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Histomorphometrical and histochemical study of small intestine development in local chicken (*Gallus gallus domesticus*) and duck (*Anas platyrhynchos domesticus*) embryos

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A B S T R A C T

This study demonstrated for the first time the histomorphometry of the small intestine (SI) and distribution of goblet and Paneth cells, collagen, and smooth muscle fibers in (SI) of the local chicken and duck during embryological development using the combined PAS and Alcian blue (pH2.5), Geimsa, and Masson's trichrome stains. One hundred and four fertilized chicken eggs and one hundred and forty fertilized duck eggs were collected from Mosul city. The sample placed in an incubator with automatic movement, ventilation, and humidity was (60%) and the temperature was (37.7 °C) for chicken and (37.5 °C) for duck. The histomorphometry showed significant differences between chicken and duck embryos at the 19th and 21st days of incubation of the villi's length, width, and apparent surface area. As for the epithelium height and the intestinal crypts, there were significant differences between chicken and duck embryos at the 18th and 20th days of incubation in the ileum and jejunum. Furthermore, the mucosa was thicker in chicken embryos in the day of hatching, the submucosa was thicker in ducks during the 18th day of the incubation in the duodenum. During day 15 of the incubation in chicken embryos in the jejunum and ileum, the muscularis layer was thicker in the duodenum and jejunum and less thick in the ileum, and the collagen fibers were less too in the duck embryos at hatching d. The serosa was thicker in the duck embryos. The percentage of the goblet cells was higher with a larger density in duck embryos than chicken at hatching day. Paneth cells were characterized by acidic granular cytoplasm. The achieved outcomes of histomorphometry and the density variances of the glycoprotein secretion are probably linked with numerous definite roles of (SI) parts in the progressions of nutrient absorption.

Introduction

The digestive system is a privileged site for interactions between the external environment and the body, therefore the lining of the mucosa is the first line of defense against viruses and bacteria and by nature renews itself at a high speed to maintain balance [1].

The histological structure of (SI) is similar along its length. The intestinal tract appears by microscopic histological examination as a tube lined with a tunica mucosa consisting of a highly differentiated epithelia surrounded and supported by loose connective tissue, followed by an irregular tunica sub mucosa composed of dense connective tissue and then the tunica muscularis layer consisting of smooth muscles consisting of two inner circular and outer longitudinal layers interspersed with blood and lymphatic vessels and nerve plexuses and from the outside the tunica serosa is a loose connective tissue covered by a simple squamous epithelium called the mesothelium [2].

The epithelium of (SI) is organized into a single layer of columnar (cylindrical) absorptive cells called intestinal absorptive cells, interspersed with mucus-secreting cells called goblet cells. Increased surface area is characterized by its impermeability to large molecules and microbes. Intestinal cells are strongly organized through tight junctions, and their lateral membranes are tightly linked. They are covering the villi and rest on a basement membrane and loose connective tissue contain nerve fibers, capillaries, and smooth muscle fibers. Capillaries deliver blood to the base of the epithelial cells [3].

The epithelium of (SI) is characterized by the rapid and continuous formation of cells, and the continuous renewal of cells takes place through the pool of active stem cells in the intestine, which are differentiated into four types of intestinal epithelial cells, where the differentiation of all types of cells occurs during their migration from the intestinal crypts to the villi except Paneth cells Paneth cells that differentiate at the bases of the intestinal crypts [4].

The goblet cells are spread between the intestinal absorptive cells and along the villi. They are considered as a gland consisting of one cell that secretes the lubricating mucus of the intestinal tract and is characterized by being highly polar secretory cells (their secretory granules gather at the apical pole and open in the apical membrane opposite the intestinal lumen), specialized in secreting a protein A sugar called mucin, which is the main component of the mucus of the gastrointestinal tract. Mucus lubricates the lining of the gastrointestinal tract to protect it from mechanical harm, pathogenic viruses, and stomach acidity. It also secretes many mineral elements such as zinc, calcium, and iron [5, 6].

The epithelial cells are interspersed with Paneth cells which present near the base of the villi within the intestinal crypts that give an antibacterial peptide called Defensins, consisting of (12-15) amino acids that it plays a role in destroying the membranes of microbes and the walls of germs [3, 7].

Paneth cells play an important role in the innate immunity of (SI) based on their secretion of peptides [4]. Mammalian Paneth cells play an important immunomodulatory role in the intestinal tract, particularly in epithelial regeneration and the initial stages of inflammation, and are secreting Lysozyme enzyme in (SI) to a limited extent [8].

