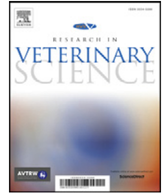
Research in Veterinary Science 103 (2015) 149–155

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc



Effect of colostrum and milk on small intestine expression of AQP4 and AQP5 in newborn buffalo calves

C. Squillacioti [a], A. De Luca [a], M.E. Pero [a], G. Vassalotti [a], P. Lombardi [a], L. Avallone [a], N. Mirabella [a], A. Pelagalli [b,c,*]

[a] Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Via Veterinaria 1, Naples 80137, Italy
[b] Department of Advanced Biomedical Sciences, University of Naples Federico II, Via Pansini 5, Naples 80131, Italy
[c] Institute of Biostructures and Bioimages, National Research Council, Via De Amicis 95, Naples 80131, Italy

ARTICLE INFO

Article history:
Received 11 April 2015
Received in revised form 16 July 2015
Accepted 17 October 2015

Keywords:
Aquaporins
Colostrum
Small intestine
Buffalo

ABSTRACT

Functional studies indicate differences in newborn gastrointestinal morphology and physiology after a meal. Both water and solutes transfer across the intestinal epithelial membrane appear to occur via aquaporins (AQPs). Given that the physiological roles of AQP4 and AQP5 in the developing intestine have not been fully established, the objective of this investigation was to determine their distribution, expression and respective mRNA in the small intestine of colostrums-suckling buffalo calves by using immunohistochemistry, Western blot, and reverse transcriptase-PCR analysis. Results showed different tissue distribution between AQP4 and AQP5 with the presence of the former along the enteric neurons and the latter in the endocrine cells. Moreover, their expression levels were high in the ileum of colostrum-suckling buffalo calves. The data present a link between feeding, intestinal development and water homeostasis, suggesting the involvement of these channel proteins in intestinal permeability and fluid secretion/absorption during this stage of development after birth.

Published by Elsevier Ltd.

1. Introduction

Aquaporins (AQP) are a family of 13 integral membrane channels (AQP0-12) that are permeable either to water alone or to water and other small solutes bi-directionally in response to an osmotic gradient. They are distributed differently among visceral organs and have various structural and physiological characteristics (King et al., 2004; Gomes et al., 2009; Isokpehi et al., 2009; Verkman, 2009). Among these organs, the digestive system plays a pivotal role in regulating fluid transport, with a magnitude of water fluxes quantitatively second only to the kidney. Water transport through the digestive system has an important role in regulating body water homeostasis and digestive and absorptive functions. In particular, as reported by Ma and Verkman (1999), the small intestine is characterized by the presence of an epithelium with low electrical resistance which regulates absorption of small solutes. This has led to the suggestion that the rapid water movement across the epithelium in small intestine could to occur by a paracellular pathway. This concept was supported by the finding of low osmotic permeability of brush border membrane vesicles from the small intestine (Tritto et al., 2007). Various studies have focused on AQP expression along this intestinal tract, showing a particular involvement of these proteins in regulating water permeability, thus providing new insights into normal gastro-intestinal (GI) physiology and disease mechanisms. Such knowledge may also yield novel therapies to regulate fluid movement in GI diseases.

In contrast, little is known concerning the functional anatomy of gastrointestinal tracts with respect to water transport across endothelial and epithelial barriers and bulk fluid movement during the neonatal period, especially in suckling animals. This applies especially to ruminant species where the intestine performs several activities due to both secretions of substances and absorption processes particularly related to tissue adaptive mechanisms occurring during the early postnatal development phase. In particular, after a meal, along the different tracts of the intestine, a large amount of water and other molecules are transported, thereby regulating the osmotic balance of intestinal contents. Such consideration is particularly important for water buffalo, a major livestock species in terms of dairy products, meat and manure in the southern Italian region of Campania (Bordi et al., 1997). The main factor that has contributed to the development of buffalo farming in the past few years has been the increase in mozzarella cheese consumption in Italy and overseas. Due to favorable climatic conditions and to considerable economic interest, most of the increase has occurred in southern Italy (Campanile et al., 2012).

Few newborn feeding studies have evaluated the changes in the gastrointestinal tract and the modulation of its specific activities (i.e. proteins, receptors) in relation to feeding (Chen et al., 2014; Schwarz and Heird, 1994). Accordingly, the present report examines AQ4 and AQP5 expression and localization along the small intestine of neonatal buffalo calves after colostrum suckling. The choice of AQP4 and AQP5 was based on their relative distribution along different regions of the

* Corresponding author at: Department of Advanced Biomedical Sciences, University of Naples Federico II, Via Pansini 5, Naples 80131, Italy.
E-mail address: alpelaga@unina.it (A. Pelagalli).

http://dx.doi.org/10.1016/j.rvsc.2015.10.004
0034-5288/Published by Elsevier Ltd.

gastrointestinal tract. In particular, mammalian AQP4 is expressed in a number of tissues but is not as ubiquitous as AQP1 (Ishibashi et al., 2009). Moreover, a significant difference between AQP1 and AQP4, concerning closing pore mechanism has been observed (Wang et al., 2010). Furthermore, a recent study conducted by our group reported that AQP1 is expressed in the small and large intestine of colostrum-suckling buffalo calves, suggesting its involvement in intestinal functions (De Luca et al., 2014). In the digestive tract AQP4 is expressed in the stomach and intestine. In contrast, AQP5 is localized particularly in salivary glands as well as in the apical membrane of the pyloric gland secretory cells and in duodenal glands in the rat (Matsuzaki et al., 2003; Parvin et al., 2002). The expression of AQP4 and AQP5 mRNA and relative proteins was studied by RT (reverse transcriptase) PCR and immunoblotting, and cellular localization was performed by immunohistochemistry.

