



## Aquaporin 3 Immunolocalization And Histological Characteristics In The Digestive Tract Of The Partridge (*Alectoris chukar*)

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**Abstract:** Aquaporins are integral membrane proteins that regulate transcellular water transport, and AQP3 is regarded as one of the major intestinal aquaporins involved in epithelial water handling, mucosal hydration, and gut homeostasis. However, information on AQP3 localization in the avian digestive tract, particularly in wild-feeding galliform species, remains limited. The aim of this study was therefore to characterize the histological structure of the digestive tract of the partridge (*Alectoris chukar*) and to determine the regional and layer-specific immunolocalization of AQP3. In this descriptive histological and immunohistochemical study, digestive tract samples were obtained from five wild partridges that died during treatment after being found injured in nature, and only tissues without gross pathological findings were evaluated. Tissue samples from the esophagus, proventriculus, gizzard, small intestine, and large intestine were processed routinely, stained with hematoxylin–eosin for histological evaluation, and examined immunohistochemically for AQP3 localization. Histologically, the digestive tract generally showed the basic structural features reported for other avian species. The most notable finding was the presence of prominent villus-like mucosal projections in the colon together with an increasing abundance of goblet cells toward the rectum. Immunohistochemically, AQP3 expression varied according to digestive segment and tissue layer. Strong immunoreactivity was detected in the epithelial layer of the esophagus and in the large intestine, particularly in the colon and rectum, whereas weaker staining was observed in the duodenum and jejunum. In the small intestine, immunopositivity was mainly confined to the epithelial layer and glandular/crypt structures. No inferential statistical comparisons were performed because the study was designed as a descriptive histological and immunohistochemical investigation based on a limited number of opportunistically obtained wild specimens. Within these limits, the findings still provide a segment-based overview of AQP3 distribution in the partridge digestive tract and establish a comparative framework for future avian studies. Overall, the predominantly epithelial distribution of AQP3 suggests that this channel may contribute to transcellular water transport, mucosal hydration, and regional fluid balance in the partridge digestive tract. These findings provide baseline data for comparative avian gastrointestinal histology and may support future studies on digestive physiology, water regulation, epithelial barrier maintenance, and adaptive responses to natural feeding environments in birds.

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**Keywords:** Aquaporin 3, immunolocalization, large intestine, small intestine.

## Kınalı Keklik (*Alectoris Chukar*) Sindirim Kanalında Aquaporin 3 İmmünolokalizasyonu Ve Histolojik Özellikleri



**Öz:** Aquaporinler, transsellüler su taşınmasını düzenleyen integral membran proteinleridir ve AQP3, epitelial su taşınması, mukozal hidrasyon ve bağırsak homeostazında görev alan başlıca intestinal aquaporinlerden biri olarak kabul edilmektedir. Bununla birlikte, özellikle doğal beslenme koşullarına sahip galliform türlerde avian sindirim kanalındaki AQP3 lokalizasyonuna ilişkin bilgiler sınırlıdır. Bu çalışmanın amacı, kınalı keklığın (*Alectoris chukar*) sindirim kanalının histolojik yapısını ortaya koymak ve AQP3'ün bölgesel ve katman-spesifik immünolokalizasyonunu belirlemektir. Bu tanımlayıcı histolojik ve immünohistokimyasal çalışmada, doğada yaralı halde bulunup tedavi sürecinde ölen beş yaban kekliginden elde edilen sindirim kanalı örnekleri kullanılmış ve yalnızca makroskopik patolojik bulgu içermeyen dokular değerlendirilmeye alınmıştır. Özofagus, proventrikulus, taşlık, ince bağırsak ve kalın bağırsaktan alınan doku örnekleri rutin histolojik işlemden geçirilmiş, histolojik değerlendirme için hematoksilin-eozin ile boyanmış ve AQP3 lokalizasyonu immünohistokimyasal olarak incelenmiştir. Histolojik olarak sindirim kanalının genel yapısının diğer kuş türleri için bildirilen temel özelliklerle uyumlu olduğu görülmüştür. En dikkat çekici bulgu, kolonda lümeneye doğru uzanan belirgin villus-benzeri mukozal çıkıntılar ile rektuma doğru artan goblet

