P*-value
Absorptive surface (cm^2)	57.58	4.91	5.83	< 0.01
Mitotic index (%)	0.28	0.52	0.02	< 0.01
Thickness of the epithelium (μm)	65.04	61.71	4.66	0.33
Thickness of the keratin layer (μm)	12.95	11.82	1.24	0.27
Thickness of the non-keratin layers (μm)	52.09	49.89	3.87	0.45
Total VFA (m*M*)	123.21	125.35	2.58	0.31
Acetate (m*M*)	56.28	56.17	1.48	0.94
Propionate (m*M*)	25.39	25.29	0.63	0.91
Butyrate (m*M*)	16.81	17.40	0.32	0.15
Valerate (m*M*)	24.73	25.32	0.31	0.23

[1] Standard error of the mean.

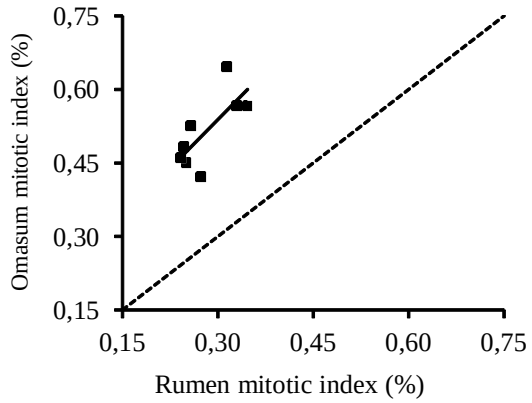

Figure 1 – Correlation between rumen mitotic index (RMI) and omasum mitotic index (OMI). OMI = 0.1463 + 1.3057 RMI; $r^2 = 0.53$; $P = 0.04$; $P = 0.57$ for slope = 1. Dashed line shows the equality line.

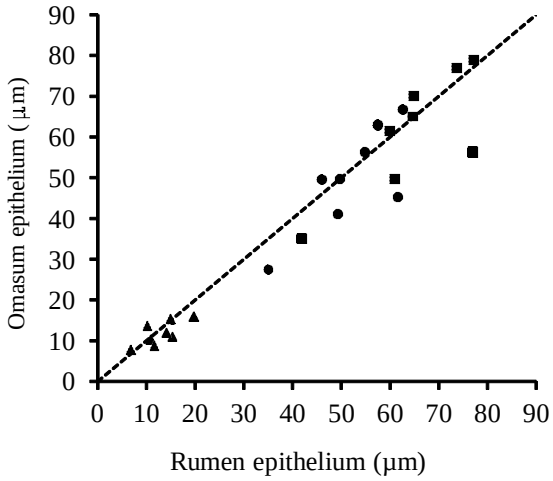

Figure 2 – Correlations between thickness of the rumen (TRE) and omasum (TOE) epithelium (■);thickness of the keratin layer of the rumen (TKRE) and omasum (TKOE) epithelium (▲); and thickness of the non-keratin layers of rumen (TNKRE) and omasum (TNKOE) epithelium (●). TOE = -2.6839 + 0.9901 TRE; $r^2 = 0.62$; $P = 0.02$; TKOE = 4.5998 + 0.5577 TKRE; $r^2 = 0.55$; $P = 0.04$; TNKOE = -6.4885 + 1.0823 TNKRE; $r^2 = 0.62$; $P = 0.02$; Dashed line shows the equality line.

Total and individual VFA concentrations did not differ among solutions used for rumen and omasum fragments (Table 1), which excludes the possibility of VFA concentration having influenced the VFA absorption (DIJKSTRA et al. 1993). Due to the addition of valeric acid to the mucosal fluid, its concentration was higher than that of butyrate and similar to that of propionate, which does not occur in physiologic conditions. Utilization of valeric acid as a marker of ruminal VFA clearance, in vivo, was proposed by Allen et al. (2000) and validated by Resende Júnior et al. (2006), but published data about its use *in vitro* is scarce.