2. Materials and methods

2.1. Animals and tissue sample collection

The present study was carried out according to the Italian legislation regarding the use of animals in the research. A total of fifteen healthy neonatal buffalo male calves from a single dairy farm in southern Italy was used in the experiment. The animals were divided into three groups (A, B and C; 5 animal each). Animals of the group A were sacrificed at birth. Animals of groups B and C were fed for 1 day with 3 L of mother colostrum using a needling bottle according to the farm's standard practice. On the second day, the animals were further divided into two groups: the animals in the first group continued to be fed with colostrum for 1 week; those in the second group were fed with buffalo milk. An additional group of five adult male buffaloes (15 months old) was used to evaluate potential age-dependent changes. The animals were killed in a public slaughterhouse. All adopted procedures were performed in accordance with European Directive 2010/63 on the protection of animals used for scientific purposes.

The abdominal cavity was opened and the gastrointestinal tracts (GIT) were removed. Tissue samples of the small intestine (duodenum, jejunum, ileum) were immediately harvested and processed for fixing (see Immunohistochemistry) or snap frozen in liquid nitrogen (LN) and subsequently stored at −80 °C until use for total RNA isolation and protein extracts.

2.2. Immunohistochemistry

Fresh segments of intestinal tissues were immersed in Bouin's fixative (6–24 h) for fixation, processed for paraffin embedding in a vacuum and cut at a thickness of 5–7 μm. The avidin–biotin–peroxidase complex (ABC) method was performed using the Vectastain ABC kit (PK-4000 — Vector Laboratories, Burlingame, CA, USA) as described more fully elsewhere (Liguori et al., 2012). Primary antisera were goat anti-AQP4 (1:200, SC-9888, Santa Cruz Biotechnology, CA, USA) and anti-AQP5 (1:200, sc-9891, Santa Cruz Biotechnology, CA, USA). Two observers without knowledge of immunocytochemical evaluation by other independently evaluated five slides (one slide selected every ten according to sequential thickness) for each intestinal tract (duodenum, jejunum and ileum) from each animal using a Leica DMRA2 microscope (Leica Microsystems, Wetzlar, Germany).

2.3. Western immunoblot analysis

Tissue homogenates were obtained in ice-cold 1× RIPA lysis buffer as well described elsewhere (Pelagalli et al., 1999). Western blot analysis were carried out as previous fully described (Scibelli et al., 2003) except that for few differences: SDS-PAGE was conducted by using 12.5% polyacrylamide gels; the primary antibodies were anti-AQP4 and anti-AQP5 antibodies (1:1000) respectively; peroxidase-conjugated donkey anti-goat IgG (1:2000, sc-2020 Santa Cruz Biotechnology, CA, USA) was used as secondary antibody; beta-actin antibody (JLA20CP01, Calbiochem, San Diego, CA, USA) was used to confirm equal loading of proteins in each lane, and detection of specific band was performed by chemiluminescence reagent (Super Signal West Pico Chemiluminescent Substrate, Thermo Scientific). Densitometric analysis of the 28 kDa band of Western blotting was performed using NIH Image J software (Ij1.46r) (National Institutes of Health, Bethesda, MD, USA); http://imagej.nih.gov/ij/) and normalized against the signal obtained by reprobing the membranes with mouse anti beta-actin. The apparent molecular weight of proteins was determined by calibrating the blots with pre-stained molecular weight markers (New England BioLabs, Herts, UK).

2.4. RNA extraction, cDNA synthesis, RT-PCR and sequencing

Samples of intestinal tracts were taken and immediately frozen by immersion in liquid nitrogen (−173 °C) for 10 s, and individually homogenized in ice-cold TRI-Reagent (Sigma) using an Ultra-Turrax homogenizer. RNA extraction, cDNA synthesis, RT-PCR and sequencing were performed as described more fully elsewhere (De Luca et al., 2014). PCR amplification was performed using specific primers for buffalo AQP4 and AQP5 designed from the published gene sequences (Bos taurus aquaporin 4 GenBank accession number NM_181003 and Bos taurus aquaporin 5 GenBank accession number NM_001191160) as described elsewhere (Squillacioti et al., 2012). The sense and antisense AQP4 primers used were 5′-GGAGGATTAGCATCGCCAAGT-3′ and 5′-AAAGCTATGGAACCGGTGACA-3′; the sense and anti-sense AQP5 primers used were 5′-ATTGGCCTGTCCGTCACACT-3′ and 5′-CCTCGTCC GGCTCATACGT-3. The two primers amplify a 250-bp and 260-bp fragment, respectively.

In order to verify the efficiency of the reverse transcription (RT) and to exclude genomic DNA contamination, a fragment of β-actin cDNA (GenBank accession no. NC_007326) was amplified and sequenced with primers designed to span an intron β-actin for 5′-CAG CTC CTC CCT GGA GAA GA-3′. β-actin rev 5′-CTG CTT GCT GAT CCA CAT CTG-3′.

3. Results

3.1. Aquaporin-4 immunohistochemistry

The results of immunohistochemistry for both aquaporins are summarized in Table 1. At birth, no AQP4 immunoreactivity (IR) was detectable in any of the tracts of the small intestine (Fig. 1A, B, C).

