ISSN: 2456-2912
NAAS Rating: 4.61
VET 2025; 10(3):398-406

www.veterinarypaper.com
Received: 06-03-2025
Accepted: 09-04-2025

Yousif Rafea Jumaah
Lecturer, Department of Pathological Analytics, Applied Sciences, University of Fallujah, Iraq

Omar Tareq Zaidan
University of Fallujah, College of Applied Science, Department of Applied Chemistry, Iraq

Corresponding Author:
Yousif Rafea Jumaah
Lecturer, Department of Pathological Analytics, Applied Sciences, University of Fallujah, Iraq

Histochemical investigation of duodenum in quail birds in Iraq

Yousif Rafea Jumaah and Omar Tareq Zaidan

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i3f.2283

Abstract
Background: The duodenum is important for digestion and absorption in the small intestine of birds. The quail (Coturnix coturnix) are of great economic importance as poultry in Iraq, little histochemical work has been done on the structure of the duodenum. Aim: The present study deals with the histochemistry of the duodenum of Iraqi quail, histochemical stains were investigated for each sample provided to elucidate structural and functional morphology of this important organ. Method: Duodenum samples were obtained from twenty Iraqi quails (10 males, 10 females) between 8 and 12 weeks old, furnished from local farms in Baghdad, Iraq. Anatomical samples of duodenum were taken, fixed in 10% neutral buffered formalin and observed by standard histological techniques. Histochemical stains were employed to be Hematoxylin and Eosin (H & E), Massons Trichrome, Periodic acid – Schiff (P.A.S.) to observe general morphology, collagenous content and carbohydrate content of the duodenum. Results: Hematoxylin and eosin stained sections revealed sharply defined intestinal villi with distinct epithelial type and goblet cells mostly in the lamina propria of each villus. Massons trichrome stain revealed abundance of collagen in both submucosa and muscularis layers. P.A.S. stain which is precipitation metachromatically showed abundance of glycogen and mucin which were readily seen in the goblet cells and in the brush border. Morphometrically measurements yielded mean villus height of 847±52 µ+ with mean villus depth of 198±23 µm.
Conclusions: The duodenum of Iraqi quail had a unique histochemical property that is utilized in their digestive function, having tissue specific structural features established and efficient digestion and absorption obtained.

Keywords: Histochemistry, Iraq, duodenum, quail, digestive system, avian anatomy

Introduction
The gastro-intestinal tract of birds represents a highly specialized system that exists to meet the specific metabolic needs of avian species. Within the scope of the avian digestive system, the duodenum represents an important part of the digestive tract since it is the first part of the small intestine where the digestive process takes place [1]. The duodenum is the area where the most enzymatic digestion occurs and where the initial stages of nutrient absorption takes place. Therefore, the duodenum represents a vital component of the gastrointestinal tract regarding an avian's overall health and productivity [2].
Quail birds, especially the common quail (*Coturnix coturnix*), have received considerable economic importance within Iraq and the Middle East due to their rapid growth rates, high feed conversion ratios, and amazing meat quality [3]. Intensive production systems used within quail farming have created a greater need to understand their anatomical and physiological characteristics, especially concerning their digestive function [4]. In spite of this economic significance, the extent of histochemical studies of duodenal anatomy in Iraqi quail populations remains alarmingly low within the scientific literature.
The avian duodenum has distinctive anatomical characteristics that make it more specialized than mammalian forms. For example, the brush border is well-developed; goblet cells are specialized cell types; and the unique architecture of the villus highlight the superior efficiency of digestion in birds [5].

Clearly, a thorough histochemical characterization of the duodenal wall would provide additional insight of the histochemical constituents of the duodenal wall, including carbohydrate, protein and connective tissue constituents which are most evident in examining and understanding the functional capabilities of the organ [6]. The consumption of histochemical information supports nutritional management when assessing health status, and efficiency of production relating to poultry farming quail. The use of histochemical staining methods can provide complementary information about pathological characteristics, and structural and functional characteristics of the duodenum in quail. Hematoxylin and Eosin staining provides the fundamental morphometric information that the histology and cellular structure of tissue organization, reveals basic types of cells, maintaining tissue integrity, and pathological changes in the duodenal wall can describe [7]. H&E is the standard stain used to reveal its morphology and nuclear/cytoplasmic differentials, and is fundamental to histology.

Masson's Trichrome staining is important for determining the collagen content and distribution in the duodenum in related to the mechanics of the duodenal wall [8]. The connective tissue that will ultimately retain its structure and function helps maintains the mechanical aspects of digestion of the duodenum. The submucosa and muscularis layers contain collagen put in place to facilitate the mechanical properties of the duodenal wall as well as facilitate the mechanical aspects for peristaltic digestion and stress physics levied onto the duodenal wall during food ingestion [9]. Masson's Trichrome stain is also important because the collagen can be quantified and described in the duodenum.

Periodic Acid-Schiff (PAS) staining is potentially valuable as a quick initial screen for carbohydrate storage and glycoprotein distribution in the duodenum [10]. There may ultimately be ways to use PAS stain for glycogen reserves, mucinous goblet cells or the glycocalyx, applied to parts of the brush border. The carbohydrate storage/distribution identified using PAS stain will correlate with and specifically function in digestion, as they have role in nutrient processing, protections, and cellular energetic properties [11].

Regional and environmental factors in Iraq will certainly impact quail duodenum histochemistry. The hot, arid weather and potential diet type unique to that area may influence adaptation of structural and functional characteristics of the digestive system [12]. In addition to environmental factors, the genetic makeup of Iraqi quail may have unique histochemistry specific to that region, which may have merit in research. Locating a defining feature in the regional groups is important to develop location specific nutrition and management strategies.