Presently, studies on the histomorphometric and histochemical features of (SI) development of local chicken (*Gallus gallus domesticus*) and duck (*Anas platyrhynchos domesticus*) embryos is very restricted. Constructed on this background, it is essential to plan a revision on the histomorphometric and histochemical appearances of (SI) of local chicken (*Gallus gallus domesticus*) and duck (*Anas platyrhynchos domesticus*) at diverse age stages during embryological development.

Materials and methods

From Mosul city and close to rural towns, one-hundred and four fertilized chicken eggs and one-hundred and forty fertilized duck eggs were collected during Autumn and Winter season, incubated in a spontaneous movement, aired, moisture (60%), incubator temperature were (37.7 °C) for chicken for 21 days, and (37.5 °C) for duck for 28 days.

Animal Ethics Committee of the Col. of Vet. Med., University of Mosul, Iraq, has permitted the current study under agreement No: UM.VET.2022.061. Embryos were detached agreeing to [9, 10], observed by a dissecting microscope (Huma scope stereo 14900/5, Germany), and permanent in the neutral buffer formalin (NBF). The embryos were examined after seventy-two hours, the intestine was detached and sited once more in (NBF) for seventy-two hours [11]. (SI) samples were treated according to the procedure of the paraffin inserting method (58-60C°).(SI) samples

were inserted vertically to get all the sheets of (SI) tissue when sectioning at (five μm) by rotary microtome [12, 13]. The slices of (SI) tissue stained with a combine PAS and Alcian blue (pH2.5) (PAS-AB) stain [14, 15], Masson's Trichrome stain [16, 17], and Geimsa stain.

Histomorphometric measurements

The images of the histological sections were taken using a digital camera equipped with an application (OMAX Toupview 16MP, china) attached to a light microscope [18], where the length and width of the villi, the virtual surface area of the villi (Fig. 1).The depth of the intestinal crypts, the thickness of the intestinal tissue layers, and the percentage of goblet cells were measured [19].The length of the villus is from the top of the villus to the neck of the crypts. The villus width was measured by taking the width of the villus from the top and bottom and taking the average width of one villus. The number of goblet cells was calculated by counting the number of nuclei in one villus and then calculating the percentage of apparent goblet cells [20].

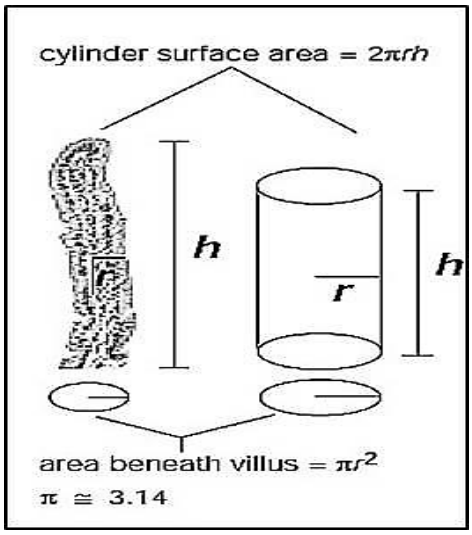

Figure 1. shows the method for measuring the surface area of the villus. h: length of the villus, r: half of the diameter of the villus, π:3.14 [21].

Statistical analysis

Mean averages and standard errors were calculated for the standard variables (using the software (IBM SPSS, v25 UK). These differences between the three parts of (SI) in each age group, in each specimen and between two types of birds, all tests were conducted at a significant value $p<0.05$ [22].