After one week of colostrum suckling, AQP4 IR was distributed in the endothelium of the vessels, in enterocytes, in enteric neurons and in lymphoid cells in the lamina propria of the duodenum (Fig. 1D), jejunum (Fig. 1E) and ileum (Fig. 1F). After one week of milk suckling, AQP4 IR was found only in lymphoid cells in the lamina propria. The density of AQP4 IR was greater in the intestine of animals suckling colostrum than animals suckling milk. In the adults, AQP4 IR was found in the endothelium, enterocytes, enteric neurons and lymphoid cells in the lamina propria (Fig. 1L, M, N). Moreover, in enterocytes the density of AQP4 IR was greater in adults than at other ages.

3.2. Aquaporin-5 immunohistochemistry

The localization of AQP5 protein in the small intestinal tracts of buffaloes was examined by immunohistochemistry because its cellular distribution is still unclear. At birth, AQP5 IR was found in the endothelium of the vessels and in the endocrine cells of all tracts of the small intestine (Fig. 2A, B, C). After one week of colostrum suckling, AQP5 IR was localized in the endothelium, endocrine cells, in enterocytes and lymphoid cells in the lamina propria of duodenum, jejunum and ileum (Fig. 2D, E, F). In contrast, after one week of milk suckling, AQP5 IR was restricted to the endothelium, the endocrine cells and the lymphoid cells in the lamina propria of all the examined tracts (Fig. 2G, H, I). The density of

Table 1
Distribution of AQP4 and AQP5 immunoreactivity in the buffalo small intestine. − undetectable; −/+ rare; + low density; ++ medium density; +++ high density.

	Duodenum				Jejunum				Ileum			
	Birth	Colostrum	Milk	Adult	Birth	Colostrum	Milk	Adult	Birth	Colostrum	Milk	Adult
AQP4-IR												
Endothelium	−/+	+	−	+	−/+	+	−	+	−/+	+	−	+
Enterocytes	−	+	−	++	−	+	−	++	−	+	−	++
Enteric neurons	−	+	−	+	−	++	−	+	−	−	−	−
Endocrine cells	−	−	−	−	−	−	−	−	−	−	−	−
Lymphoid tissue	−	+	+	+	−	+	−	+	−	+	−	+
AQP5-IR												
Endothelium	+	+	+	++	+	+	+	++	+	+	+	++
Enterocytes	−	−/+	−/+	−/+	−	−/+	−	−/+	−	−/+	−	−/+
Enteric neurons	−	−	−	−	−	−	−	−	−	−	−	−
Endocrine cells	+	++	+	++	+	++	+	++	+	++	+	++
Lymphoid tissue	−	+	+	+	−	+	+	+	−	+	+	++

AQP5 IR in the lymphoid cells in the lamina propria was greater in the ileum than duodenum and jejunum. In the adults, AQP5 IR was distributed in the endothelium, endocrine cells and lymphoid cells in the lamina propria (Fig. 2L, M, N). All the examined intestinal tracts of adult buffaloes exhibited more strong density of AQP5 IR than at other ages.

3.3. Western blotting

Results of AQP4 expression in small intestine tracts of buffalo calves in different conditions (birth, colostrum, milk) and in adult buffaloes are shown in Fig. 3A. Different bands were observed in immunoblot probed with anti-AQP4 antibody at 28–30 kDa corresponding to its molecular mass and other bands with higher molecular masses. At birth, no band was observed at the corresponding molecular mass of the protein while only a faint band was observed between 45 and 60 kDa. By contrast, in colostrum-suckling buffalo calves, in all examined tissues AQP4 was expressed both as a band at 28–30 kDa and as multiple bands respectively of about 45 and 60 kDa. A similar band profile was observed in animals which had received milk and in adult buffaloes with a different intensity for each band that increased from milk-suckling calves to adult buffaloes. The Western blotting data were also confirmed by densitometric analysis using beta-actin as protein loading control (Fig. 3B). This analysis showed a growing band intensity in tissues of colostrum-suckling buffalo calves with a maximum for the ileum.

Western profile for AQP5 was similar to AQP4, albeit with some differences (Fig. 4A). At birth a faint band was observed for 28–30 kDa while different bands both at 28–30 kDa and 45–60 kDa were observed for all different conditions, though with a weaker signal as reported by densitometry (Fig. 4B). In particular, a more intense band for AQP5 was observed for colostrum-suckling buffalo calves in the ileum with lower intensity than for AQP4.

3.4. RT-PCR analysis

RT-PCR was carried out to identify AQP4 and AQP5 mRNA in the small intestine of buffalo calves, according to different animal conditions (birth, colostrum, milk), and of adult buffaloes. The reaction revealed a single band of the expected size (250 bp AQP4; 260 bp AQP5) in all the examined intestinal tracts. Both the efficiency of the reverse transcription (RT) and the exclusion of genomic DNA contamination were verified by amplification and sequencing of a β-actin cDNA (GenBank accession no. NC_007326) with primers designed to span an intron β-actin for 5′-CAG CTC CTC CCT GGA GAA GA-3′. β-actin rev 5′-CTG CTT GCT GAT CCA CAT CTG-3′.

4. Discussion

In this study AQP4 and AQP5 mRNAs and their relative proteins were detected in all the tracts of the small intestine of buffalo calves at different conditions (birth, colostrum and milk suckling) and in adult buffaloes. This conclusion was obtained on the basis of data from RT-PCR, Western blotting and immunohistochemistry (Table 1 and Figs. 1–4). In particular, immunohistochemical analysis supported by Western blotting showed the presence of proteins AQP4 and AQP5 in different regions of the small intestine, albeit with a more intense distribution and expression in colostrum-suckling buffalo calves. RT-PCR experiments confirmed AQP4 and AQP5 presence in all animal groups, confirming the immunohistochemical staining data. Moreover, results obtained by Western blot analysis showed the simultaneous presence of different bands (28–60 kDa) for both AQP4 and AQP5 assuming, as reported elsewhere (Cutler et al., 2012; Parvin et al., 2005; Tiwari et al., 2014), the presence of glycosylated forms of the same protein.

