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MILK PROTEIN SYNTHESIS AND THEIR ABSORPTION IN BUFFALO: THE ROLE OF GAMMA-GLUTAMYLTRANSFERASE

P. M. Elena, D. L. Adriana, M. Vincenzo, d'A. Danila, C. Anna, M. Nicola, L. Pietro,. A. Sattar, Z. Fabio and V. Domenico

Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università di Napoli Federico II, Napoli, Italy; Dipartimento di Medicina Sperimentale e Clinica, Università Magna Græcia, Catanzaro, Italy; Dipartimento di Scienze Zootecniche e Ispezione degli Alimenti, Università di Napoli Federico II, Napoli, Italy. Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

ABSTRACT

The aim of this study was to investigate gamma-glutamyltransferase (GGT) activity in mammary gland during early lactation and its influence on gastrointestinal (GIT) calves proteins absorption. Twelve buffalo dams and their calves were included in the trial. Biopsies were obtained from mammary gland after delivery and GGT activity were evaluated. The highest GGT levels were found after delivery. Histochemistry confirmed these findings and revealed that GGT reactivity was distributed throughout the cytoplasm of alveolar epithelial cells. Concerning GIT studies, calves were divided in two groups according to age. The activity found in the first group (<36 hours of life) was significantly higher ($P<0.01$) compared to that of the second group (>36 hours of life) in the descending and ascending duodenum and in the jejunum. Histochemistry also showed a greater enzyme activity during the first 36 hours of life. Results support the hypothesis that GGT is involved in milk production in water buffalo by supporting milk protein synthesis. Moreover, they also confirm the hypothesis that such enzyme may influence the adsorption process in the newborn calf.

Key words: Absorption, Milk Protein, Gamma-Glutamyltransferase

INTRODUCTION

Gamma-glutamyltransferase (GGT) is a membrane bound enzyme that is widely distributed in mammalian tissues which are involved in absorption and secretion, its expression seems to be regulated in a tissue specific manner and it is known that high levels of GGT activity can be found in epithelial cells of organs that are involved in the transport of amino acids and peptides across cell membranes (Braun *et al.,* 1986). In particular, in the mammary gland GGT expression during lactation suggests that this enzyme plays a role in both amino acid translocation and uptake by mammary epithelial cells (Pero M.E., et al., 2006). The role of GGT has been investigated in calves of several ruminant species, including water buffalo. In the gastrointestinal tract (GIT) GGT activity has been shown to be mainly distributed along the small intestine. In ruminants GGT activity has been determined in the small intestine of sheep and related to protein absorption. The mechanisms by which GGT may influence protein absorption in ruminants, in particular from colostrum, are, however, still unclear. In this respect, by studying the site of expression and the levels of GGT activity in the gastrointestinal tract of buffalo calves during the postnatal period it could be possible to get important information in this field. For these reasons, we analyzed the GGT activity in the mammary gland and in the GIT by using histochemical and biochemical methods.

MATERIALS AND METHODS

Twelve buffalo dams and their calves from a single dairy farm were used. Two groups of calves were included in the study (6 to 36 hours and 36 hours up to one week of age). Tissue samples from abomasum, duodenum, jejunum, ileum, caecum and colon were obtained by biopsy. All procedures were approved by Italian laws regarding animal use in research. GGT activity was measured in mammary tissues and in the GIT with a kinetic procedure by Spinreact, Spain. All results were expressed as means ± standard deviations (M±SD). Differences within groups were calculated by one-way ANOVA (SAS Institute Inc). Differences were considered significant for $p<0.01$. Hystochemistry samples were prepared according to Rutenburg *et al.* (1969). Samples for RNA analysis were kept at -196°C until required. Total RNA was extracted by using TRIzol. Quantification assays were performed to detect the relative expression of GGT mRNA among the two groups of calves at different ages. The first group of animals aged < 36 hours with higher expression of GGT was chosen as the calibrator sample in order to evaluate the putative differential mRNA expression of target genes in the second group (> 36 hours of life). This expression analysis was carried out by using the ABI 7300 Real-Time PCR System.