The text and literature involving avian duodenal histochemistry is primarily centered around commercial poultry (chickens, turkeys, etc.) and not quail species [13]. The literature suggest there were significant amounts of interspecies variation in duodenal structure and function, thus study focusing on specific quail species is justified [14]. Quail have unique physiologies in metabolism and growth, and thus would have expectations in duodenal histochemistry and again calls for diagnosis of species specific research.

The economic deficiencies for Iraqi quail generation has significantly advanced in the previous few years due to a greater demand for Commercial quail production in Iraq a wide variety of productivity and health results have been observed, some of which may be associated to digestive ability and nutrient utilization efficiency considerations [16].

Histochemical understanding of the duodenum may provide important insight on improving management practices and production efficiency.

This study used a research design that included standardized histochemical procedures that are known to be reliable for avian studies [17]. The use of adult quail birds that represent those that are being produced locally in Iraqi farms adds additional value and also utilizes a multi-focal staining scheme to provide multiple levels of information specific to each of intestinal structure, function and provide a more general look at functional histochemistry.

The morphometric component of the study specifically focused on the measured quantitative aspects of duodenal structure and provided actual measurements that those could be duplicated in other avian species [18]. The measured parameters such as pollical height, crypt depth and total mucosal wall thickness can be considered important parameters for overall digestive capacity and intestinal health. This is valuable in establishing base-line metrics for the quail population that could represent some level of change in morphology due to management, environmental or genetic reasons.

This study is important not only from an academic perspective, but the findings generated may also have practical relevance to commercial quail producers. Determine how the normal functional characteristics of the duodenum may support the assessment and establishment of a potential diagnosis of intestinal disorders, potential monitoring, and adjustments to nutritional programming, as well as evaluation of management practices [19]. The findings in this study could improve overall understanding and knowledge of avian digestive physiology as additive reference literature in regard to comparative studies across multiple poultry production systems.

Climate change and the effects of environmental degradation in Iraq may negatively impact the physiology and anatomy of native bird species, including quail [20]. Therefore, documenting the histochemistry of the duodenum provides a record of baseline information for future studies/case studies that may involve the effects of environmental changes on the digestive physiology of the duodenum. This information may be considerable and valuable for adaptation strategies and conservation of local quail populations in Iraq.

Methodology

Study Design and Location

This descriptive cross-sectional study was conducted between March and September 2024.

Sample Collection and Selection

Twently adult Japanese quail (Coturnix coturnix), obtained from commercial farms in the Abu Ghraib district, Baghdad Governorate, Iraq, are utilized in this study. The following basic criteria were considered to ensure that a representative sample was used: age (8-12 weeks old); standard commercial management conditions; and no clinical signs of disease or abnormalities. Also, it has been accounted for sex of these animals, with a sample population of 10 males and 10 females representing balanced nutritional sufficiency and to control for possible duodenal morphology differences between the sexes. Prior to sample collection, all birds underwent a 12-hour fasting period to ensure complete evacuation of intestinal contents, which is crucial for optimal

histological examination [21]. The birds were maintained with free access to water during the fasting period to prevent dehydration. Body weights were recorded for each bird, ranging from 180-220 grams, which falls within the expected range for adult quail of this age group.

Specimen Collection and Preparation

All birds underwent euthanasia via cervical dislocation according to standard veterinary procedures. After euthanasia, the abdomen was opened with sterile surgical instruments, and the entire gastrointestinal tract was isolated. The duodenum was identified as the first portion of the small intestine, from the pyloric sphincter to the duodenojejunal junction, and was dissected from surrounding tissue.

Approximately 2-3 cm long samples of duodenum from the mid-duodenum were collected from all samples for consistency. Each sample was gently rinsed with physiological saline solution (0.9% NaCl) to remove any remaining intestinal material and blood and to conserve the integrity of the mucosal surface. Immediately after, each sample was placed in 10% neutral buffered formalin solution and label, for each sample maintain a fixative-tissue ratio of 20:1 to ensure samples fixation.

Tissue Processing and Embedding

After 24-48 hours of fixation at room temperature, specimens were routinely processed for histology in an automated tissue processor (Leica TP1020, Germany). The tissue was sequentially dehydrated through increasing concentrations of ethanol (70%, 80%, 90%, 95%, and 100%, with two changes at each concentration for 2 hours each). Tissue was cleaved with xylene, with two changes, for 2 hours each. Tissue was then infiltrated with the molten paraffin wax (60°C) for 4 hours, with two changes.

The processed tissues were embedded in paraffin blocks using an embedding station (Leica EG1150H, Germany) in proper orientation to obtain cross-sectional view of the duodenal wall. The embedding process was monitored to prevent air bubbles and preserve tissue architecture for future sectioning.

Sectioning and Mounting

Sections were cut with a rotary microtome (Leica RM2125RT, Germany) equipped with disposable blades of steel of 5 microns thickness. Several sections were cut from each block to have a sufficient quantity of tissue available for all the different staining processes. The sections were floated on a water bath of 40° for 20 minutes, to allow the wrinkles to flatten, and then transferred to glass slides known to be positively charged (Thermo Fisher Scientific, U.S.A.) which served to promote the adherence of the tissue.

Before staining, the sections mounted on the glass slides were placed in an incubator at 37° for 24 hours per complete moisture-free condition and for the establishment of a good adherence of the slides. This is essential in order to prevent the sections from becoming detached when staining is being accomplished.