To date, thirteen AQPs have been divided into three groups of orthodox AQPs, aquaglyceroporins and unorthodox AQP according to their characteristics (Borgnia et al., 1999; Ishibashi 2006; Rojek et al., 2008). AQP4 and AQP5 belongs to the orthodox group of AQPs and their activities seem to diversify within the GI tract. In particular, our immunohistochemistry results detected AQP4 and AQP5 generally in endothelium, enterocytes and lymphoid cells. Under greater scrutiny, their localization along the tracts of small intestine showed a peculiar differentiation with an expression of the former protein in the enteric neurons and the latter in the endocrine cells. This phenomenon, accompanied by a more extensive tissue distribution and intense expression in tissues of colostrum-suckling buffalo calves, suggests a different involvement of these proteins in important processes that occur along the intestinal tract, especially at a critical stage of its development soon after birth.

The maturation of intestine physiology related to a wide range of mechanisms (myenteric system development, immune mechanisms and absorptive/secretory hormone-dependent processes) requires careful and systematic analysis that allows for the possible effects of diets or food intake.

In this respect, a recent paper (Wolinski et al., 2012) regarding the effect of colostrum on enteric neurons during intestinal development demonstrated a particular expression of such structures in pigs, suggesting its effect in maintaining the activity of the small intestine nitroergic myenteric neurons. Other reports strengthen this hypothesis by evidencing a modification in density and number of enteric neurons in preterm pigs in response to enteral food (colostral or formulae diets) (van Haver et al., 2008; Oste et al., 2005). Thus, AQP4 distribution along these structures appears well correlated with the role of this system in regulating fluid homeostasis and with the positivity in lymphoid tissue. Interaction between the enteric nervous and immune systems remains

