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Original Research Article

Localization of Aquaporin-1 in the Small and Large Intestines of Geese (*Anser anser*)

Key words

geese;
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Abstract: Aquaporins are selective water channels that serve transportation of water across cell membranes, which play a vital role in all cells. In this study, using the immunohistochemical method, the authors intended to investigate the localization of Aquaporin-1 in the small and large intestines of geese. In this study, small and large intestine tissue samples taken from healthy adult geese (*Anser anser*) (n = 10) were used as materials. After fixation for 24 hours at 10% formaldehyde, the tissue samples were passed through graded series of ethanol and xylol and embedded in paraffin. Mallory's modified triple-staining method was used to examine the general structure of the intestine. The Avidin-Biotin-Peroxidase Complex (ABC) method was applied to determine the immunoreactivity of Aquaporin-1. The apical parts of crypt epithelial cells showed strong Aquaporin-1 immunoreactivity in the duodenum and moderate Aquaporin1 immunoreactivity in the jejunum and ileum. Strong Aquaporin-1 immunoreactivity was determined in vascular endothelial cells in the duodenum, jejunum, and ileum, and weak immunoreactivity was found in smooth muscle cells. However, a weak Aquaporin-1 immunoreactivity was detected only in the smooth muscle cells of the cecum and rectum but not in vascular endothelial cells and crypt epithelial cells. The intestine tissue regulates salt transport and hydrostatic pressure differences, enabling the transportation of water. It was suggested that the duodenum and jejunum sections in particular are permeable to high levels of water for balancing the osmotic pressure of the intestinal content. Consequently, with this study, Aquaporin-1 immunoreactivity was detected in the crypt epithelial cells, smooth muscle cells, and vascular endothelium of the small intestines of geese.

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Introduction

Water passes through the plasma membrane by simple diffusion. Channels consisting of specialized membrane proteins are required for the rapid and intense passage of water (1). Aquaporins (AQPs), a family of water channel proteins, are small hydrophobic and integral membrane channel proteins that facilitate the transportation and velocity of water and are responsible for water balance regulation by ensuring continuous and rapid permeability of water with low activation energy throughout the epithelial cells (2, 3). Water molecules that pass through AQP channels move very quickly. In one second, 109 water molecules pass through the AQP channel. The speed of water that transit through the AQP channel is even faster than the catalase enzyme, which is known metabolically as the fastest. This speed is a high speed for metabolic events (4).

Aquaporins have been reported to be hydrophobic proteins with six transmembrane domains whose molecular weight ranges from 28 kDa (unglycosylated form) to 40-50 kDa (glycosylated form), and they are mostly found as a homotetramer (5). Depending on their permeability, AQPs in mammals are divided into three groups 1): Water-selective Aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8), 2); Aquaglyceroproteins which mediate the passage

of glycerol, urea, and some neutral molecules besides water (AQP3, AQP7, AQP9, and AQP10), and unorthodox or super-aquaporins (AQP11 and AQP12) (6, 7).

AQP 1, 2, 4, and 5 show the widespread distribution in every tissue and organ where water is crucial (8). At least eleven varieties of Aquaporin are expressed in various tissues in the gastrointestinal tract. AQP1 is expressed in duodenum, ileum, large intestine, liver, pancreas and gallbladder, AQP2 in small intestine, AQP3 in small intestine, colon and liver, AQP4 in duodenum and colon, AQP5 in duodenum and pancreas, AQP7 in small intestine and colon, AQP8 in large intestine, liver, pancreas, and gallbladder, AQP9 in duodenum, ileum and liver, AQP10 in small intestine, AQP11 in small intestine, colon and liver and AQP12 in pancreas (9, 10).

Aquaporin-1 (AQPl) was first discovered by chance during studies of the human red cell Rh protein as a homologous protein to MIP (Major Intrinsic Protein of Bovine lens) and was labelled as CHIP28 (Channel Forming Intrinsic Protein of 28 kDa). After that CHIP was designated Aquaporin-1 (abbreviated AQPl) by the Human Genome Committee (11, 12).