RESULTS AND DISCUSSION

The levels of GGT in tissue homogenates were higher in the younger calves (group 1) compared to the older ones (group 2) (P<0.01). Differences were detected for descending duodenum (0.35±0.08 vs 0.18±0.04 U/g), ascending duodenum (0.42±0.05 vs 0.10±0.08 U/g) and jejunum (1.03±0.03 vs 0.21±0.11 U/g) while in the abomasum, caecum, ileum and colon the GGT activities were similar. In the gland the highest activity was found at first days of lactation (32·57 ± 7·41U per g) and was higher (P < 0·05) than second period of lactation (10·76 ± 3·6U per g) or the non-lactating period (9·86 ±7·94U per g). In the abomasum (Figure 1a, 1b), the mucosal epithelial cells were moderately reactive. GGT activity was observed at the apex of the intestinal villi. In the cranial duodenum, a strong GGT reaction was observed in the submucosal duodenal (Lieberkuhn's) glands (Figure 1c, 1d). The GGT was expressed in the cytoplasm of enterocytes as red-purple coloured granules. In the cranial duodenum, the intensity of the reaction was similar in both the submucosal glands and the villi between the two groups. In the descending part of the duodenum not all the villi showed reactivity (Figure 1e, 1f). In the ascending duodenum the GGT activity was uniformly distributed among the villi (Figure 1g, 1h). In the jejunum, GGT activity as was localized along the entire length of the villi and almost all the villi expressed some enzyme activity (Figure 1i, 1l). In the ileum GGT activity was greatest at the apex of the villi in the younger calves (Figure 1m,1n). In cecum (Figure 1o, 1p) and colon (Figure 1q,1r) the GGT activity was difficult to detect in both groups (Figure 2d, e and f). In the gland GGT was expressed in the cytoplasm of these cells as red-purple coloured granules of different size. During the non-lactating period (Figure 2a, b and c) as well as at the end of lactation (Figure 2g, h and i), a reduced enzymatic activity was observed. Real-time PCR experiments confirmed that GGT RNA levels were significantly lower (P<0,05) in group 2 (>36h). We showed evidence of high GGT activity in the first hours of life during the maximal absorption capacity of protein from colostrums. During this period the intestine of neonatal calves endures morphological and physiological modification and protein are replaced by and the cells that exist in the very early phase of ontogeny are replaced by more mature cell population with enzymes that characterized the microvillus surface.

Our results show that GGT plays a role in the absorption of amino acids in the newborn calf and, being its highest activity during the best period for IgG absorption, they also suggest a possible role of the enzyme in IgG absorption soon after birth.