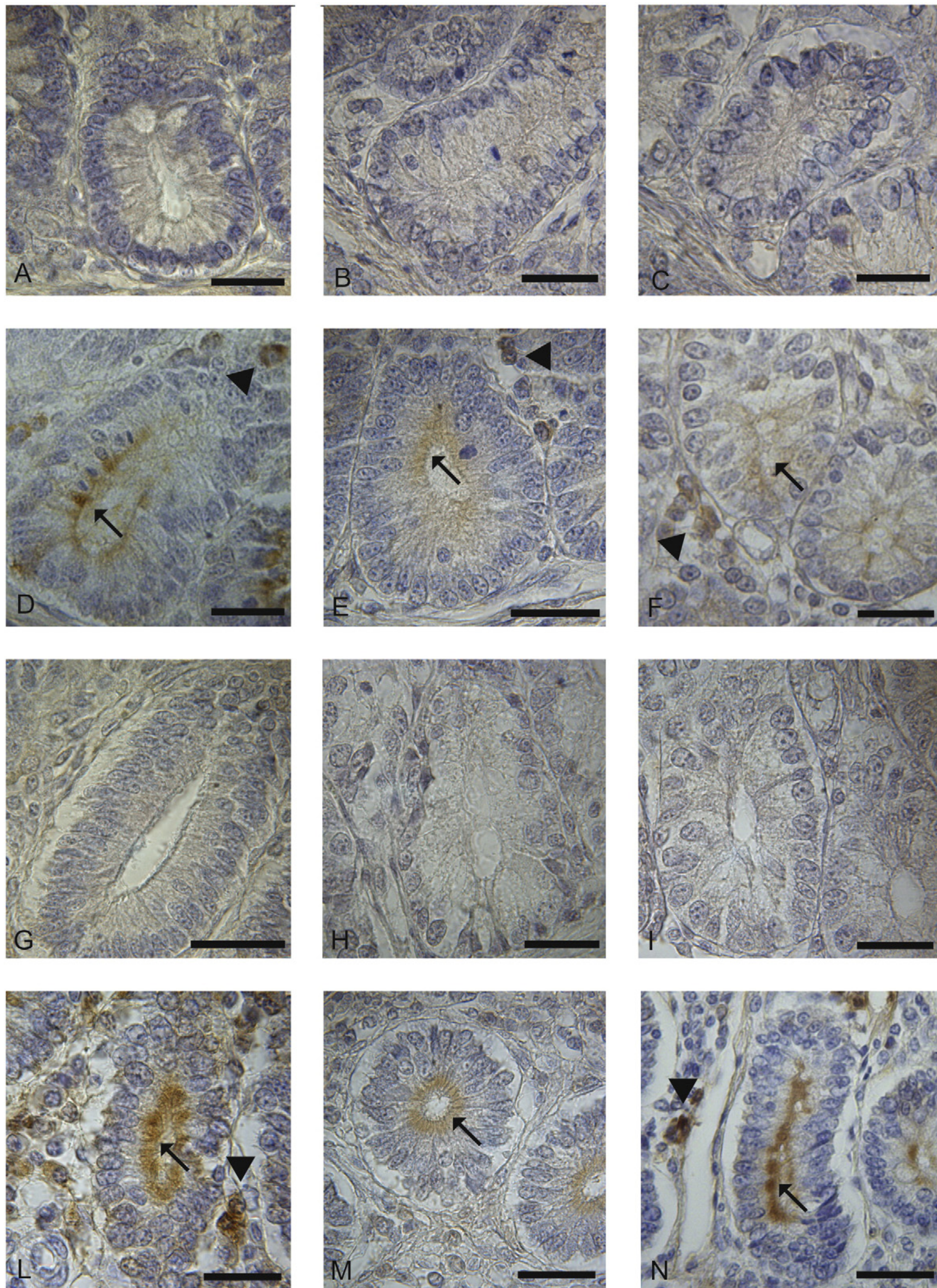


Fig. 1. Distribution of AQP4-immunoreactivity in the small intestine of the buffaloes at birth (A, B, C), after 1 week of colostrum (D, E, F) and milk (G, H, I) suckling and adult (L, M, N). Duodenum (A, D, G, L), jejunum (B, E, H, M) and ileum (C, F, I, N). arrows positive enterocytes; arrow head positive lymphoid cells in the lamina propria; scale bar 25 μm.

an important topic and progress has been achieved to shed some light on the neuro-immune axis in the human gut. Moreover, the observed positivity of AQP4 at the level of enteric neurons was in agreement with data from Thi et al. (2008), albeit in the colon of the adult rat, while high immunostaining in the ileum has already been confirmed (Jiang et al., 2014).

In addition, AQP4- IR was distributed in the lymphoid cells located in the intestine lamina propria. These positive cells belong to the GALT ("Gut Associated Lymphoid Tissue") which includes lymphoid cells in the epithelium, lymphoid and reticuloendothelial cells in the lamina propria and organized lymphoid aggregates in the mucosa (Peyer's patches) (Parsons et al., 1989). Aquaporins were found to be expressed

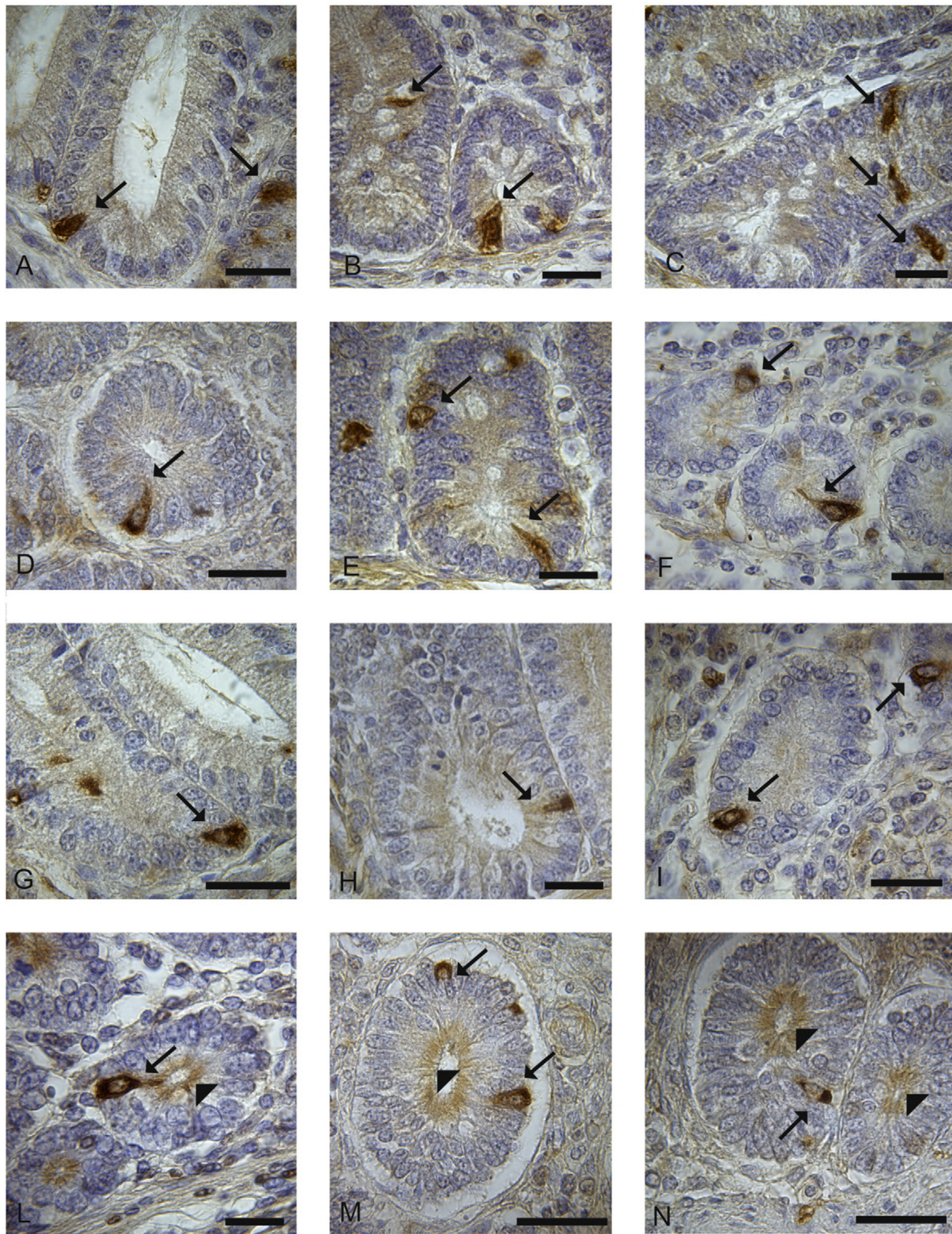


Fig. 2. Distribution of AQP5-immunoreactivity in the small intestine of the buffaloes at birth (A, B, C), after 1 week of colostrum (D, E, F) and milk (G, H, I) suckling and adult (L, M, N). Duodenum (A, D, G, L), jejunum (B, E, H, M) and ileum (C, F, I, N). arrows positive endocrine cells; arrow head positive enterocytes; scale bar 25 μm.

in the human immune system (Moon et al., 2004) and involved in the cutaneous primary immune response (Hara-Chikuma et al., 2012) supporting the hypothesis that these proteins play a role in immune response regulation.