VFA fractional absorption rate by surface of the omasum epithelium was 11 times greater than that of the rumen (Table 2). This finding demonstrates the higher absorptive potential of omasum, even considering the fact that the absorptive surface of reticulorumen is approximately 4 times higher than that of omasum (DANIEL et al. 2006). The reason for this high magnitude of difference could be related to absorption mechanisms in the organ wall. In the rumen, VFA are absorbed under dissociated (through bicarbonate exchange) and non-dissociated forms (lipophilic and highly permeable) (GÄBEL et al. 2002). According to VFA pKa is minor than 4.9, over 95% of the VFA should be in ionized form at the ruminal pH around 6 to 7. In the omasum, VFA seems to be absorbed predominantly under protonated form (ALI et al. 2006). Furthermore, unlike the rumen, the omasum absorbs bicarbonate, through chloride exchange (ALI, et al. 2006).

Correlation between rumen and omasum VFA fractional absorption rates was positive (Figure 3), indicating that variation in the absorptive capacity of those organs is unidirectional. Thus, the use of nutritional strategies to prevent digestive disturbances related to ruminal VFA accumulation could to improve the absorption capacity of the whole forestomach.

VFA metabolism in rumen epithelium, as a proportion of absorbed VFA, was higher than in omasum epithelium (Table 2), probably because of its larger mass of active metabolic cells. Since thicknesses of non-keratin layers of the rumen and omasum epithelia were similar (Table 1), the absorptive surface can be used as an indicator of active metabolic cells mass.

Thickness of the rumen epithelium keratin layer ($r^2 = 0.76$; $P = 0.03$) and the mitotic index ($r^2 = 0.68$; $P = 0.06$) were positively correlated with rumen metabolism rate. In the omasum, correlation between VFA absorption and mitotic index was positive ($r^2 = 0.80$; $P = 0.02$).

Table 2 – Fractional absorption and metabolism rates of volatile fatty acids in bovine rumen and omasum fragments incubated for 2.08 hours in a tissue diffusion chamber.

	Rumen				Omasum					*P*-value[1]		
	A[2]	P[3]	B[4]	V[5]	A	P	B	V	SEM[6]	Comp	Acid	Comp *Acid
Fractional absorption rate (%/h)	19.70	23.75	18.36	22.11	18.48	25.79	23.57	22.82	4.05	0.57	0.51	0.86
Fractional absorption Rate/surface (%/h.cm^2)	0.42	0.49	0.34	0.45	3.76	5.25	4.80	4.67	0.52	<0.01	0.45	0.50
Metabolism (%)	11.10	33.21	43.12	46.46	10.83	28.94	36.49	37.59	2.21	0.01	<0.01	0.09
Metabolism rate (μmol/h)	262.11	400.98	348.95	544.10	253.57	342.64	292.37	445.06	36.33	0.06	<0.01	0.54
Metabolism rate/surface (μmol/h.cm^2)	4.96	7.96	6.90	10.81	51.66	69.80	59.56	89.97	4.68	<0.01	<0.01	<0.01

[1] Comp: compartment effect; Acid: Acid effect; Comp*Acid: Interaction between compartment and acid effect.
[2] A: acetate; [3] P: propionate; [4] B: butyrate; [5] V: valerate.
[6] Standard error of the mean.

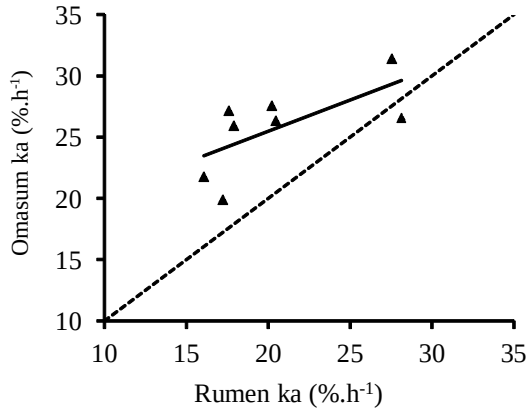

Figure 3 – Correlation between VFA fractional absorption rate in the rumen (Rumen ka) and in the omasum (Omasum ka). Omasum ka = 15.2422 + 0.5117 Rumen ka; $r^2 = 0.45$; P = 0.07; Dashed line shows the equality line.