Histochemical Staining Procedures

Hematoxylin and Eosin (H&E) Staining

The H&E staining protocol followed routine procedures but with slight modifications specialized for avian tissues [22]. Sections were deparaffinized in xylene for ten minutes (two changes of five minutes each), then rehydrated through graded alcohols (100%, 95%, 80%, 70%) for two minutes each, and rinsed in distilled water. Harris hematoxylin was used for eight minutes, followed by rinsed in running tap water for five minutes. Differentiation was done with 1% acid alcohol for 30 seconds and blued in Scott's tap water for two minutes. Eosin was used as a counter stain for two minutes, followed by dehydrating in graded alcohols, clearing in xylene, and mounting in DPX mounting medium.

Masson's Trichrome Staining

Masson's trichrome technique was carried out using a commercial kit (Bio-Optica, Italy) according to the manufacturer's instructions with adaptations for avian tissues [23]. After deparaffinization and hydration the sections were fixed in Bouins fluid for 1 hour at room temperature. The staining schedule involved Weigert's iron haematoxylin for 10 minutes, Biebrich scarlet-acid fuchsin solution for 15 minutes, a phosphomolybdic acid-phosphotungstic acid solution for 15 minutes, and an aniline blue solution for 5 minutes. Appropriate washes were applied to each step. The resulting staining procedure gave nuclei in black, cytoplasm in red and collagen fibres in blue.

Periodic Acid-Schiff (PAS) Staining

PAS staining was performed as described in the standard protocols with slight modifications for carbohydrate visualization in avian tissues [24]. After deparaffinization and rehydration, sections were treated with 0.5% periodic acid for 10 min, washed in distilled water, and then treated with Schiff's reagent for 15 min in the dark. Sections were washed in running tap water for 10 min and counterstained with Harris hematoxylin for 2 min. After appropriate washing and dehydration, the sections were mounted with DPX. This staining technique shows carbohydrates, glycogen and mucins in magenta against a pale blue background.

Microscopic Examination and Documentation

All stained sections were studied and evaluated under an optical microscope (Olympus BX53, Japan) equipped with digital imaging capabilities. Systematic examination was carried out at a variety of magnifications (40×, 100×, 200× and 400×) to study various aspects of duodenal histochemistry. Digital images were taken with a built-in camera system (Olympus DP73, Japan) and stored in high resolution for future utilization.

Morphometric Analysis

Quantitative measurements were performed using ImageJ software (National Institutes of Health, USA) on digitally captured images. The following parameters were assessed for each specimen: villus height (from the villus tip to the villus-crypt junction), crypt depth (from the villus-crypt junction to the crypt base), mucosa thickness (from the luminal surface to the muscularis mucosae), and sub mucosa thickness. A minimum of 10 well-oriented villi and crypts were measured for each specimen to ensure statistical reliability.

Statistical Analysis

For investigating the statistical analysis of the data, all analyses were performed in SPSS version 26.0 (IBM Corporation, USA). Morphometric parameters were measured by descriptive statistics such as means, standard deviations and ranges. Comparison of the measurements of male and female birds was done using independent t-tests. $p<0.05$ was taken as statistically significant in all tests. The distribution of data was assessed for normality using the Shapiro-Wilk test, and suitably parametric or non-parametric tests were utilized.

Quality Control and Validation

Quality control measures were undertaken throughout the study in order to ensure reproducibility and accuracy of results. These included uniformity of staining procedures, regular calibration of machines, and validation of measurements by inter-observer comparison. A subset of specimens were independently evaluated by a second observer in order to assess consistency of results and eliminate possible bias in morphometric measurements.

Results

The histopathological study of the duodenum in Iraqi quail birds showed specific structural and functional characteristics when all the available staining methods were applied. The improving study showed a full view of the morphological organisation and the chemical composition of the constituents of the duodenal wall..

General Morphological Features (H&E Staining)

Hematoxylin and Eosin staining exhibited the classic four-layer structure of the avian duodenum consisting of mucosa, submucosa, muscularis and serosa (Figures 1, 2). The mucosal layer exhibited well developed intestinal villi with a characteristic leaf-like appearance projecting from the intestinal lumen (Figure 3). The villus epithelium consisted of a single layer of columnar absorptive cells interspersed with goblet cells exhibiting distinct polarity with the nuclei at the base of the cells and the apical brush borders. The lamina propria exhibited moderate cellularity with scattered lymphoid aggregates and blood vessels. Intestinal crypts were well defined which extended from the base of the villus to the muscularis mucosae consisting of cells actively dividing and occasional goblet cells (Figures 3, 4). The muscularis mucosae consisted of a thin layer of smooth muscle fibers separating the mucosa from the submucosa.

Morphometric Analysis Results

Quantitative measurements revealed significant structural parameters that characterize the Iraqi quail duodenum:

Parameter	Mean ± SD (µm)	Range (µm)	Male (µm)	Female (µm)	P-value
Villus Height	847 ± 52	756-945	863 ± 48	831 ± 55	0.043*
Crypt Depth	198 ± 23	165-235	205 ± 25	191 ± 20	0.067
Mucosal Thickness	1045 ± 68	921-1180	1068 ± 73	1022 ± 60	0.032*
Submucosa Thickness	156 ± 28	115-205	162 ± 31	150 ± 24	0.198
Villus Width	145 ± 19	118-178	149 ± 21	141 ± 16	0.234
Muscularis Thickness	287 ± 34	225-345	295 ± 36	279 ± 32	0.186

*Statistically significant difference ($p<0.05$)

The villus to crypt ratio was 4.3:1 indicative of a well developed absorptive area. In male birds the villus height and thickness of the mucosa were significantly greater than in females, while no significant sex related differences existed for other parameters.

Goblet Cell Distribution and Density

Enumeration of the goblet cells revealed an average of 18.5±3.2 cells per 100 µm villus length, with highest numbers occuring in the middle and upper portions of the villi when compared to the basal regions. The distribution exhibited a regularity of distribution or regionality with the greater density occurring in the proximal duodenum when compared to the distal portions.