Interestingly, the presence of AQP5 in the endocrine cells, particularly in the small intestine tracts of colostrum-suckling buffalo calves, was not previously established for this animal species although already revealed in the rat digestive tract (Matsuzaki et al., 2003). More recently, a study on sheep salivary glands showed an analog AQP5 immunoreactivity regulation related to the pasture vegetative cycle (Scocco et al., 2011), suggesting the possible influence of environmental changes.

Moreover, the different tissue distribution of AQP4 and AQP5 according to animal condition (birth, colostrum, milk and adult) could be associated to their trafficking to and from membranes as a powerful mechanism to regulate epithelial and cellular water flux both in the short and long term (Valenti et al., 2005). Results from different studies report AQP5 redistribution from inside the cells to the apical membrane on stimulation with secretagogues (Parvin et al., 2005). Thus, the different involvement of AQ4 and AQP5 in intestinal function during early

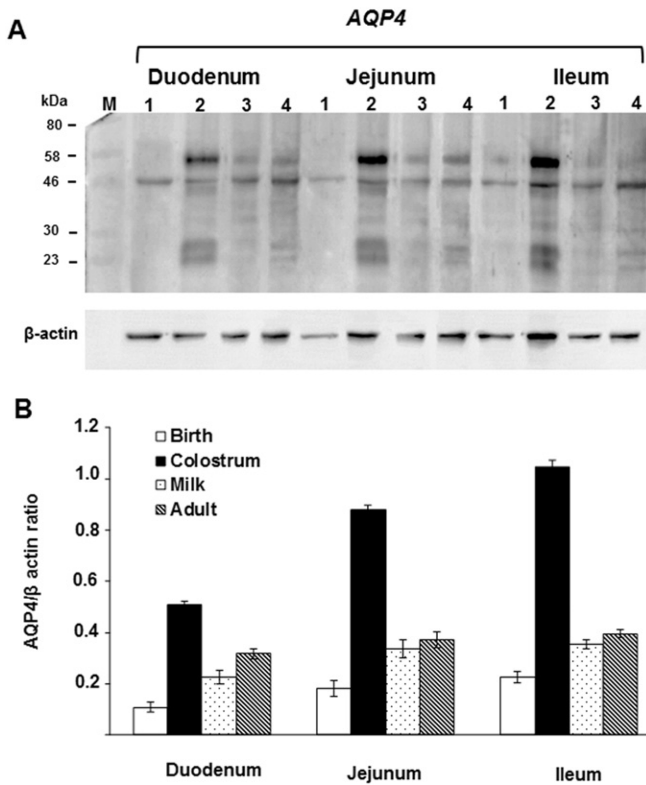


Fig. 3. (A) Western blot analysis of AQP4 expression in the small intestinal tracts (duodenum, jejunum and ileum) of the buffaloes at birth (lane 1), after 1 week of colostrum (lane 2) and milk suckling (lane 3) and of adult buffaloes (lane 4). M: molecular weight standard (kDa). β-Actin was used as a loading control. (B) Semi-quantitative analysis of AQP4 expression. Averaged data from five animals from each group on three different blots for each intestine tracts are expressed as ratios with the corresponding value for β-actin. Data are presented as means ± standard error (SE).

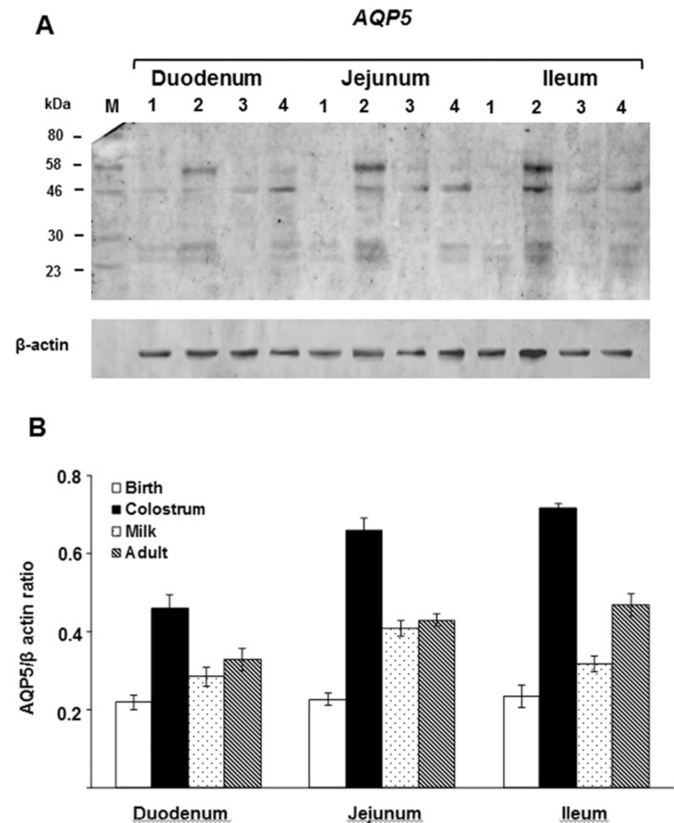


Fig. 4. (A) Western blot analysis of AQP5 expression in the small intestinal tracts (duodenum, jejunum and ileum) of the buffaloes at birth (lanes 1), after 1 week of colostrum (lane 2) and milk suckling (lane 3) and of adult buffaloes (lane 4). M: molecular weight standard (kDa). β-Actin was used as a loading control. (B) Semi-quantitative analysis of AQP5 expression. Averaged data from five animals from each group on three different blots for each intestine tracts are expressed as ratios with the corresponding value for β-actin. Data are presented as means ± standard error (SE).