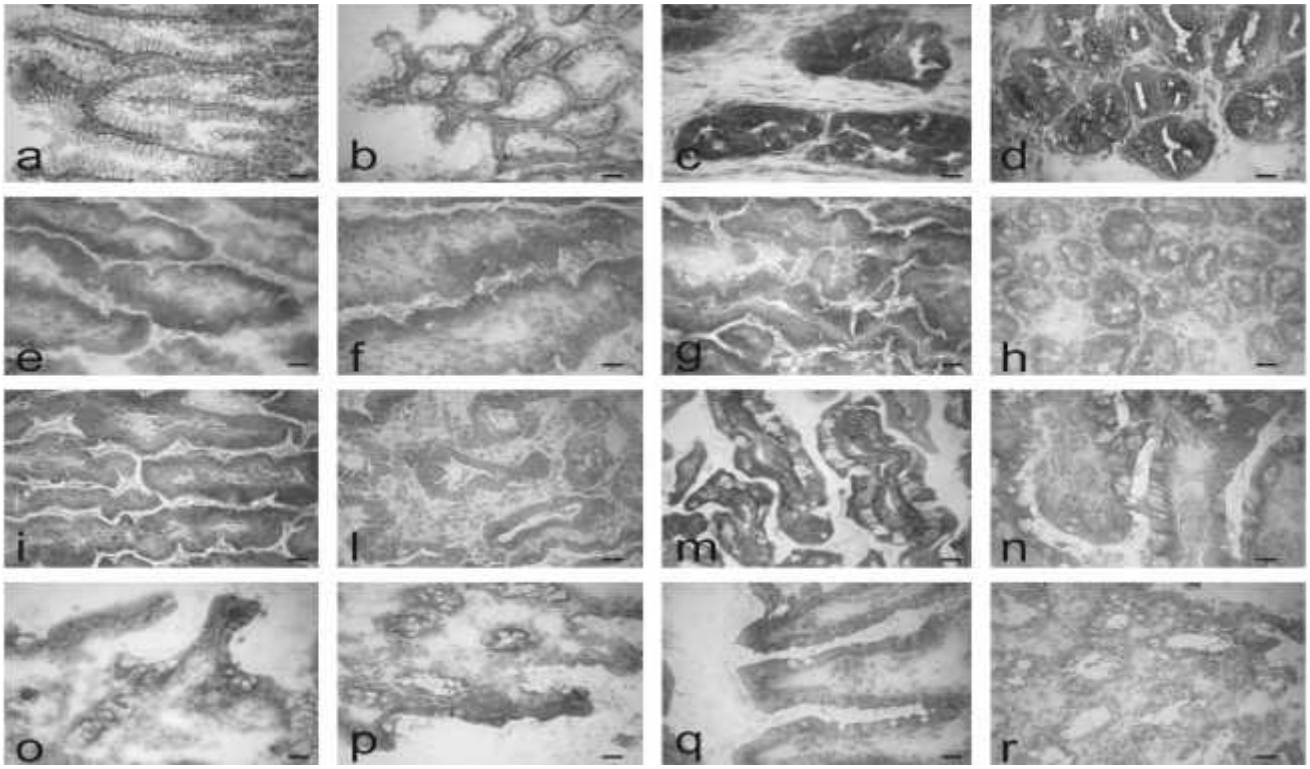

Figure 1. Histochemistry of GGT in samples of newborn calves GIT. a) abomasum at 6h; b) abomasums at 72h. c) Lieberkuhn gland at 6h; d) Lieberkuhn gland at 72h. e) ascending duodenum at 6h; f)ascending duodenum at 72h. g) discending duodenum at 6h; f) discending duodenum at 72h.

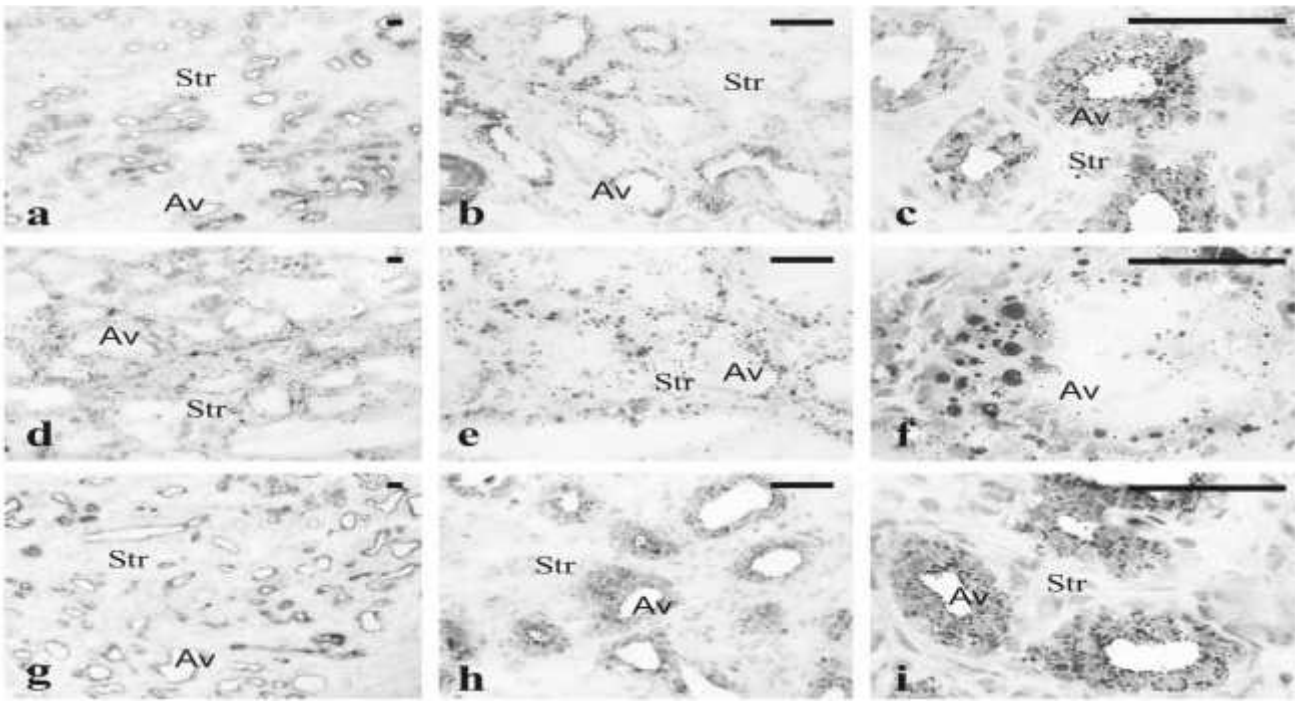

Figure 2. Histochemistry of GGT in samples of mammary glands from non-lactating (a, b, c), first days (d, e, f) and 180 days (g, h, i) of lactation water buffalo dams. Av = alveolar epithelial cells; Str = stromal tissue. At first days of lactation, all the alveolar epithelial cells were reactive. Large GGT reactive granules were widespread distributed throughout the cytoplasm (f). During the non-lactating period or at the end of lactation, a reduced enzymatic activity was observed. Not all the alveoli were reactive (a, g) and alveolar epithelial cells showed a variation (c) in the density of GGT reactive granules. These granules were small and located all around the nuclei (i).

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