Non-physiologic concentrations of valerate on the mucosal side of both rumen and omasum may have affected butyrate metabolism (KRISTENSEN; HARMON 2005). Kristensen and Harmon (2005) observed that the portal appearance of the butyrate that disappeared from the rumen increased from 25% to 52% when ruminal concentration of valerate increased from 1.2 to 8.0 mmol kg^{-1}. Moreover, the same authors observed that the increase in ruminal butyrate concentration (4 to 36 mmol kg^{-1}) resulted in an increase of portal recovery of butyrate (18 to 52%) and valerate (16 to 54%), reinforcing the theory of competing metabolism routes in the ruminal epithelium between those two VFA (KRISTENSEN; HARMON 2004).

The rumen VFA metabolism mean rate (389.03 μmol h^{-1}) was higher (P = 0.06) than that of omasum (333.41 μmol h^{-1}) (Table 2), reflecting the largest percentage of metabolized VFA, since amounts of VFA absorbed from mucosal side were similar. The rumen and omasum valerate metabolism mean rate (494.58 μmol/h) was higher than that of butyrate (320.66 μmol/h) and of propionate (371.81 μmol/h), which were higher than that of acetate (257.84 μmol/h) (Table 2).

Increase of mucosal fluid pH and its reduction in serosal fluid (Figure 4) reflected VFA transfer from the mucosal to serosal direction. Rumen and omasum mucosal pH have increased during incubation, but there was a tendency (P = 0.08) of interaction between compartment and time. Rumen mucosal pH rose faster than omasum mucosal pH. This may have happened because of bicarbonate secretion by rumen epithelium and bicarbonate absorption by omasum epithelium, seeing as VFA absorption rates were similar among compartments.

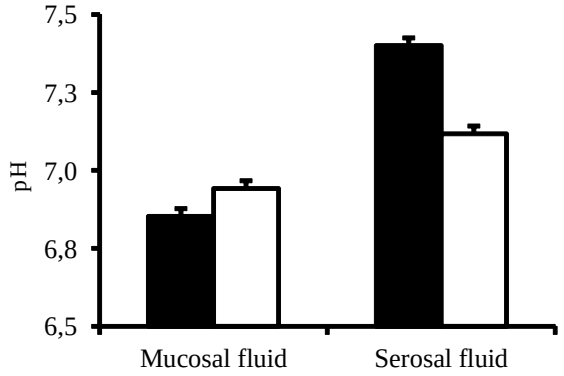

Figure 4 – Means of initial (black bar) and final (white bar) pH of mucosal and serosal fluid of rumen and omasum fragments incubated for 2.08 hours in a tissue diffusion chamber. $P = 0.30$ for compartment effect; $P < 0.01$ for solution effect; $P = 0.17$ for interaction between compartment and solution effect; $P < 0.01$ for time effect; $P = 0.27$ for interaction between compartment and time effect; $P < 0.01$ for interaction between solution and time effect; $P = 0.16$ for interaction between compartment, solution and time effect.

CONCLUSIONS

VFA metabolism and absorption potential per surface of the omasum is higher than that of the rumen, showing that there are important physiological differences between these organs and highlighting the necessity of more research on omasum physiology.

Rumen and omasum absorption capacities vary in the same way, and there are indications that factors capable of stimulating rumen wall proliferation also are capable of stimulating omasum wall growth.

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REFERENCES

ALI, O.; Z. et al. Transport of acetate and sodium in sheep omasum: mutual, but asymmetric interactions. **Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology**.Heidelberg, v.176, n.5, p. 477-87, jun. 2006.

ALLEN, M.S., L.E. et al. Method to measure fractional rate of volatile fatty acid absorption from the rumen. In: **Proceedings of the 25th Conference on Rumen Function.** Chicago, IL, p.26. 2000.