Results

The present study involved the examination of the histomorphometric and histochemical constructions of (SI) development of local chicken (*Gallus gallus domesticus*) and local duck (*Anas platyrhynchos domesticus*). The study of histomorphometric measurements of (SI) of chicken embryos (14th -21st) and (18th - 28th) which showed that the length and width of the villi increase with the progression of incubation days until the day of hatching in the three parts of (SI). As a result the apparent surface area of the villi increased. Hence there were significant differences in the length and width of the villi and its apparent surface area between chicken and duck embryos at the age of 19th and 21st from the days of incubation in the duodenum, jejunum and ileum at a significant level of $p\leq0.001$. The epithelium also increases in height gradually until the day of hatching in the duodenum and jejunum in chicken embryos except for the ileum. It was increased gradually during days 18th and 20th of the age of the duck embryo where significant differences were observed between chicken and duck embryos at a significant level of $p\leq0.001$. As for the intestinal crypts, they were small and gradually increasing in depth until the day of hatching in the duodenum and ileum in chicken embryos, except for the jejunum, where they were gradually increasing in depth only during days 18th and 20th of the age of the duck embryo, where significant differences were observed between chicken and duck embryos at a significant level of $p\leq0.001$. As for the thickness of the four layers of the intestine, it also increases in thickness with the progression of incubation days until the day of hatching, but there were significant differences between the chicken and duck embryos at a significant level of $p\leq0.001$, where mucosa layer was thicker in chicken embryos on the day of hatching. As for the submucosal layer, it was thicker in ducks during the 18th day of the incubation in the duodenum, and during the 15th day of the incubation in chicken embryos in the jejunum and ileum. The thickness of the muscularis layer increased gradually until the day of hatching in both types of birds and the three parts of the intestine, but significant differences were observed between the chicken and duck embryos at a significant level of $p\leq0.001$, where it was thicker in the duodenal and jejunal parts, and less thick in the ileum of the duck embryos at hatching day. As for the serosa layer, its thickness increased gradually until the hatching day in both types of birds and the three parts of the intestine, but

significant differences were observed between the chicken and duck embryos at a significant level of $p \leq 0.001$, where it was thicker in the duck embryos. As for the percentage of the numbers of goblet cells, there was a gradual increase in their numbers in both types of birds and the three parts of the intestine, but significant differences were observed between chicken and duck embryos at a significant level of $p \leq 0.001$, as it was higher in duck embryos than in chicken embryos in the three parts of the intestine at hatching day (Tables 1, 2, 3, 4 & Figures 2- 5).

The histochemical study used to detect the types of carbohydrates and their locations within the layers of (SI). The PAS-AB (pH 2.5) stain was used to investigate the neutral and acidic glycoprotein during the developmental stages of (SI) in each of the local chicken and duck embryos. The mucous membrane consists of epithelial and goblet cells that secrete mucous substances which help in absorbtion of substances from the intestine, which gives a different response in the three parts of (SI). The percentage of goblet cells in the three parts differed in each of the local chicken and duck embryos. In 14th days incubated chicken embryo, neutral glycoprotein (pink color) was found in medium amounts in the jejunum, small amounts in the ileum, and very small amounts in the duodenum, while acidic glycoprotein (blue color) was found in medium amounts in the duodenum and ileum, and in small amounts in the jejunum. As for 21st days incubated chicken embryo, neutral glycoprotein was found in very large amounts in the jejunum and ileum, and small amounts in the duodenum, while acidic glycoprotein was found in very large quantities in the three parts of (SI). While in 18th days incubated duck embryo, neutral glycoprotein was found in medium amounts in the duodenum and small amounts in both the jejunum and the ileum, while acidic glycoprotein was found in small amounts in the duodenum and jejunum, and medium amounts in the ileum. As for 28th days incubated duck embryo, neutral glycoprotein was found in very large amounts in the duodenum, moderate amounts in the ileum, and very small amounts in the jejunum, while acidic glycoprotein was found in very large quantities in the jejunum, in small quantities in the duodenum, and very small amounts in the ileum (Table 4 & Figures 2-4).

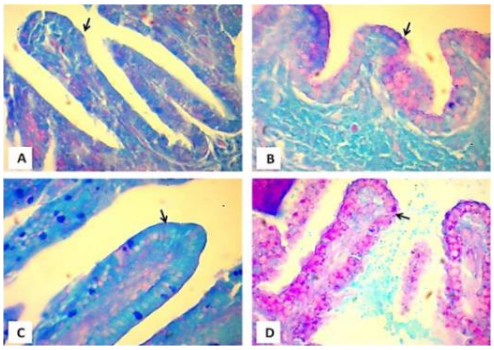

Figure 2. A cross histological section of the duodenum, (A) 14th days incubated duck embryo, (B) 18th days incubated duck embryo, (C) 21st days incubated chicken embryo, (D) 28th days incubated duck embryo, the black arrow shows the intensity of stain reaction in the epithelium, PAS-AB (pH 2.5), magnification 400X.