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hücreyi yoğunluğu olmuştur. İmmünohistokimyasal olarak AQP3 ekspresyonunun sindirim kanalının bölümleri ve katmanları arasında farklılık gösterdiği belirlenmiştir. Güçlü immünreaktivite özofagus epitelinde ve özellikle kolon ile rektumu içeren kalın bağırsakta saptanırken, duodenum ve jejunumda daha zayıf boyanma görülmüştür. İnce bağırsakta immünpozitiflik esas olarak epitel tabakası ile glandüler/kript yapılarla sınırlı bulunmuştur. Genel olarak, AQP3'ün ağırlıklı olarak epitelde yerleşim göstermesi, bu kanalın kekklik sindirim kanalında transsellüler su taşınmasına, mukozal hidrasyona ve bölgesel sıvı dengesine katkı sağlayabileceğini düşündürmektedir. Bu bulgular, avian gastrointestinal histoloji için karşılaştırmalı temel veriler sunmakta ve kuşlarda sindirim fizyolojisi, su düzenlenmesi ve doğal beslenme koşullarına adaptif yanıtların anlaşılmasına katkı sağlayabilecek nitelik taşımaktadır.

**Anahtar Kelimeler:** Akuaporin 3, immünolokalizasyon, kalın bağırsak, ince bağırsak.

## INTRODUCTION

Partridge (*Alectoris chukar*) is not only a hunting and wild animal, but also intensive and semi-intensive breeding is practiced today (Sariyel et al., 2015). Nutritional conditions are closely linked to the structural and functional organization of the avian digestive tract, and differences in feeding ecology may influence epithelial architecture, mucosal specialization, and intestinal physiology (Dunel-Erb et al., 2001; Karasov et al., 2004; Kuzmina et al., 2024). Recent reviews on avian intestinal physiology likewise emphasize that digestive structure and function should be interpreted in relation to feeding biology and physiological adaptation (Kuzmina et al., 2024). The digestive system is necessary for living organisms to maintain their life functions and is responsible for the breakdown and absorption of nutrients in the body and the elimination of non-organic residues (Duke, 1997; Denbow, 2000; Çelik & Açıkgöz, 2006). In poultry, the digestive tract starts with the oral cavity, continues with the pharynx, esophagus, glandular stomach (proventriculus), muscular stomach (gizzard), small and large intestine and ends with the cloaca. Just like in mammals, the digestive system has accessory organs such as salivary gland, liver, pancreas and gall bladder (Karadağ & Nur, 2004).

The aquaporins (AQPs) are water channel membrane proteins with a molecular weight of approximately 30kDa that mediate the cellular transport of water (Laforenza, 2012). So far, 13 AQPs (0-12) have been identified in mammals. Of these, AQP 0,1,2,4,5,6,8 are aquaporins selectively permeable only to water, while AQP 3,7,9,10 are selectively permeable to water and glycerol. Even AQP9 is reported to be permeable to larger substances other than glycerol. Although the physiological and pathological transport properties of AQP11 and 12 are not fully known, they are grouped as super AQP containing asparagine-proline- alanine (Da Silva et al., 2006; Gorelick et al., 2006; Benga, 2012; Laforenza, 2012).

The transepithelial transport of fluid in the intestine is mediated by paracellular and cellular pathways. In paracellular transport, tight junctions in the intestinal epithelium mediate the regulation of passage according to the size and load of the substances to be transported (Fischbarg, 2010). Cellular transport is mediated by AQP

(Laforenza, 2012). The localization of AQP in the gastrointestinal tract varies according to the functions of the organ. In addition to mediating the transport of water, glycerol, and other small solutes in the intestines, AQP3 is also involved in maintaining intestinal homeostasis (Zhu et al., 2016). It has been reported that AQP3 is localized in the basolateral membranes of epithelial cells in the gastric secretory epithelium and in the epithelial cells of the small intestine and colon mucosa, while functioning as an apical water channel in absorptive epithelial cells (Laforenza, 2012; Ikarashi et al., 2011). Recent reviews have further emphasized that intestinal AQP3 is involved not only in water transport but also in mucosal homeostasis, barrier maintenance, and gut protection under physiological and pathological conditions (Zhu et al., 2016; Zhu et al., 2023). Since different AQP isoforms may show distinct tissue- and species-specific distribution patterns, determining their localization in different avian species is important for physiological interpretation (Takata et al., 2004). Previous avian studies have demonstrated intestinal or gastrointestinal expression of different aquaporin isoforms, including AQP5 in the small and large intestine of chicken, AQP4 in the gastrointestinal tract of chicken, and AQP1 in the small and large intestines of geese, indicating that water-channel distribution in birds is region-specific and isoform-dependent (Ramírez-Lorca et al., 2006; Keiji et al., 2011; Karadağ Sarı et al., 2024). Nevertheless, data specifically addressing AQP3 in the avian digestive tract remain very limited. Therefore, the aim of this study was to determine the histological characteristics and AQP3 immunolocalization of the digestive tract of wild partridges (*Alectoris chukar*).

