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SHORT COMMUNICATION

Glutathione concentration and gamma-glutamyltransferase activity in water buffalo colostrum

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Summary

Evidence is presented that the buffalo mammary gland contains enzymes that catalyse the synthesis and utilization of glutathione. A significant, inverse correlation (r = 0.79) was detected between colostrum gamma-glutamyltransferase (GGT) and glutathione (GSH), suggesting that the enzyme uses GSH as a substrate for its activity. A similar trend was shown in mammary gland homogenates (r = 0.75). Our results show that GSH is secreted into buffalo colostrum and suggest that the enzyme GGT degrades it. To the authors' knowledge, this is the first demonstration of the involvement of GGT-mediated GSH metabolism in the synthesis of colostrums, which elucidates the role of the enzyme that has always been reported very high in colostrum.

Glutathione (GSH) is a tripeptide that contains the three amino acids, glutamine, cysteine and glycine. It is widely distributed in various tissues (Meister and Anderson, 1983) and performs several important physiological functions. It serves as the major cellular reductant and plays a fundamental role in protecting against free radical carcinogens, heavy metals and other toxic chemicals. It is involved in amino acid transport and maintenance of the protein sulphydryl reduction status (Sies, 1999). Glutathione is produced in almost all cells, but the cellular capacity to synthesize GSH *in vivo* is not well known. Breast milk feeding represents the principal source of GSH for neonatal animals. Both the mammary gland and milk contain enzymes that catalyse the synthesis and utilization of GSH. Maintaining GSH concentrations in the gland is essential for milk production and their decrease leads to apoptosis and involution of the mammary tissue (Zaragozà et al., 2005).

Gamma-glutamyltransferase is a cell membrane-bound enzyme located on the outer surface of cell membranes. It transports and uses GSH as a source of amino acids for cell metabolism (Owen et al., 1979). It is widely distributed among epithelial tissues involved in absorption and secretion processes. Under physiological conditions, the relationships between mammary gland GGT activity and lactation have been investigated in rodents as well as in ruminants. It has been suggested that the enzyme GGT participates in the amino acid translocation and uptake by mammary cells (Viña et al., 2001). High levels of GGT activity are known to be found in epithelial cells of organs that are involved in the transport of amino acids and peptides across cell membranes. Moreover in the mammary gland, GGT expression during lactation suggests that this enzyme plays this twofold role.

This study examined the hypothesis that GSH is secreted in buffalo milk and that some of it is degraded by the enzyme GGT within the milk. We therefore assayed GSH and GGT activity in colostrum and in serum from neonatal buffalo after colostrum ingestion as well as their concentration in buffalo mammary tissue at the first day of lactation.

Water buffalo dams (n = 8) at delivery and their neonatal calves (n = 8) were included in the trial. The calves were born between January and March 2008 and sucked colostrum directly from the udder.

Blood samples of buffalo dams were taken from the caudal vein at delivery; colostrum was taken directly from the udder within 6 h of delivery. The samples were centrifuged at 3500 *g* for 30 min to remove most of the fat and then ultracentrifuged at 20 000 *g* for 30 min. The supernatant with a small residual fat was removed and the intermediate layer was withdrawn for analysis. All calves started suckling within 6 h from birth and blood was taken from the jugular vein of calves at 12 h after delivery. Blood samples were centrifuged at 2500 *g* and the serum was used for GGT and GSH determination. To obtain a specimen from the mammary gland, the udder was thoroughly cleaned and disinfected, and a subcutaneous anaesthesia was performed using a 2% lidocaine injection. All procedures were compliant with Italian laws regarding animal use in research. The mammary samples (10–20 mg) were taken from the gland at the first day of lactation using a Magnum biopsy needle (Bard, Covington, GA, USA). Samples were thoroughly washed and homogenized with a Ultra-Turrax (IKA®-Werke GmbH & Co., Staufen, Germany) (10–20 mg/200 μl phosphate-buffered saline) and centrifuged at 10 000 *g* for 15 min at 4 °C and the supernatant was used for analysis. Each sample was separated into aliquots to evaluate GSH and GGT. Gamma-glutamyltransferase activity was measured with a kinetic procedure by Spinreact Reagents (Spinreact, SA, St. Esteve de Bas, Girona, Spain) (100 mM TRIS buffer, pH 8.25, 100 mM glycylglycine and L-g-glutamyl-3-carboxy-*p*-nitroanilide as substrate) using a spectrophotometer (550 SE; Perkin-Elmer, Wellesley, MA, USA) at 405 nm.

As regards total GSH determination, as much protein as possible was removed from the samples to avoid interference due to particles and sulphydryl groups by using a metaphosphoric acid solution (Sigma-Aldrich, Milano, Italy) (Baker et al., 1990; Owens and Belcher, 1965). GSH concentration was determined using an ELISA GSH assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) at 405 nm. The glutathione disulphide (Cat No. 703014) from Cayman Chemical Company was used as a standard to quantify GSH. Regression and correlation coefficients were calculated by the simple linear regression (SAS, 2000). Each test was run in duplicate and the average of the two results was used for calculation. Correlations were considered significant at $p < 0.05$.