AQP1 was determined to have important roles in physiological processes such as water homeostasis, neuro-homeostasis, digestion, body temperature regulation, and reproduction by contributing to fluid release and fluid absorption in the body (13). It has been suggested that AQP1 may be involved in angiogenesis, wound healing, organ regeneration, and tumor metastasis (14). A positive correlation has been established between endometrial adenocarcinoma progression and AQP1, microvascular density, as well as vascular endothelial growth factor (VEGF) (15).

Chicken ceca and rectum were determined to have AQP4 immunoreactivity (16). Also, jejunum, ileum, and colon have ck-AQP5 mRNA (17), and the lower intestinal tract of a sparrow has AQ1 distribution (18), but no study of AQ1 immunoreactivity in the small and large intestines of geese was encountered in the literature. In this study it was aimed to determine immunolocalization of Aquaporin-1, that is important in terms of physiological and pathological roles it assumes in the small and large intestines of geese which has economic importance.

Material and Methods

Animal Material

Tissue samples were harvested in compliance with an approved Kafkas University Animal Care and Use Committee Protocol (No. 2018/04, dated 26.04.2018 and coded KAÜHADYEK/2018-049) for this study. The small and large intestine tissue samples taken from 10 female geese (*Anser anser*) at the age of 8 months that local breeders slaughtered for consumption purposes were used as materials.

Histological Procedure

The small and large intestinal tissue samples were fixed for 24 hours in a 10% formaldehyde solution. Afterwards, they were dehydrated (ethanol), cleared (xylol), and embedded in paraffin. Paraffin blocks were cut into 5-µm thick sections on a rotary microtome (LIECA) and stained with Mallory's modified triple staining to examine the general structure of the tissues.

Immunohistochemical procedure

The Avidin-Biotin Peroxidase complex (ABC) technique was used to determine the localization of Aquaporin 1 (AQP 1) immunohistochemically in small and large intestine tissues. 4-µm cross-sections were fixed to lamellas covered with chrome alum gelatin and were subjected to deparaffinization and dehydration. They were then incubated for 20 minutes in the solution of hydrogen peroxide in methanol (3%) to prevent endogenous peroxide activity. Then they were kept in the microwave oven at 600 watts for 20 minutes within a sodium citrate buffer (pH 6.0) solution to release antigenic receptors. Sections incubated for 10 minutes with Blocking Solution A (Invitrogen-Histostatin Plus Bulk Kit) were kept at room temperature for 1 hour after dripping the Aquaporin 1 primary antibody [1/500] (abcam: ab9566) without a PBS wash. The sections were then incubated for 30 minutes with the biotinylated secondary antibody and 30 minutes in Streptavidin Peroxidase solution. To demonstrate the antibody reaction, the DAB (3.3 ' -Diaminobenzidine) chromogen solution was added to the cross-sections, and they were examined with a light microscope. The reaction was stopped with PBS by checking the condition of immunoreactivity. Distilled water-washed sections were subjected to Harris hematoxylin stain for reverse staining and were dehydrated and covered with entellan.

The evaluation was made by two independent observers using the semi-quantitative method by taking the degree of staining in the cross-sections as a criterion. Depending on the staining properties, the slides were scored within the range of 0–3 during their evaluation: no immunoreactivity 0(-), weak immunoreactivity 1(+), moderate immunoreactivity 2(++), and strong immunoreactivity 3(+++). To determine whether immunohistochemical staining is specific, the sections were subjected to an immunohistochemical staining procedure without adding a primary antibody (negative control), provided that all processes were identical. The preparations prepared for histological and immunohistochemical examinations were then photographed and assessed under the light microscope (Olympus Bx53 JAPAN).

Statistical analysis

The data were analyzed with the IBM Statistical Package for Social Sciences (SPSS) program. In analysis, minimum-maximum values, mean, and standard deviation were used and median was calculated to evaluate the data.