Region	Goblet Cells/100µm	Percentage of Total Epithelial Cells
Proximal Duodenum	21.3±4.1	24.5±3.8
Mid Duodenum	18.5±3.2	21.2±2.9
Distal Duodenum	15.7±2.8	18.6±2.4

Collagen Distribution (Masson's Trichrome Staining)

The interstitial fibrils seen on a trichrome stain (Masson) showed a considerable deposition of collagen in the wall of the duodenum, in marked distribution. The collagen was most abundantly found in the fibromuscular coats, where the blue connective tissue was in dense network surrounding the blood-vessels and the nerves (Figs. 3 and 4) . The muscular coat was moderately infiltrated with collagen, this being between the bundles of muscular fibres. Little if any collagen was found in the lamina propria, which only showed slight deposits, especially around blood-vessels and lymphoid structures.

Quantitative analysis of collagen content using digital image analysis showed:

Layer	Collagen Area (%)	Fiber Density (arbitrary units)
Lamina Propria	8.3±2.1	2.4±0.6
Submucosa	34.7±5.8	8.9±1.3
Muscularis	18.2±3.4	5.1±0.9
Serosa	42.1±6.2	11.2±1.8

Carbohydrate Content (PAS Staining)

The reaction by the PAS was intense among various constituents of the duodenum. The brush borders of the absorptive cells gave vigorous PAS reactions, indicating large quantities of glycocalyx. The goblet cells indicate intense magenta staining, showing abundance of mucin, which is essential for protective and lubricating functions (Fig. 5, 6).

The basement membrane showed moderate PAS positivity, while blood vessel walls demonstrated variable staining intensity. Glycogen deposits were identified in the cytoplasm of absorptive cells, particularly in the basal regions.

Structure	PAS Intensity	Distribution Pattern
Brush Border	+++	Uniform, intense
Goblet Cell Mucin	+++	Cytoplasmic granules
Basement Membrane	++	Continuous linear
Absorptive Cell Cytoplasm	+ to ++	Basal predominance
Blood Vessel Walls	++	Variable intensity

Intensity scale: + (weak), ++ (moderate), +++ (strong)

Morphometric evaluation showed greater villus heights and greater mucosal thickness in males than in females which may indicate increased absorptive capacity. The villus-to-crypt ratio of 4.3:1 indicates a well-maintained equilibrium between absorptive area and cellular turnover characteristic of healthy intestinal function. The regional variation in goblet cell distribution with a greater concentration in the proximal duodenum was found to be in keeping with what would be expected from the pattern of digestive enzyme secretion and the pH buffering requirements in the same area.

Discussion

The histochemical profiling of the morphology and physiology of the duodenum in Iraqi quail birds gives us an abundance of data although this is beyond the scope of this study. It did show us that the duodenum of Iraqi quen birds contains unique histochemical variability, which ultimately determines the evolutionary development of these birds for optimal processing and absorption of nutrients. Our morphological descriptions of the duodenum with H&E staining were consistent to what has been shown in more general studies of avian intestinal morphology; some that were distinctive however, would make sense for this species of quail[25]. Using a similar small-stained villus length of 847 ±52 µm to what was shown for villus length in Japanese quail (Coturnix japonica), while it appeared different from what has been reported for domestic European quail[26]. This suggests that duodenal morphology of quail species may differ based on genetic origins or environmental adaptations. The villi of the Iraqi quail seemed to be quite well developed with an adequately formed brush border structure which best indicated a functional absorptive role. This is significant in the scope of the extreme growth potential of quail species as a whole. The significantly difference in villus height between males and females(863 ± 48 µm vs 831±55 µm), P=0.043, seems also appropriate and could potentially maximize the feeding program development and feed efficiency[27]. Male quail may develop a duodenum where the absorptive surface area is maximized, given their faster growth rates and metabolic needs [27]. The findings provide additional justification for having a sex specific feeding program for commercially raised quail.

The goblet cell distribution patterns in the present study; the increased density observed between proximal duodenum (21.3±4.1 cells/ 100µm); followed normal physiological requirements for that region of the GI tract. Proximal duodenum is the first region that food enters after the gizzard, when the acidic chyme enter the proximal duodenum, it's neutralized from carbonate base mucin secretion [28]. Goblet cell density (18.5±3.2 cells/ 100µm villus length) reported in the distal duodenum of the quail, is comparable to values for other gallinaceous birds, and shows regional distribution variation is most likely adaptations for digestive processing that occurs in quail[29]. The dense collagen matrix seen with Masson's Trichrome stain on GI tissue wall gave much insight about structural integrity and mechanical properties of duodenum wall. The consistent collagen amount for submucosa (34.7±5.8%) and serosa collagen (42.1±6.2%), provide strong structure adaptations to deal with rheological changes occurring with peristalsis and food processing [30]. The ramifications of these findings has implications for commercial quail with varying degree of mechanical loading that occur with the housing systems and types of diets that are used.