development after birth together with the activity of AQP1 already shown (De Luca et al., 2014) suggests a model of action in the GI in which aquaporins are involved to varying degrees and upregulated in relation to colostrum intake. Their respective activation in relation to water transport and hence osmoregulation could be activated by trophic substances and enzymes such as colecystokinin (CCK) that regulate intestinal cell activities (Zabielski et al., 1999). In addition, it is possible to hypothesize the presence of a simultaneous series of events and factors (nutrients, local hormones, immunomodulatory factors and pH variations) that modulate expression of these channel proteins as well as the capacity to secrete intestinal enzymes.

5. Conclusions

To our knowledge, this is the first study reporting the presence of AQP4 and AQP5 along the buffalo calf small intestine, providing an anatomical basis for their expression in the intestine during calf development after delivery, especially after colostrum assumption. Further functional studies are warranted to verify our findings and to fully elucidate their specific role during the specific adaptation mechanisms occurring along the tissues during the early postnatal period.

Acknowledgments

The authors thank Ms. Sabrina Ali for her technical assistance. This work was in part supported by the University of Naples Federico II (FARO project 2010).

References

Bordi, A., De Rosa, G., Grasso, F., Napolitano, F., Fortunato, M., 1997. It is time “to think” about buffalo as a quality meat producer. Bubalus Bubalis 3, 31–44.
Borgnia, M., Nielsen, S., Engel, A., Agre, P., 1999. Cellular and molecular biology of the aquaporin water channels. Annu. Rev. Biochem. 68, 425–458.
Campanile, G., Neglia, G., Vecchio, D., Zicarelli, L., 2012. Buffalo dairying industry in Italy. In 24th Annual Convention of the National Association of Animal Breeders, September 20–21, 2012; Milwaukee, WI, USA. National Association of Animal Breeders (NAAB), Columbia, Missouri, pp. 120–130.
Chen, R.F., Guo, A.M., Geng, D., Zhang, H.Y., Wang, S.C., 2014. Effect of colostrums powder and matura milk powder of buffalo on small intestine tissue morphological structure in newborn piglets. China Animal Husbandry Veterinary Medicine 41, 137–143.
Cutler, C.P., Harmon, S.J., Walsh, S., Burch, K., 2012. Characterization of aquaporin 4 protein expression and localization in tissues of the dogfish (*Squalus acanthias*). Front. Physiol. 3, 1–13.
De Luca, A., Vassalotti, G., Pelagalli, A., Pero, M.E., Squillacioti, C., Mirabella, N., Lombardi, P., Avallone, L., 2014. Expression and localization of aquaporin-1 along the intestine of colostrum suckling buffalo calves. Anatomia, Histologia, Embryologia http://dx.doi.org/10.1111/ahe.12157.
Gomes, D., Agasse, A., Thiebaud, P., Delrot, S., Geros, H., Chaumont, F., 2009. Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochim. Biophys. Acta 1788, 1213–1228.
Hara-Chikuma, M., Sugiyama, Y., Kabashima, K., Sohara, E., Uchida, S., Sasaki, S., Inoue, S., Miyachi, Y., 2012. Involvement of aquaporin-7 in the cutaneous primary immune response through modulation of antigen uptake and migration in dendritic cells. FASEB J. 26, 211–218.
Ishibashi, K., 2006. Aquaporin superfamily with unusual npa boxes: S-aquaporins (superfamily, sip-like and subcellular-aquaporins). Cell. Mol. Biol. 52, 20–27.
Ishibashi, K., Hara, S., Kondo, S., 2009. Aquaporin water channels in mammals. Clin. Exp. Nephrol. 13, 107–117.
Isokpehi, R.D., Rajnarayanan, R.V., Jeffries, C.D., Oyeleye, T.O., Cohly, H.H., 2009. Integrative sequence and tissue expression profiling of chicken and mammalian aquaporins. BMC Genomics 10, S7.
Jiang, L., Li, J., Liu, X., Burnstock, G., Xiang, Z., 2014. Expression of aquaporin-4 water channels in the digestive tract of the guinea pig. J. Mol. Histol. 45, 229–241.

King, L.S., Kozono, D., Agre, P., 2004. From structure to disease: the evolving tale of aquaporin biology. Nat. Rev. Mol. Cell Biol. 5, 687–698.

Liguori, G., Assisi, L., Squillacioti, C., Paino, S., Mirabella, N., Vittoria, A., 2012. Presence, distribution and steroidogenic effect of the peptides orexin A and receptor 1 for orexins in the testis of the South American camelid alpaca (Vicugna pacos). Gen. Comp. Endocrinol. 179, 137–142.

Ma, T., Verkman, A.S., 1999. Aquaporin water channels in gastrointestinal physiology. J. Physiol. 517, 317–326.