Table 1: Statistical analysis of Aquaporin-1 immunoreactivity in geese small and large intestine

	Cells	N	Min	Max	Mean±SD	Median
Duodenum	Crypt epithelial cells	8	2	3	2,56±0,42	2,50
	Vascular endothelial cells	8	2	3	2,56±0,42	2,50
	Smooth muscle cells	8	0	2	1,1875±0,65	1
Jejenum	Crypt epithelial cells	8	1	3	2,06±0,62	2
	Vascular endothelial cells	8	2	3	2,625±0,44	2,75
	Smooth muscle cells	8	0	2	1,0625±0,582	1
Ileum	Crypt epithelial cells	8	1	3	1,88±0,69	2
	Vascular endothelial cells	8	2	3	2,63±0,44	2,75
	smooth muscle cells	8	0,5	2	1,0625±0,49	1
Cecum	Crypt epithelial cells	8	0	1	0,25±0,38	0
	Vascular endothelial cells	8	0	1	0,19±0,37	0
	Smooth muscle cells	8	0	2	1,0625±0,67	1
Rectum (Colon)	Crypt epithelial cells	8	0	1	0,31±0,46	0
	Vascular endothelial cells	8	0	1	0,25±0,46	0
	Smooth muscle cells	8	0,5	2	1,0625±0,49	1

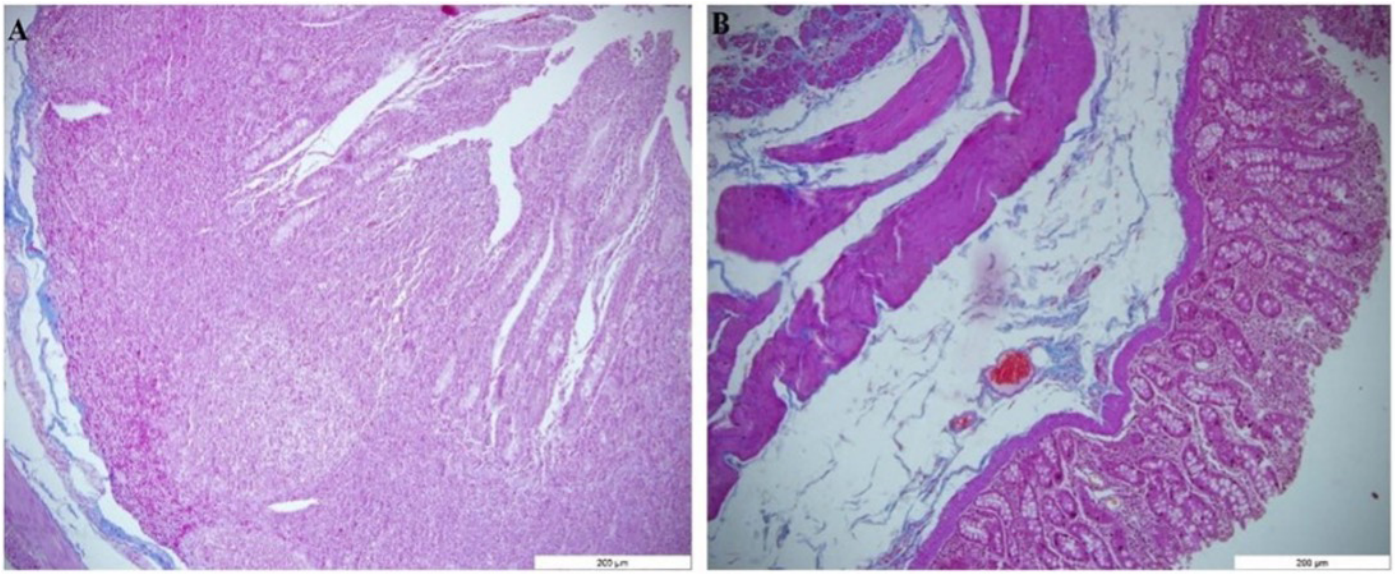

Figure 1: Goose intestine tissue. A; İleum, B; Cecum. Mallory's modified triple staining. A and B; Bar: 200 µm, original magnification, X10