We hypothesized that AQP3 would show segment- and layer-dependent immunolocalization along the digestive tract of partridges and that these regional differences would be associated with histological features relevant to epithelial water handling and mucosal adaptation.

## MATERIAL AND METHOD

**Animal Material:** This study was conducted with Harran University Animal Experiments Ethics Committee (Decision no: 2024/005/03) ethical approval. Five wild partridges (*Alectoris chukar*) found injured in nature and

subsequently dying during treatment were included in the study. Exact age, sex, and body-weight records were not available for all specimens because the material was obtained opportunistically; this limitation is acknowledged in the Discussion. The organs of the digestive tract were removed as a whole by necropsy and the different organs of this system were dissected. Tissue samples were taken from each of the organs without any pathological findings and placed in 10% buffered formaldehyde solution.

**Tissue Processing:** The tissues, which were fixed in 10% buffered formalin solution, were subjected to routine tissue follow-up after removal of formaldehyde in running tap water. After dehydration and clearing, the tissue samples were infiltrated and embedded in paraffin. Each paraffin block was sectioned with a rotary microtome onto 4 thick normal and adhesive slides. These tissue sections were stained with hematoxylin–eosin (HE) for histological examination and processed immunohistochemically for AQP3 localization.

**Histological Examination:** The tissues were stained with hematoxylin and eosin for histological examination. For this purpose, tissue sections were heated, deparaffinized, and rehydrated through xylol and graded alcohol series. After rinsing in distilled water, the sections were stained with hematoxylin and eosin, washed, dehydrated, cleared in xylol, and mounted with entellan. In histologic examinations under light microscope, images were taken from the relevant organs.

**Immunohistochemical Staining:** The tissues deparaffinized and rehydrated in xylol and alcohol series were kept in distilled water and then kept in 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes. Washing was performed with PBS. Tissue sections were boiled 3 times in citrate buffer (0.01 M, pH 6.0) and cooled. Washing was done with PBS (PBS; 0.01 M, pH 7.4). Tissues were demarcated with Pap pen. Protein block was dripped and incubated for 20 min. Washing was performed with PBS. Sections were incubated with the primary anti-AQP3 antibody (Thermo Fisher Scientific, USA, Cat. No. PA5-78811) at a dilution of 1:50 overnight at 4°C. After washing with PBS, sections were incubated with the secondary antibody/detection system for 20 min, followed by streptavidin conjugate for 20 min. Immunoreactivity was visualized with DAB chromogen, and Mayer's hematoxylin was used for counterstaining. Finally, the tissues dehydrated with alcohol were cleaned with xylol and covered with a coverslip by dropping entellan. Immune reactions were visualized under light microscope.

**Statistical Analysis:** The preparations were examined under a light microscope (Olympus BX51, Tokyo, Japan), and AQP3 immunoreactivity was assessed semi-quantitatively as absent (–), mild (+), moderate (++), or strong (+++) according to staining intensity and

distribution in each digestive segment and tissue layer (Dortbudak et al., 2025). Histological observations and immunohistochemical scores were summarized descriptively. Because the study was designed as a descriptive histological and immunohistochemical investigation with a limited number of specimens, no inferential statistical comparisons were performed.