The orientation of collagen fibers which may appear to have more closely woven and established alignment around the blood vessels and nerves in the submucosa demonstrates an organized and a vascular and nerve supportive system. This organization is essential to relay blood resupply and nerve control for digestive function, including birds (like quail) that have high metabolic needs and feeding changes (31). The composite collagen population (18.2±3.4%) in the muscularis layer allows for proper balance of structural supports necessary for duodenum; along with the muscle functional supports required during peristalsis. Furthermore, the positive PAS staining reaction in brush border and goblet cells indicated there would be appropriate amounts of carbohydrates needed for digestive function. The robust appearance of the glycocalyx indicated surface membrane bound enzymes and transport proteins were adequate to continue the digestion and absorption mechanisms [32]. This would justify the gut efficiency and feed conversion efficiencies of the quail and feed conversion efficiencies achieved Commercially. Glycogen in the cytoplasm of absorptive cells, particularly in the basal areas, indicates active carbohydrate metabolism and energy for duodenal epithelium [33]. The high energy requirement of the intestinal epithelium which undergoes rapid cellular turnover and has a high energetic cost involved in active transport processes is consistent with the detected glycogen deposits. The specific pattern of glycogen deposition could indicate the cells are preparing for increased absorptive activity immediately after feeding. The environmental characteristics in Iraq, which are largely associated with extreme temperatures and a desert-like climate, could very possibly be related to the histochemical characteristics outlined in this study. Goblet cells and the glycocalyx appear to be highly developed, and this very well could be adaptations to promote and preserve the intestinal barrier through thermal stress [34]. The adaptive capabilities of these cells may be beneficial in the large scale production of quail produced under Iraqi environmental conditions.

The results of this study provide a number of useful considerations for quail production in Iraq. The observed sexual dimorphism in duodenal structure implies places where male and female birds may respond to different nutritional strategies and derive their digestive capacity from separate sources. The strong collagen matrix implies a good level of structural integrity allows feeding in pellet form and in high feeding frequency without compromising the intestine [35]. The abundant carbohydrates observed at PAS staining suggests that Iraqi quail are completely adapted to feeding on high carbohydrate diets that is particularly important because it enables the feed to be cost effective with local grains. The differences in the goblet cell densities indicates that quail may be more susceptible to acidic injury in the most proximal duodenum. This considered the need for appropriate feed processing and pH monitoring in commercial feeds [36].

Concerning other birds species, the Iraqi quail duodenum has both similar anatomical features to other gallinaceous species, but it also contains unique features. Villus crypt ratio 4.3:1 is comparative to other studies with chickens, but greater than that reported in turkeys, suggesting a proper equilibrium between absorptive surface area of the villus, to the cell turnover of the crypts [37]. The ratio indicates normal function of the intestinal tract and digestibility.

The histochemical profiles reported in this study can serve as a baseline to assess intestinal health in Iraqi quail populations. Any differences from the normal values reported may represent nutritional deficiencies, pathological state, or adverse environmental factors. Also, establishing the baseline

values will be beneficial to veterinary diagnoses and monitoring performance and production in a commercial operation [38].

The effects from climate change would also impact on Iraqi agriculture and livestock production products; which would ultimately affect the digestive physiology of quail birds. The histochemical adaptations demonstrated in this research highlight active current adaptability to the local environment and monitoring in the future may show alterations in duodenal structure and function if the environmental conditions continue to change [39]. This baseline information will be useful going forward to monitor the effect of changing environmental conditions and population adaptation on quail physiology.

The design used in this study makes use of several staining methods that are complementary which provides a comprehensive research methodology for duodenal analysis, by combining the results of H&E, Masson's Trichrome, and PAS staining, an overall duodenal structure and function can be correlated [40]. This multi-staining model has the potential to be used in further avian digestive architecture and physiology studies.

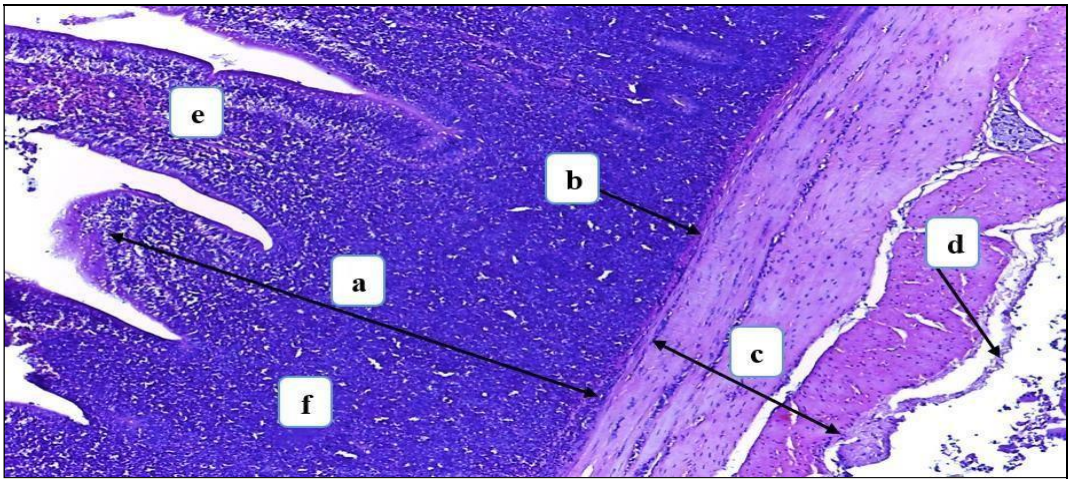

Fig 1: photography of duodenum of quail fowl show the, (a) Mucosa, (b) Submucosa, (c) *Muscularas externia*, (d) Serosae, (e) Villi, (f) Laminae prepare, X40, H&E