Matsuzaki, T., Tajika, Y., Takeshi, S., 2003. Immunolocalization of the water channel, aquaporin-5 (AQP5), in the rat digestive system. Arch. Histol. Cytol. 66, 307–315.

Moon, C., Rousseau, R., Soria, J.C., Hoque, M.O., Lee, J., Jang, S.J., Trink, B., Sidransky, D., Mao, L., 2004. Aquaporin expression in human lymphocytes and dendritic cells. Am. J. Hematol. 75, 128–133.

Oste, M., Van Ginneken, C.J., Van Haver, E.R., Bjornvad, C.R., Thymann, T., Sangild, P.T., 2005. The intestinal trophic response to enteral food is reduced in parenterally fed preterm pigs and is associated with more nitrergic neurons. J. Nutr. 135, 2657–2663.

Parsons, K.R., Howard, C.J., Jones, B.V., Sopp, P., 1989. Investigation of bovine gut associated lymphoid tissue (GALT) using monoclonal antibodies against bovine lymphocytes. Vet. Pathol. 26, 396–408.

Parvin, M.N., Tsumura, K., Akamatsu, T., Kanamori, N., Hosoi, K., 2002. Expression and localization of AQP5 in the stomach and duodenum of the rat. Biochim. Biophys. Acta 1542, 116–124.

Parvin, M.N., Kurabuchi, S., Murdiastuti, K., Yao, C., Kosugi-Tanaka, C., Akamatsu, T., Kanamori, N., Hosoi, K., 2005. Subcellular redistribution of AQP5 by vasoactive intestinal polypeptide in the Brunner's gland of the rat duodenum. Am. J. Physiol. Gastrointest. Liver Physiol. 288, G1283–G1291.

Pelagalli, A., Belisario, M.A., Squillacioti, C., Della Morte, R., d'Angelo, D., Tafuri, S., Lucisano, A., Staiano, N., 1999. The mycotoxin fumonisin B1 inhibits integrin-mediated cellmatrix adhesion. Biochimie 81, 1003–1008.

Rojek, A., Praetorius, J., Frøkiaer, J., Nielsen, S., Fenton, R., 2008. A current view of the mammalian aquaglyceroporins. Annu. Rev. Physiol. 70, 301–327.

Schwarz, S.M., Heird, W.C., 1994. Effects of feeding on the small intestine mucosa of beagle pups during the first 5 d of life. Am. J. Clin. Nutr. 60, 879–886.

Scibelli, A., d'Angelo, D., Pelagalli, A., Tafuri, S., Avallone, L., Della Morte, R., Staiano, N., 2003. Expression levels of the focal adhesion-associated proteins paxillin and p130CAS in canine and feline mammary tumors. Vet. Res. 34, 193–202.

Scocco, P., Aralla, M., Catorci, A., Belardinelli, C., Arrighi, S., 2011. Immunodetection of aquaporin 5 in sheep salivary glands related to pasture vegetative cycle. Folia Histochem. Cytobiol. 49, 458–464.

Squillacioti, C., De Luca, A., Alì, S., Paino, S., Liguori, G., Mirabella, N., 2012. Expression of urocortin and corticotropin-releasing hormone receptors in the horse thyroid gland. Cell Tissue Res. 350, 45–53.

Thi, M.M., Spray, D.C., Hanani, M., 2008. Aquaporin-4 water channels in enteric neurons. J. Neurosci. Res. 86, 448–456.

Tiwari, A., Hadley, J.A., Ramachandran, R., 2014. Aquaporin 5 expression is altered in ovarian tumors and ascites-derived ovarian tumor cells in the chicken model of ovarian tumor. J. Ovarian Res. 25, 99.

Tritto, S., Gastaldi, G., Zelenin, S., Grazioli, M., Orsenigo, M.N., Ventura, U., Laforenza, U., Zelenina, M., 2007. Osmotic water permeability of rat intestinal brush border membrane vesicles: involvement of aquaporin-7 and aquaporin-8 and effect of metal ions. Biochem. Cell Biol. 85, 675–684.

Valenti, G., Procino, G., Tamma, G., Carmosino, M., Svelto, M., 2005. Minireview: aquaporin 2 trafficking. Endocrinology 146, 5063–5070.

Van Haver, E.R., de Vooght, L., Oste, M., Sangild, P.T., Thymann, T., Weyns, A.L., Van Ginneken, C.J., 2008. Postnatal and diet-dependent increases in enteric glial cells and VIP-containing neurones in preterm pigs. Neurogastroenterol. Motil. 20, 1070–1079.

Verkman, A.S., 2009. Aquaporins: translating bench research to human disease. Journal of Experimental Biology 212, 1707–1715.

Wang, Y., Shaikh, S.A., Tajkhorshid, E., 2010. Exploring transmembrane diffusion pathways with molecular dynamics. Physiology 25, 142–154.

Wolinski, J., Slupecka, M., Westrom, B., Prykhodko, O., Ochniewicz, P., Arciszewski, M., Ekblad, E., Szwiec, K., Ushakova, Skibo, G., Kovalenko, T., Osadchenko, I., Goncharova, K., Boertmans, J., Pierzynowski, S., 2012. Effect of feeding colostrum versus exogenous immunoglobulin G on gastrointestinal structure and enteric nervous system in newborn pigs. J. Anim. Sci. 90, 327–330.

Zabielski, R., Le Huerou-Luron, I., Guilloteau, P., 1999. Development of gastrointestinal and pancreatic functions in mammalians (mainly bovine and porcine species): influence of age and ingested food. Reprod. Nutr. Dev. 39, 5–26.