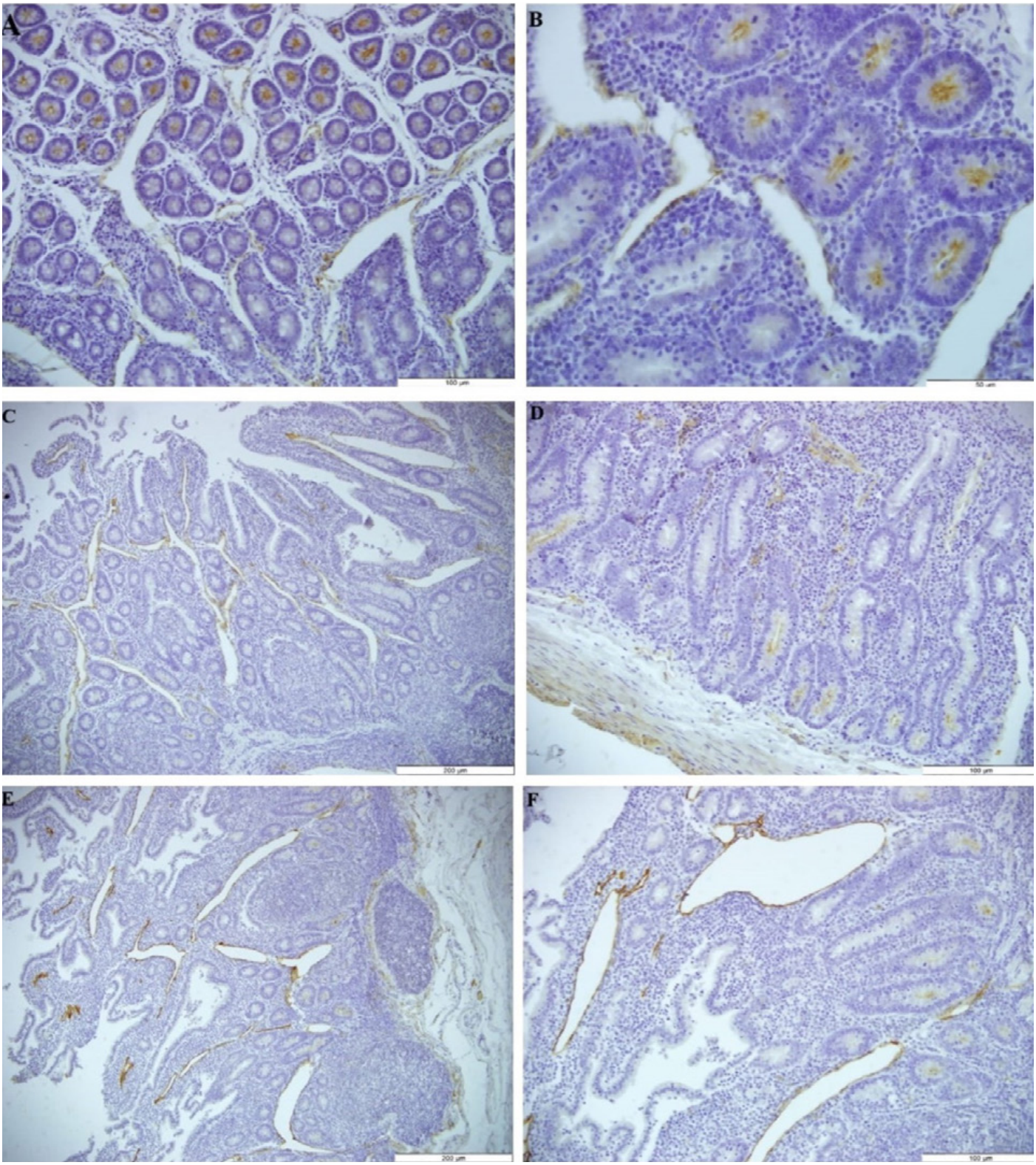

Figure 2: Goose small intestine tissue. AQ1 immunoreactivity. A, B; duodenum, C, D; jejenum E, F; ileum. The Avidin-Biotin-Peroxidase Complex (ABC) method. B; Bar: 50 µm, original magnification, X40, A, D, F; Bar: 100 µm, original magnification, X20, C, E; Bar: 200 µm, original magnification, X10