## RESULTS

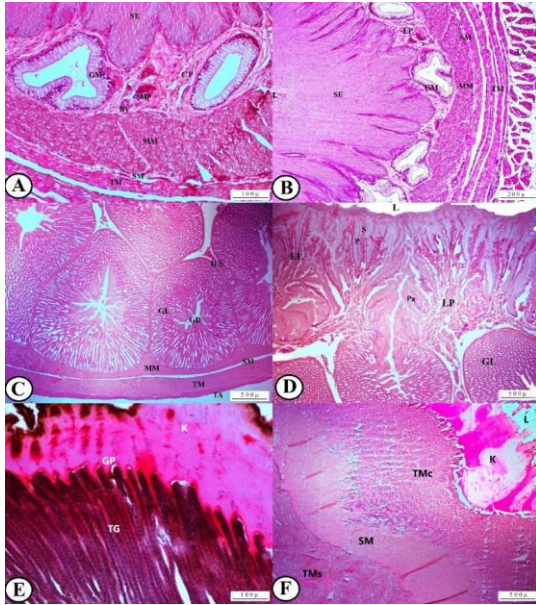
**The Histologic Results:** The histologic examination revealed that the esophagus consisted of 3 main layers: mucosal, muscular and serosal. The luminal surface of the esophagus was lined by non-keratinized stratified squamous epithelium. Numerous mucus glands were seen in the lamina propria under a large epithelial layer and the muscular structure of the mucosa was well developed. The lamina muscularis was well developed and surrounded by loose serosa (Figure 1A, B).

The histologic structure of the glandular stomach consisted of mostly lobular glandula with papillae originating from the glands and plicae as finger-like extensions of these papillae. The sulcus between the plicae, which extended laminally from the conical shaped papillae, was quite prominent. Under this epithelial layer, glands of various formations were seen extending to the base of the mucosa. Loose connective tissue and lamina propria rich in vessels were observed between the epithelial layer and glands. It was determined that the glands with fluid ducts in the center showed a density of cells towards the periphery. Beneath the mucosa, a distinct tunica submucosa composed of connective tissue containing blood vessels and nerves was observed before the tunica muscularis. Under these structures, a large muscular structure and serosa were observed (Figure 1C, D).

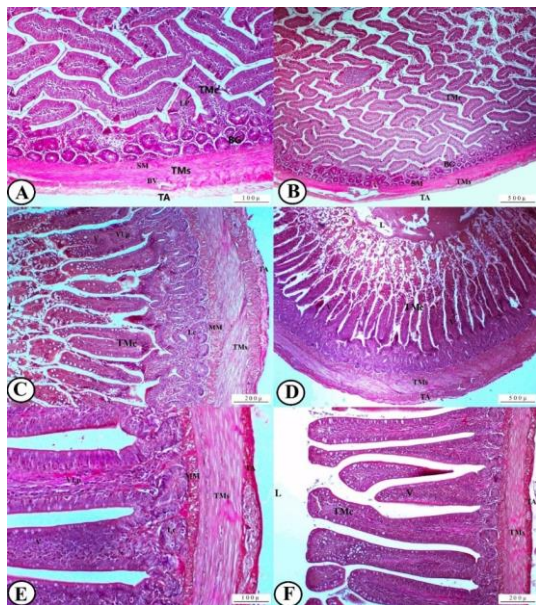
In the luminal surface of the muscular stomach, a thick cuticle layer was observed. This cuticle, composed of the keratin-like carbohydrate–protein complex koilin, protects the mucosa from gastric secretions and contributes to the mechanical grinding of food. Most of the mucosal structure underneath this layer was composed of tubular glands that produced the main substance of koilin. A connective tissue tunica submucosa was present between the mucosa and the thick tunica muscularis. The large muscular layer under the mucosa was surrounded by serosa (Figure 1E, F).

The small intestine consists of the duodenum, jejunum, and ileum, which share a similar basic histological organization, characterized by prominent finger-like villi extending into the lumen. Villus height and crypt depth decreased from the duodenum to the ileum, whereas the number of goblet cells on the villi increased. The lamina propria under the mucosal layer was rich in lymph and blood vessels and consisted of loose connective

tissue. There was a developed muscular layer under a thin submucosa and an serosa surrounding it from the outside (Figure 2A-F).