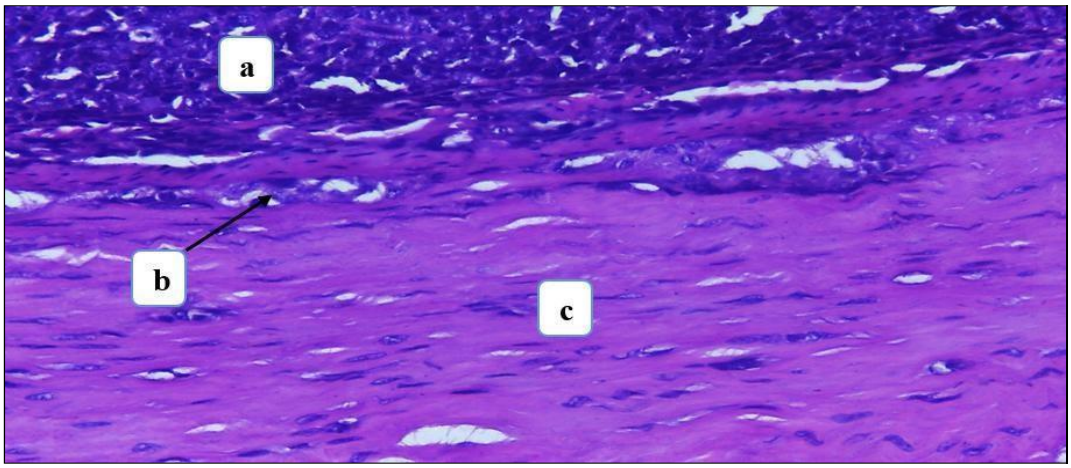

Fig 2; photography of duodenum of quail fowl show the, (a) Laminae prepare, (b) Submucosa, (c) *Muscularas externia*, X400, H&E

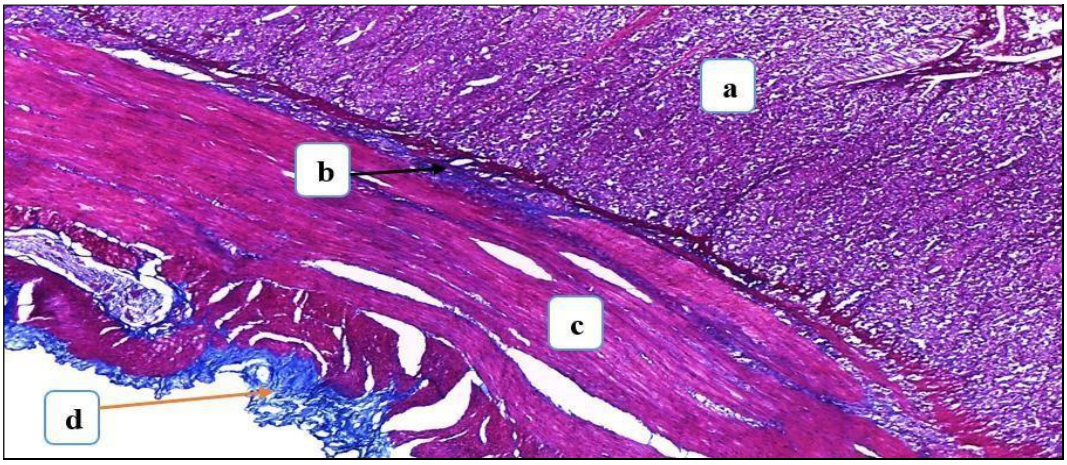

Fig 3: photography of duodenum of quail fowl show the, (a) Mucosa, (b) Submucosa, (c) *Muscularas externia*, (d) Serosae, X40, Masson Trichrome stain

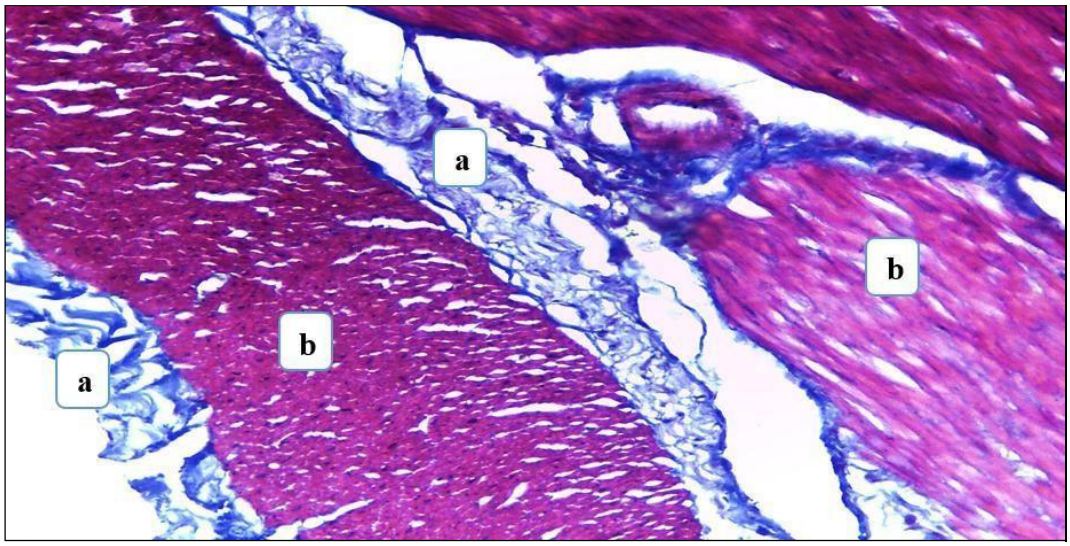

Fig 4: photography of duodenum of quail fowl show the, (a) Connective tissue, (b) Smooth muscles, X400, Masson Trichrome stain