Results

The normal histological structure of the small and large intestines of geese is shown in Figure 1. The apical parts of crypt epithelial cells in the duodenum showed strong, and the apical parts of crypt epithelial cells in the jejunum and ileum showed moderate AQP1 immunoreactivity. In the duodenum, jejunum, and ileum, strong AQP1 immunoreactivity was determined in vascular endothelial cells and weak immunoreactivity in smooth muscle cells. Weak AQP1 immunoreactivity was detected in the smooth muscle cells of the

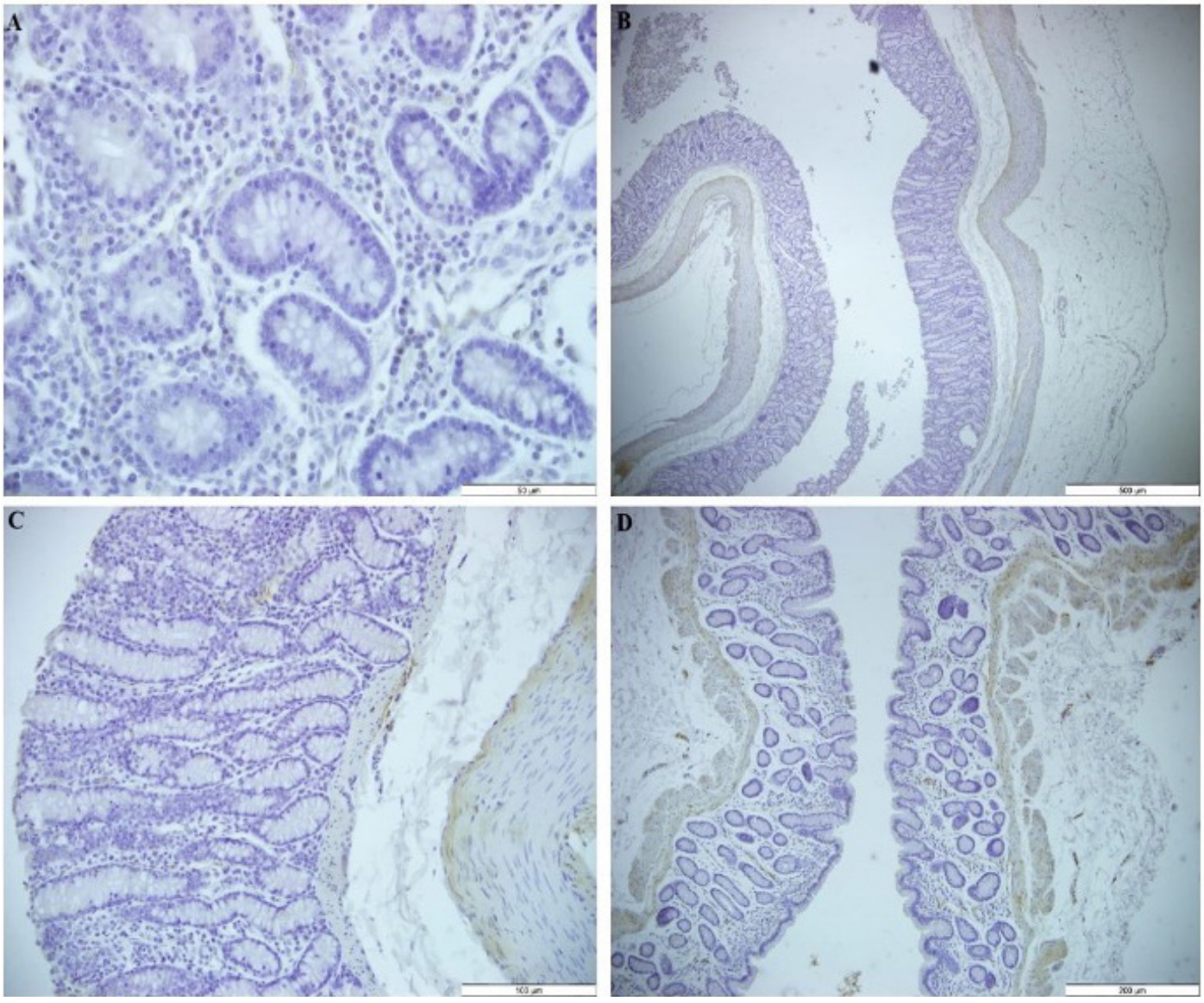

Figure 3: Goose large intestine tissue. AQ1 immunoreactivity. A; cecum, B, C, D; rectum (colon). The Avidin-Biotin-Peroxidase Complex (ABC) method. A; Bar: 50 µm, original magnification, X40, B; Bar: 500 µm, original magnification, X4, C; Bar: 100 µm, original magnification, X20, D; Bar: 200 µm, original magnification, X10