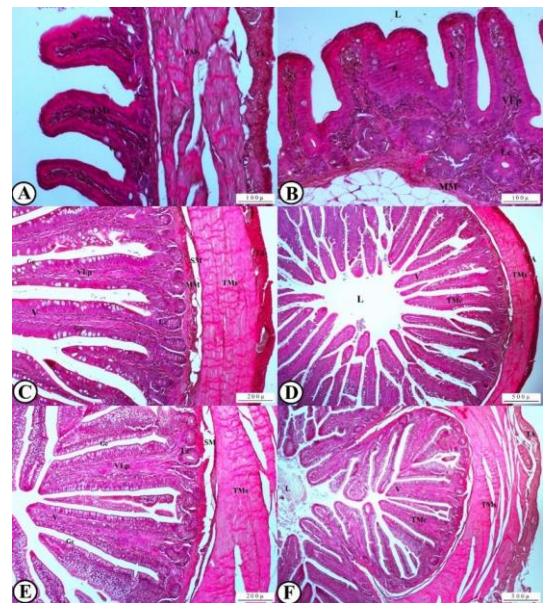
**Figure 1.** The histological appearance of partridge (*Alectoris chukar*) upper digestive tract with hematoxylin-eosin staining. A. Esophagus, SE; Stratified epithelium, MP; Meissner's pleksus, GM; Glandular Mukoza, CT; Connective tissue, BV; Blood vessel, MM; Musculer Mukosa, SM; Submukosa, TM; Tunica mukosa B. Esophagus, SE; Stratified epithelium, L; Lumen, LP; Lamina propriya, GM; Glandular Mukoza, MM; Musculer Mukosa, SM; Submukosa, TM; Tunica mukosa, TA; Tunica adventisia C. Bezli mide, İLS; İnterlobüler septa, GL; Glanduler lobul, GD; Glanduler duct, MM; Musculer mukosa, SM; Submukosa, TM; Tunica mukosa, TA; Tunica adventisia D. Bezli mide, L; Lumen, S; Sulcus, P; Plika, EL; Epitelyal lamina, Pa; Papilla, LP; Lamina propriya, GL; Glanduler lobul E. Taşlı mide, K; Kollin, GP; Gastrik pits, TG; Tubuler glands F. Taşlı mide, L; Lumen, K; Kollin, TMc; Tunica mukoza, SM; Submukoza, TMs; Tunica muskularis.



**Figure 2.** The histologic appearance of partridge (*Alectoris chukar*) small intestine by hematoxylin-eosin staining. A. Duodenum, TMc; Tunica mukoza, LP; Lamina propriya, BG, Brunner Gland, SM; Submukosa, TMs; Tunica muskularis, BV; Blood vessel, TA; Tunica adventisia B. Duodenum, TMc; Tunica mukoza, BG, Brunner Gland, SM; Submukosa, TMs; Tunica muskularis, TA; Tunica adventisia C. Jejunum, L; Lumen, V; Villus, VLp; Villöz lamina propriya, TMc; Tunica mukoza, Lc; Lieberkuhn kript, MM; Musculer mukoza, TMs; Tunica muskularis, TA; Tunica adventisia D. Jejunum, L; Lumen, TMc; Tunica mukoza, V; Villus, TMs; Tunica muskularis, TA; Tunica adventisia E. Ileum, V; Villus, VLp; Villöz lamina propriya, Lc; Lieberkuhn kript, MM; Musculer mukoza, TMs; Tunica muskularis, TA; Tunica adventisia F. Ileum, V; Villus, VLp; Villöz lamina propriya, Lc; Lieberkuhn kript, MM; Musculer mukoza, TMs; Tunica muskularis, TA; Tunica adventisia.

Lieberkuhn kript, MM; Musculer mukoza, TMs; Tunica muskularis, TA; Tunica adventisia D. Jejunum, L; Lumen, TMc; Tunica mukoza, V; Villus, TMs; Tunica muskularis, TA; Tunica adventisia E. İleum, V; Villus, VLp; Villöz lamina propriya, Lc; Lieberkuhn kript, MM; Musculer mukoza, TMs; Tunica muskularis, TA; Tunica adventisia F. L; Lumen, TMc; Tunica mukoza, V; Villus, TMs; Tunica muskularis, TA; Tunica adventisia.