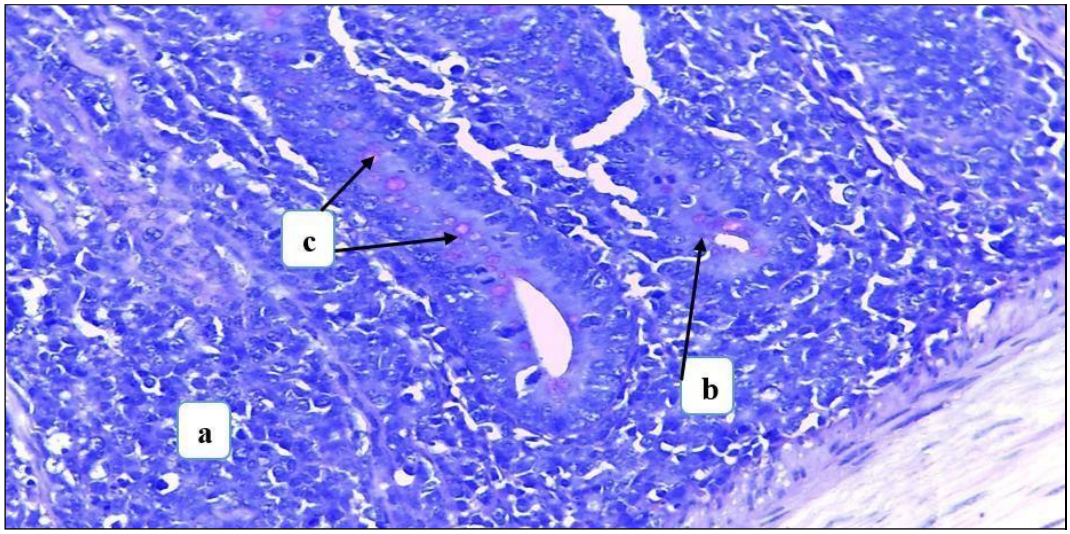

Fig 5: photography of duodenum of quail fowl show the, (a) Laminae propre, (b) Crypts, (c) Mucin with goblet cells , X400, PAS

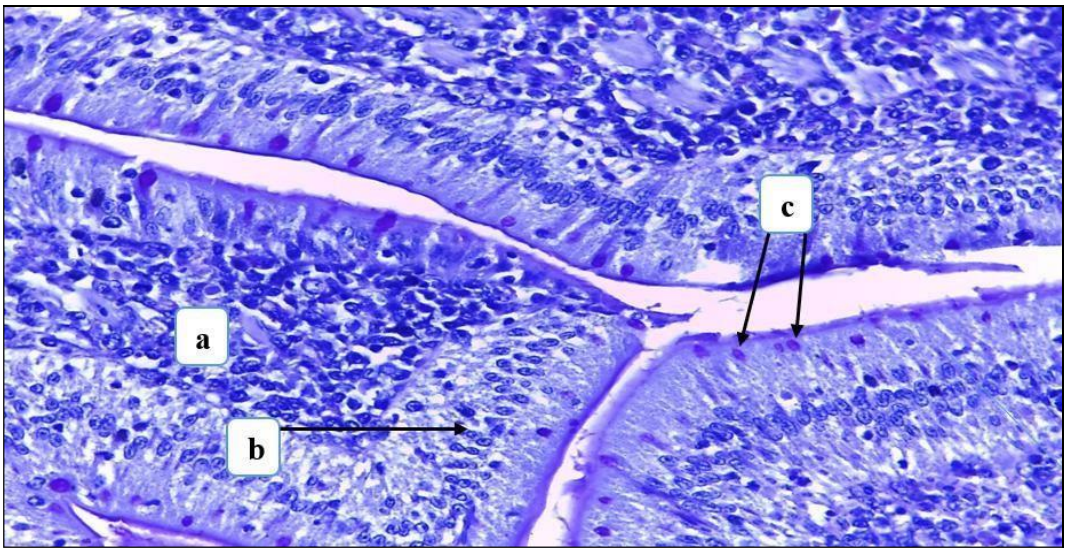

Fig 6: photography of duodenum of quail fowl show the: (a) Villi, (b) epithelium cells, (c) Mucin with goblet cells, X400, PAS

Conclusion

This histochemical investigation on the duodenum of Iraqi quail birds has generated useful information on this important organ structure and function. The study showed that Iraqi quail had extensive duodenal architecture with unique features for the efficient processing and absorption of nutrients.

Notable findings included well-developed intestinal villi with a mean height of 847±52 µm providing an excellent absorptive capacity necessary for their fast growth nature. The

sex dimorphism noted with male birds possessing greater villus height and mucosal thickness suggests differences in nutrition for commercial production. Goblet cell distributions were highest in the proximal duodenum, which we might expect as a natural and biological response expects as a biological and natural response due to the increased demands of digestion in the proximal duodenum area of the anatomy. Masson's trichrome stain presented a significant structural characteristic of a collagen mesh work, demonstrating ample structural ability to handle mechanical demands of both ingest and digest volume. The PAS staining presented indication of polysaccharides absorbance in the intestinal tract indicating carbohydrates present in the digest and protective agents, which suggested adaptations to carbohydrate diets which a commercial Iraqi quail farmer likely incorporating. Importantly, the current observation will serve as at minimum a baseline presentation for the Iraqi quail population and general recommendations for increasing the development of better production systems. These now outlined histochemical and structural-functional representations would serve to support and grow the evolving Iraqi quail industry and preferably lend some subsequent global perspectives of avian digestive physiology. Future studies will continue to indicate differential effects of dietary curriculum and management strategies for the histochemical variances for production and welfare efficiency.