BALDWIN, R.L. V. I.; MCLEOD, K.R.; CAPUCO, A. V. Visceral tissue growth and proliferation during the bovine lactation cycle. **Journal of Dairy Science**, Champaign, v. 87, n.9, p.2977-86, sep. 2004.

BINNERTS, W.T.; VAN'T KLOOSTER, A.T.; FRENS, A.M. Soluble chromium indicator measured by atomic absorption in digestion experiments. **Veterinary Record**, London, v.82, p.470, 1968.

DANIEL, J.L.P.; RESENDE JÚNIOR, J.C.; CRUZ, F.J. Participação do ruminoretículo e omaso na superfície absortiva total do proventrículo de bovinos. **Brazilian Jounal of Veterinary Research and Animal Science**, São Paulo, v.43, n.5, p.688-94, jul. 2006.

DIJKSTRA, J. et al. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. **Britsh Journal of Nutrition**, Cambridge, v.69, n.2, p.385–96, Mar. 1993.

DIRKSEN, G. et al. Morphologie der pansenschleimhaut und fettsäureresorption beim rind: bedeutende faktoren für gesunkheit und leistung. **Zentralblatt für Veterinärmedizin. Reihe A**, Berlin. v.31, n.6, p.414–30, Jul. 1984.

EDRISE, B.M.; SMITH, R.H.; HEWITT, D. Exchange of water and certain water-soluble minerals during passage of digesta through the stomach compartments of young ruminating bovines. **British Journal of Nutrition, Cambridge**, v.55, n.1, p.157-67, Jan. 1986.

GÄBEL G.; ASCHENBACH, J.R.; MULLER, F. Transfer of energy substrates across the ruminal epithelium: implications and limitations. **Animal Health Research Reviews**, Wallingford, v.3, n.1, p.15-30, Jun. 2002.

GÁLFI, P.; NEOGRÁDY, S.; KUTAS, F. Dissimilar ruminal epithelial response to short-term and continuous intra-ruminal infusion of sodium n-butyrate. **Journal of Veterinary Medicine Series A,** v.33, n.1, p.47-52, Jan. 1986.

KRISTENSEN, N.B.; HARMON, D.L. Effects of adding valerate, caproate, and heptanoate to ruminal buffers on splanchnic metabolism in steers under washed-rumen conditions. **Journal of Animal Science**, Champaign, v.83, n.8, p.1899-907, Aug. 2005.

KRISTENSEN, N.B.; HARMON, D.L. Effect of increasing ruminal butyrate absorption on splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. **Journal of Animal Science**, Champaign, v.82, n.12, p.3549-59, Dec. 2004.

LITTELL, R.C.; HENRY, P.R.; AMMERMAN, C.B. Statistical analysis of repeated measures data using SAS procedures. **Journal of Animal Science**, Champaign, v.76, n.4, p.1216-31, Apr. 1998.

RESENDE JÚNIOR, J.C. et al. Comparison of techniques to determine the clearance of ruminal volatile fatty acids. **Journal of Dairy Science,** Champaign, v.89, n.8, p.3096-106, Aug. 2006.

SAKATA, T. et al. Stimulatory effect of insulin on ruminal epithelium cell mitosis in adult sheep. **British Journal of Nutrition**, Cambridge, v.44, n.3, p.325-31, Nov. 1980.

SAKATA, T.; TAMATE H. Effect of intraruminal injection of n-sodium butyrate on the mitotic index in sheep ruminal epithelium. **Tohoku Journal Agricultural Research**, v.27, p.133-135. 1976.

SAS INSTITUTE, SAS/STAT users guide. Version 6.12. Cary, NC. 1999.

SHEN, Z. et al. An energy-rich diet causes rumen papillae proliferation associated with more IGF type 1 receptors and increased plasma IGF-1 concentrations in young goats. **Journal of Nutrition**, Philadelphia. v.134, n.1, p.11-117, Jan. 2004.

TAMATE, H.; FELL B.F. Cell deletion as a factor in the regulation of rumen epithelial populations. **Veterinary Science Communications**, v.1, p.359-64. 1977.