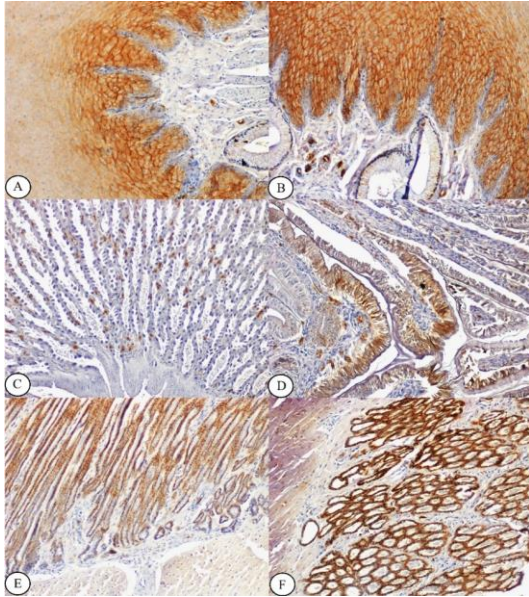
The large intestine consisting of the cecum, colon and rectum had some histologic differences compared to the small intestine. Some differences were also observed in the sections within the large intestine. Compared to the small intestine, the number of villi was decreased, while the villi appeared thicker, and the number of goblet cells increased. The increase in the number of goblet cells from the cecum to the rectum was quite prominent. Underneath the mucosal layer, as in the other intestinal segments, there was a muscular layer and this layer was surrounded by serosa from the outside. It was determined that the villi of the colon developed towards the lumen as in the small intestine and there was a significant presence of goblet cells on the villi and even an increase towards the rectum (Figure 3A-F).



**Figure 3.** The histologic appearance of partridge (*Alectoris chukar*) large intestine with hematoxylin-eosin staining. A. Sekum, TMc; Tunica mukoza, V; Villus, Gc; Goblet cell, TMs; Tunica muskularis, TA; Tunica adventisia B. Sekum, L; Lümen, V; Villus, VLp; Villöz lamina propriya, Lc; Lieberkuhn kript, MM; Musculer mukoza C. Colon, V; Villus, VLp; Villöz lamina propriya, Lc; Lieberkuhn kript, MM; Musculer mukoza Gc; Goblet cell, TMs; Tunica muskularis, SM; Submukoza, TA; Tunica adventisia D. Colon, L; Lümen, TMc; Tunica mukoza, V; Villus, TMs; Tunica muskularis, TA; Tunica adventisia E. Rectum, V; Villus, Gc; Goblet cell, VLp; Villöz lamina propriya, Lc; Lieberkuhn kript, SM; Submukoza, TMs; Tunica muskularis F. Rectum, L; Lümen, V; Villus, TMc; Tunica mukoza, TMs; Tunica muskularis, TA; Tunica adventisia.

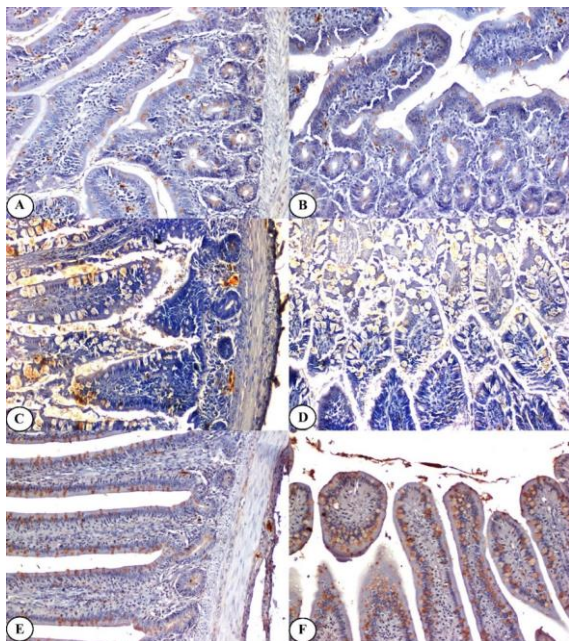
**Immunohistochemical Results:** In the immunohistochemical examination, it was noted that AQP 3 expression differed between the organs and layers of the digestive system. High expression was observed in the epithelial layer of the esophageal mucosa. However, no immune positivity was recorded in other layers. While overexpression was observed in the epithelial layer of the

glandular stomach, very little AQP 3 expression was observed in the glands and lamina propria. In the muscular stomach, severe AQP3 expression was observed in the tubular glandulae in the mucosal layer and less in the muscular layer (Figure 4A-F).