A significant, inverse correlation ($r = 0.79$, $p < 0.05$) was detected between colostrum GGT and GSH, thus suggesting that the enzyme uses GSH as a substrate for its activity (Fig. 1). A similar relationship was shown for mammary gland homogenates ($r = 0.75$, $p < 0.05$) (Fig. 2). Our results show that GSH is secreted into buffalo colostrum and suggest that the enzyme GGT degrades it. We found high levels of GSH concentrations in colostrum (894 ± 39 μM). Barber et al. (1999) reported that rat milk collected at the peak of lactation, days 11–14, contains almost 100 μM total GSH. Ankrah et al. (2000) also reported that human breast milk obtained from healthy lactating mothers contains almost 200 μM of GSH, while no data are available on colostrum. Colostrum levels of GSH and GGT were much higher than those detected in serum (data not shown), thus showing that they do not reach colostrum via the paracellular pathway but are directly secreted by the mammary epithelial cells. The significant relationship detected in mammary homogenates suggests that mammary GGT and GSH are involved in colostrogenesis and probably in colostrum protein synthesis. In mammary tissue, at the first day of lactation,

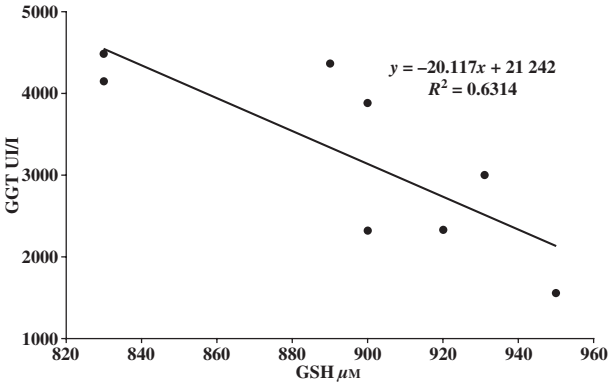

Fig. 1 Scatter plot and regression lines illustrating the relationship between gamma-glutamyltransferase (GGT, UI/l) and GSH (μm) in water buffalo colostrum at first day of lactation.

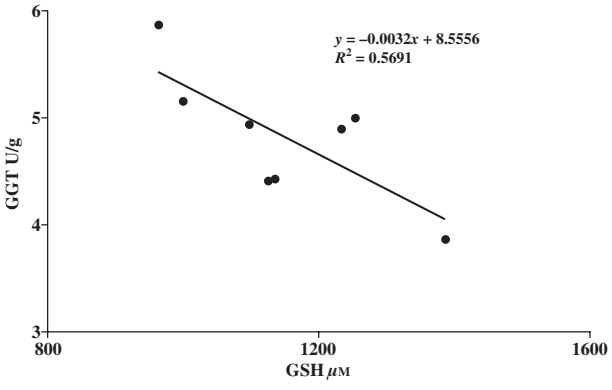

Fig. 2 Scatter plot and regression lines illustrating the relationship between gamma-glutamyltransferase (GGT, U/g) and GSH (μm) in water buffalo mammary tissue at first day of lactation.

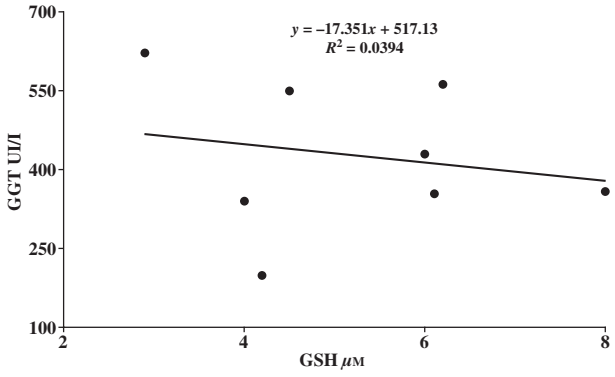

Fig. 3 Scatter plot and regression lines illustrating the relationship between gamma-glutamyltransferase (GGT in U/g) and GSH (μm) in neonatal calf serum at first day of delivery.

we found a concentration of 1150 ± 125 μM of total GSH. In the mammary gland of rat, a similar GSH concentration has been found, but no data are available at the first day of lactation. Mammary tissue also showed a relatively high activity (4.6 ± 4.8 U/g of tissue) of the GSH catabolic enzyme GGT. Most of the enzyme activity in buffalo mammary tissues has been shown in reactive granules distributed throughout the cytoplasm of alveolar epithelial cells (Pero et al., 2006). These findings are in contrast with those reported in humans where GGT has been found, by histochemistry and immunohistochemistry, to be primarily localized at the luminal surface of normal breast cells rather than the basolateral surface (Hanigan et al., 1996). Gamma-glutamyltransferase catalyses the breakdown of circulating plasma GSH, thus facilitating the uptake of the constituent amino acids of GSH into mammary gland cells.

Finally, no significant correlation between GGT and GSH was detected in buffalo calf serum, where GSH concentration was very low (5.9 ± 2.9 μM). This agrees with findings elsewhere (Meister and Anderson, 1983) and suggests that the GGT-mediated GSH metabolism is completed at intestinal level (r = 0.19) (Fig. 3). However, additional studies are needed to evaluate the functional roles of GSH and other antioxidants in colostrum to improve our understanding of their effects upon neonatal buffalo health.

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