Conflict of Interest
Not available
Financial Support
Not available

References

1. Uni Z, Ferket PR. Methods for early nutrition and their potential. World Poult Sci J. 2004;60(1):101-111.
2. Iji PA, Saki A, Tivey DR. Body and intestinal growth of broiler chicks on a commercial starter diet. Br Poult Sci. 2001;42(4):505-513.
3. Al-Daraji HJ, Al-Hassani AS. Effect of dietary supplementation with different levels of turmeric (*Curcuma longa*) on broiler performance. Iraqi J Agric Sci. 2011;42(1):35-43.
4. Jameel YJ, Saheb AF. Morphological and histological study of digestive system in local quail (*Coturnix coturnix*). Basrah J Vet Res. 2010;9(2):75-85.
5. Yamauchi K, Kamisoyama H, Isshiki Y. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorn hens. Br Poult Sci. 1996;37(5):909-921.
6. Pelicano ERL, Souza PA, Souza HBA, Figueiredo DF, Boiago MM, Carvalho SR, *et al*. Intestinal mucosa development in broiler chickens fed natural growth promoters. Braz J Poult Sci. 2005;7(4):221-229.
7. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 6th ed. Edinburgh: Churchill Livingstone, 2008.
8. Masson P. Some histological methods. Trichrome staining's and their preliminary technique. J Tech Methods. 1929;12:75-90.
9. Gartner LP, Hiatt JL. Color Textbook of Histology. 3rd Ed. Philadelphia: Saunders Elsevier, 2007.
10. McManus JFA. Histological and histochemical uses of periodic acid. Stain Technol. 1948;23(3):99-108.
11. Suvarna KS, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. 7th Ed. Edinburgh: Churchill Livingstone, 2013.
12. Kassie ALGAM. The role of peppermint (Mentha piperita) on performance in broiler diets. Agric Biol J North Am. 2010;1(5):1009-1013.
13. Montagne L, Pluske JR, Hampson DJ. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim Feed Sci Technol. 2003;108(1-4):95-117.
14. Iji PA, Saki AA, Tivey DR. Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. Asian-Australas J Anim Sci. 2001;14(1):54-60.
15. Taha AH, Abbas RJ. Effect of different protein levels on productive performance of Japanese quail. Iraqi J Agric Sci. 2010;41(4):69-76.
16. Mohammed AA, Abbas RJ, Al-Shuhaib MBS. Molecular identification and phylogenetic analysis of Eimeria species infecting domestic quails (*Coturnix coturnix* domesticus) in Iraq. Vet World. 2017;10(9):1107-1111.
17. Luna LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Ed. New York, McGraw-Hill, 1968.
18. Incharoen T, Yamauchi K, Erikawa T, Gotoh H. Histology of intestinal villi and epithelial cells in chickens fed low protein or low fat diets. Ital J Anim Sci. 2010;9(4):429-434.
19. Awad WA, Ghareeb K, Raheem AS, Böhm J. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci. 2009;88(1):49-56.
20. Obaidi ALQT, Shadeedi ALSMJ. Climate change impacts on livestock production in Iraq. Iraqi J Agric Sci. 2019;50(1):285-295.
21. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. 4th Ed. Bloxham: Scion Publishing, 2008.
22. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc. 2008;2008:pdb.prot4986.
23. Goldner J. A modification of the Masson trichrome technique for routine laboratory purposes. Am J Pathol. 1938;14(2):237-243.
24. Hotchkiss RD. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch Biochem. 1948;16(2):131-141.
25. Hodges RD. The Histology of the Fowl. London: Academic Press, 1974.
26. Nasrin M, Siddiqi MNH, Masum MA, Wares MA. Gross and histological studies of digestive tract of broilers during postnatal growth and development. J Bangladesh Agric Univ. 2012;10(1):69-77.
27. Boleli IC, Maiorka A, Macari M. Estrutura funcional do trato digestório. In: Macari M, Furlan RL, Gonzales E, editors. Fisiologia Aviária Aplicada a Frangos de Corte. Jaboticabal: FUNEP, 2002. p. 75-95.
28. Ferreira AJP, Pizarro LDC, Lemos MJ, Roxo E, Macari M. Desenvolvimento e distribuição das células caliciformes no intestino delgado de frangos. Arq Bras Med Vet Zootec. 1990;42(5):377-382.
29. Uni Z, Ganot S, Sklan D. Posthatch development of mucosal function in the broiler small intestine. Poult Sci. 1998;77(1):75-82.

30. Shamoto K, Yamauchi K. Recovery responses of chick intestinal villus morphology to different refeeding procedures. Poult Sci. 2000;79(5):718-723.
31. Yamauchi K, Tarachai P. Changes in intestinal villi, cell area and intracellular autophagic vacuoles related to intestinal function in chickens. Br Poult Sci. 2000;41(4):416-423.
32. Sklan D, Uni Z. The effect of essential fatty acid deficiency on mucin dynamics in the small intestine of the chick. Br J Nutr. 2004;91(6):877-882.
33. Uni Z, Noy Y, Sklan D. Posthatch development of small intestinal function in the poult. Poult Sci. 1999;78(2):215-222.
34. Star L, Arsiwalla T, Molist F, Leushuis R, Dalsgaard TK, Christensen CV. Gradual provision of live black soldier fly (*Hermetia illucens*) larvae to broilers did not impact their growth performance and welfare. J Insects Food Feed. 2020;6(2):179-190.
35. Geyra A, Uni Z, Sklan D. The effect of fasting at different ages on growth and digestive tract development in the chick. Br Poult Sci. 2001;42(4):515-522.
36. Baurhoo B, Phillip L, Ruiz-Feria CA. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult Sci. 2007;86(6):1070-1078.
37. Sell JL, Angel CR, Piquer FJ, Mallarino EG, Batshan ALHA. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poult Sci. 1991;70(5):1200-1205.
38. Choct M. Managing gut health through nutrition. Br Poult Sci. 2009;50(1):9-15.
39. Lara LJ, Rostagno MH. Impact of heat stress on poultry production. Animals. 2013;3(2):356-369.
40. Mowry RW. The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins, with revised directions for the colloidal iron stain, the use of Alcian Blue G8X and their combinations with the periodic acid-Schiff reaction. Ann NY Acad Sci. 1963;106(2):402-423.

How to Cite This Article
Jumaah YR, Zaidan QT. Histochemical investigation of duodenum in quail birds in Iraq. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(3):398-406.