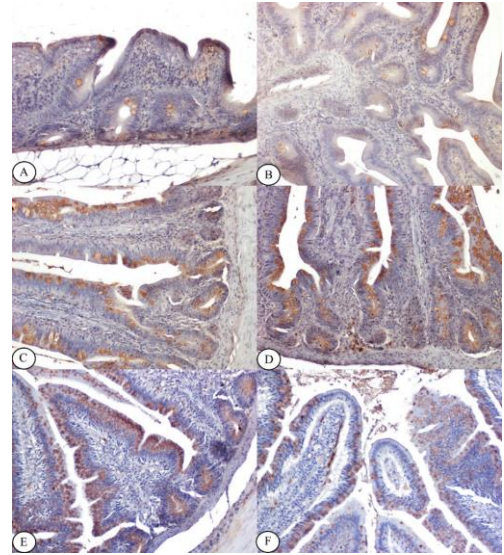
**Figure 4.** AQP3 distribution in partridge (*Alectoris chukar*) upper digestive tract by immunohistochemical staining A, B. AQP3 expression in the esophagus C, D. AQP3 expression in the glandular stomach E, F. AQP3 expression in the muscular stomach.

In the small intestine, immunopositivity was observed in the epithelial layer of the duodenum and in Brunner's glands. In the jejunum, immunopositivity was noted in the epithelial layer and mainly in the crypts of Lieberkühn. In the ileum, immunopositivity was observed in the epithelial mucosa and crypt glands (Figure 5A-F).



**Figure 5.** Partridge (*Alectoris chukar*) small intestine AQP3 distribution by immunohistochemical staining A, B. AQP3 expression in duodenum C, D. AQP3 expression in jejunum E, F. AQP3 expression in ileum.

In the large intestine, severe AQP 3 expression was observed in the epithelial layer and glands, while partial immunoreaction was observed in the lamina propria and lamina muscularis mucosae. Similarly, severe AQP 3 expression was observed in enterocytes and especially in goblet cells in the colon and rectum (Figure 6A-F). AQP 3 expression and its severity in the organs of the digestive system are tabulated (Table 1).



**Figure 6.** The distribution of AQP3 in partridge (*Alectoris chukar*) large intestine by immunohistochemical staining A, B. AQP3 expression in the cecum C, D. AQP3 expression in the colon E, F. AQP3 expression in the rectum.

**Table 1.** Distribution and expression intensity of AQP3 in the organs of the digestive system.

	Tunica Mucosa				Tunica Muscularis	Tunica Adventitia
	Lamina Epithelialis	Lamina Propriya	Muscule r Mucosa	Glandula r Mucosa		
Esophagus	+++	-	-	-	-	-
Proventrikulus	+++	+	-	++	-	-
Gizzard	+	-	+	+++	++	-
Duodenum	+	-	-	++	-	-
Jejunum	++	-	-	++	-	-
Ileum	+++	-	-	++	-	-
Cecum	+++	+	+	+++	-	-
Colon	+++	-	-	+++	-	-
Rectum	+++	-	-	+++	-	-

## DISCUSSION

The avian digestive tract shows species-specific structural variation that is closely related to feeding biology and digestive function (Taşçı, 2018). In the present study, the general histological organization of the partridge digestive tract was largely consistent with the basic avian pattern reported for other birds, supporting the view that the esophagus, proventriculus, gizzard, and intestinal segments share a conserved structural framework across poultry species. At the same time, some regional findings in the large intestine suggested a degree of species- or ecology-related specialization.

In the general histologic structure of the digestive tract, the mucous membrane is the innermost layer. This structure is involved in the transmission and digestion of

food. Beneath the mucosal layer is a muscular layer of varying thickness that provides motility. At the outermost part of the digestive tract is the serosa layer, which is mostly composed of connective tissue and provides integrity to the digestive tract (Taşçı, 2018; Hamdi et al., 2013; Kadhim & Mohamed, 2015). In this study, it was determined that the general histological characteristics of the partridge (*Alectoris chukar*) digestive tract were compatible with the literature. It was determined that the part of the partridge used in the study up to the large intestine (esophagus, glandular stomach, muscular stomach, duodenum, jejunum and ileum) was similar to other poultry species (Taşçı, 2018; Zaher et al., 2012; North et al., 2016; Taşçı et al., 2018; Deniz, 2022; Pandit et al., 2018).