cecum and rectum but no immunoreactivity was observed in either the crypt epithelial cells or the vascular endothelial cells (Table 1 and Figures 3).

Discussion

In the transportation of water, the intestines are the second most important organ after the kidneys. Water transport occurs in the digestive tract because of hydrostatic pressure and osmotic pressure caused by the transport of salt. Much of the transported water is used to regulate saliva, gastric juice, bile, pancreatic fluid, and intestinal fluid, and to adjust water and ion balance (19, 20).

The feed material is ingested, moisturized, ground into small particles, acidified, and attacked by endogenous enzymes in the digestive tract of poultry similar to other animal species (21). In poultry, the intestine is an important organ where enzymatic digestion takes place, and nutrients are absorbed via numerous ion channels and transporters present on the apical intestinal epithelial border. Materials consisting of indigestible food and waste are mixed up with urine in the cloaca and are excreted from the body as feces (22). Intestine composed of duodenum, jejunum, ileum and ceca, rectum (colon), and cloaca. The wall structure of the poultry intestines consists of mucosa, submucosa, muscularis, and serosa layers, like the mammalian intestine (23).

AQP-1 gene sequences of chicken, human, and toad exhibit 94%, 88%, and 78% homology, respectively (18). Specific AQP1 labeling was seen in the endothelia of central lacteals in the villi of the porcine small intestine (24). In the calf of adult buffalo, AQP-1 was detected in the endothelium,

enterocytes, lymphoid tissue, and enteric neurons of both the small and large intestines (25). AQP1 was demonstrated on endothelial cells of lymphatic vessels in the submucosa and lamina propria and capillary endothelial cells in the smooth muscle layer throughout the rat gastrointestinal tract and villus intestinalis and crypt epithelium cells, vascular endothelium, erythrocytes and connective tissue within the small intestine and serosa layer, vascular endothelium and erythrocytes in the large intestines of the mice (26, 27).

Strong AQP4-immunoreactivity was demonstrated in a fiber network in the enteric plexus in chicken ceca and rectum (16). In poultry, ck-AQP5 mRNA was found in the crypt cells of the jejunum, ileum, and colon, but not in the cells that cover the villi (17). Goose testis and vas deferens capillaries were reported to have AQP-1 immunoreactivity in endothelial cells (28). In bird and mammal kidneys, AQP-1,2 and 4 were expressed (29). In this study it was determined that there was AQP1 immunoreactivity in crypt epithelial cells, vascular endothelial cells, and smooth muscle cells in geese duodenum, jejenum and ileum like rat (26), mice (27), and porcine (24) whereas only smooth muscle cells showed a reaction in cecum and rectum.

AQP1 distribution was determined on the apical membrane of the enterocytes, especially in the crypts, and on the cell membrane of erythrocytes of bottlenose dolphin's small intestine. Strong immunostaining was reported in the apical membrane of enterocytes in the mid and bottom regions of the crypt, also comparatively moderate immunostaining was demonstrated at the apical membrane and cytoplasm of enterocytes in the villi and upper region of the crypt (30). It was observed the apical parts of crypt epithelial cells in the duodenum showed strong AQP1 immunoreactivity, and the apical parts of crypt epithelial cells in the jejunum and ileum showed moderate AQP1 immunoreactivity. Also, strong AQP1 immunoreactivity was determined in vascular endothelial cells in the duodenum, jejunum, and ileum and a weak immunoreactivity in smooth muscle cells of geese intestine.