In wildlife, as in intensive feeding, the animal may go through a long period of starvation because it cannot find feed when it is hungry. Therefore, unlike domestic or commercially farmed species, some changes occur in the gastrointestinal system. These changes are reported to be an increase in intestinal length and volume, decrease in mucosal weight, changes in villus length and thickness, and phenotypic changes in enterocytes (Dunel-Erb et al., 2001; Karasov et al., 2004; Zeng et al., 2012). One of the most notable findings of this study was the presence of prominent villus-like mucosal projections in the colon together with abundant goblet cells, increasing toward the rectum (Zaher et al., 2012; North et al., 2016; Pandit et al., 2018; Karadağ Sarı et al., 2024). This pattern may be compatible with enhanced epithelial surface specialization, mucosal protection, and regional fluid handling under natural feeding conditions; however, because no morphometric or functional measurements were performed, this interpretation should be considered tentative (Dunel-Erb et al., 2001; Karasov et al., 2004; Zeng et al., 2012).

Aquaporins play important roles in transepithelial water transport in the gastrointestinal tract, and AQP3 has been reported to be a functionally important channel in epithelial water movement, mucosal hydration, and intestinal fluid balance (Laforenza, 2012; Ikarashi et al., 2011; Ikarashi et al., 2016; Kon et al., 2018; Zhu et al., 2016; Zhu et al., 2023). In the present study, the predominantly epithelial distribution of AQP3 supports this general functional interpretation. Strong immunoreactivity in the esophageal epithelium may be associated with epithelial hydration and luminal surface protection, whereas the marked expression observed in the colon and rectum suggests that AQP3 may contribute to distal intestinal fluid regulation and maintenance of mucosal integrity. In contrast, the weaker immunoreactivity detected in the duodenum and jejunum indicates that the contribution of AQP3 to water transport

may differ among digestive segments in partridge. In addition, the localization of staining mainly in epithelial and glandular compartments, rather than in vascular or muscular structures, suggests that AQP3 may be more closely related to mucosal surface regulation than to deeper tissue components in this species.

Comparative avian data on aquaporins in the digestive tract remain limited, but the available studies support the view that their distribution is both isoform-specific and region-specific. In geese, weak AQP1 immunoreactivity has been reported in the muscular tissue of the cecum and rectum, with no immunoreaction in the crypt epithelium or vascular endothelium (Karadağ Sarı et al., 2024). In chicken, ck-AQP5 expression has been reported mainly in crypt cells of the jejunum, ileum, and colon rather than in villus-lining cells (Ramírez-Lorca et al., 2006), whereas AQP4 immunoreactivity has been described in the gastrointestinal tract, especially in the glandular stomach and in the epithelial layers of the cecum and rectum (Keiji et al., 2011). In the present study, AQP3 immunoreactivity was strongest in epithelial and glandular compartments, particularly in the cecum, colon, and rectum, while the muscular layer showed weaker staining. Similarly, AQP3 immunopositivity was observed in the epithelial layers of the cecum and rectum, whereas expression in the glandular structure of the glandular stomach was weaker than in its epithelial layer. Taken together, these findings suggest that water movement in the avian digestive tract may be regulated by different aquaporin isoforms in different cellular compartments; therefore, the present comparisons are intended to support physiological interpretation rather than to imply direct equivalence among AQP types or avian species (Takata et al., 2004). This study has some limitations. First, the number of specimens was limited. Second, the study was descriptive and semi-quantitative, and no morphometric or functional analysis was performed. Third, only AQP3 was evaluated; therefore, the possible coordinated roles of other aquaporin isoforms in the partridge digestive tract remain unclear. These limitations should be considered when interpreting the present findings.

Future studies combining immunohistochemistry with morphometric, histochemical, or ultrastructural approaches such as SEM may further clarify whether the distal intestinal specializations observed in partridge are linked to functional adaptation.

## CONCLUSION

In conclusion, the distribution pattern of AQP3 immunoreactivity observed in the epithelial structures of the digestive tract suggests that AQP3 may contribute to transcellular water transport and the regulation of mucosal hydration in the partridge intestine. Its presence in

absorptive epithelial regions supports a role in maintaining fluid balance, which is essential for digestion, nutrient absorption, and protection of the mucosal surface. These findings provide baseline data for comparative studies on avian digestive physiology and may contribute to a better understanding of the functional significance of aquaporins in the gastrointestinal tract of birds (Laforenza, 2012; Zhu et al., 2016; Zhu et al., 2023).

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