The presence of AQP-1 in the distal rectum of sparrows was reported in large intestines from the ceca to coprodeum with limited distribution. It was suggested that the AQP-1 was present within the cecae especially in the lamina propria and in the mucosa and the muscularis of the proximal rectum and in the epithelium, the lamina propria and muscularis of the distal rectum of house sparrows (18). In this study it was seen that a weak Aquaporin-1 immunoreactivity was detected only in the smooth muscle cells of the geese cecum and rectum. Furthermore, identification of AQP-1 in the mucosa of the large intestine suggested that AQP1 may play a role in water transportation, while localization of AQP-1 in the distal rectal epithelium drew more attention to the importance of retrograde peristalsis for water conservation (18).

In conclusion, indicating that Aquaporin 1 immunoreactivity was observed especially in the apical membranes of crypt enterocytes in geese small intestines as in dolphins (30) and that Aquaporin 1 was localized in endothelial cells of lymph vessels in lamina propria and in the submucosa from the esophagus to the colon of rats (26) suggests that Aquaporin 1 release may be similar in poultry and mammalian intestines.

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Ethical approval. Ethics Committee approval was obtained from the Experimental Animal Ethics Committee of Kafkas University (No. 2018/04, dated 26.04.2018 and coded KAÜ-HADYEK/2018-049) for this research.

Conflict of interest. There are no conflicts of interest to be declared by the authors.

Author's Contribution. EKS conceptualized the study. methodology, E.K.S., B.B., G.F.A., and S.E.Y; software, E.K.S, G.F.A. and S.E.Y; validation, E.K.S., B.B., G.F.A., and S.E.Y; formal analysis, E.K.S., B.B., G.F.A., and S.E.Y; resources, E.K.S. and B.B. ; data curation, E.K.S., B.B., G.F.A., and S.E.Y; writing—original draft preparation, E.K.S., B.B., G.F.A., and S.E.Y; writing—review and editing, E.K.S., B.B., G.F.A., and S.E.Y; visualization, E.K.S., B.B., G.F.A., and S.E.Y; supervision, E.K.S., B.B. and S.E.Y; All authors have contributed to the final version of the manuscript. All authors approved the final manuscript.

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Določanje mesta nahajanja akvaporina-1 v tankem in debelem črevesu gosi (*Anser anser*)

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Izvleček: Akvaporini so selektivni vodni kanali za prenos vode skozi celične membrane in imajo pomembno vlogo v vseh celicah. V tej študiji smo z imunohistokemično metodo ugotavljali mesto nahajanja akvaporina-1 v tankem in debelem črevesju gosi. Uporabili smo vzorce (n = 10) tankega in debelega črevesa odraslih, zdravih gosi (Anser anser). Po 24 urni fiksaciji v 10% formaldehidu smo vzorce dehidrirali v zaporednih stopnjah etanola in ksilola ter jih vpeli v parafin. Za pregled splošne strukture črevesa smo uporabili Malloryjevo modificirano metodo trojnega barvanja. Za določanje imunoreaktivnosti akvaporina-1 je bila uporabljena metoda kompleksa avidin-biotin-peroksidaza (ABC). Močno imunoreaktivnost akvaporina-1 smo ugotovili na apikalnih delih epitelijskih celic kript dvanajstnika ter žilnih endotelijskih celicah v dvanajstniku, jejunumu in ileumu. Zmerna imunoreaktivnost akvaporina-1 je bila prisotna v jejunumu in ileumu. Imunoreaktivnost je bila šibka v celicah gladkih mišic, vendar le v celicah slepega črevesa in danke, ne pa tudi v žilnih endotelijskih celicah in epitelijskih celicah kripte. Črevesno tkivo omogoča prenos vode z uravnavanjem prenosa soli in razlik v hidrostatičnem tlaku. Predpostavljeno je, da sta zlasti odseka dvanajstnika in jejunuma prepustna za velike količine vode za namen uravnavanja osmotskega tlaka črevesne vsebine. Posledično je bila tudi v tej študiji ugotovljena imunoreaktivnost akvaporina-1 v epitelijskih celicah kript, gladkih mišičnih celicah in žilnem endoteliju tankega črevesa gosi.

Ključne besede: gosi; črevo; akvaporin-1