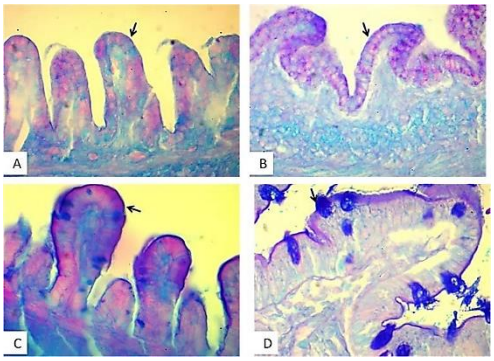

Figure 3. A cross histological section of the jejunum, (A) 14th days incubated duck embryo, (B) 18th days incubated duck embryo, (C) 21st days incubated chicken embryo, (D) 28th days incubated duck embryo, the black arrow shows the intensity of stain reaction in the epithelium, PAS-AB (pH 2.5), magnification 400X.

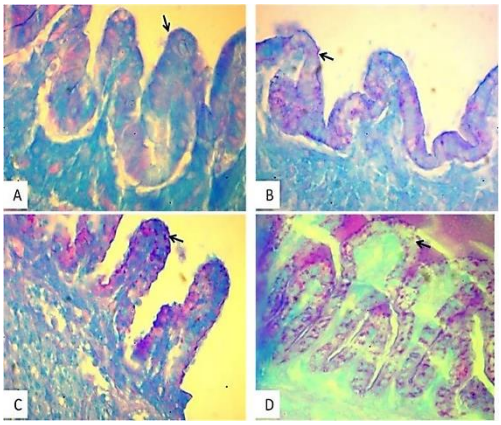

Figure 4. A cross histological section of the ileum, (A) 14th days incubated duck embryo, (B) 18th days incubated duck embryo, (C) 21st days incubated chicken embryo, (D) 28th days incubated duck embryo, the black arrow shows the intensity of stain reaction in the epithelium, PAS-AB (pH 2.5), magnification 400X.

Masson's Trichrome stain was used to investigate the type of fibers in the four layers of (SI) in each of the local chicken and duck embryos, where the connective fibers took a green color and the smooth muscle fibers a red color, while the nucleus took the black color, and the goblet cells a green color. The thickness of the muscularis layer was thicker in the duodenal and jejunal parts, and less thick in the ileum, and the collagen fibers were less too in the duck embryos at hatching day (Figure 5).

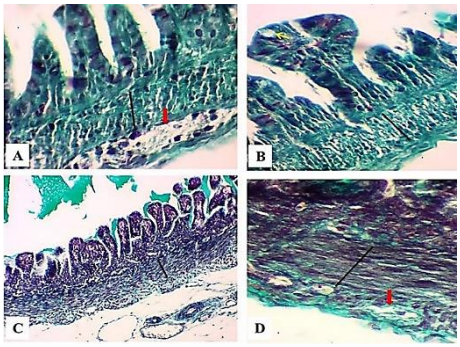

Figure 5. A cross histological section of the jejunum, (A&B) 21st days incubated chicken embryo, (C&D) 28th days incubated duck embryo, the black line shows the smooth muscle and collagen fibers, the red arrow shows nerve plexuses and the yellow arrow shows goblet cells. Masson's Trichrome stain, magnification (A, B&D) 400X, (C) 100X.

While Geimsa stain was used for the first time to detect Paneth cells in (SI) of both local chicken and duck embryos, where these cells appeared at the base of the villi and intestinal crypts (Lieberkühn crypts) and were characterized by their triangular-shape with a central nucleus and acidic cytoplasm (acidic granules) (Figure 6).

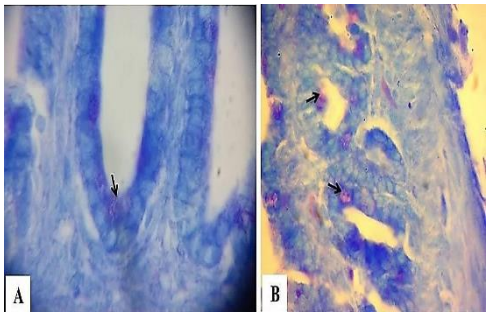

Figure 6. A cross histological section of the duodenum of a chicken embryo incubated for 21st days, the black arrow shows Paneth cells at the base of the villi (A), and in intestinal crypts (Lieberkühn crypts) (B), Geimsa stain, magnification 1000X, (B) 400X.

Discussion

The present histological revision presented that the wall of (SI) with its three portions (duodenum, jejunum, and ileum) comprises of four tunicae: the mucosa, the submucosa, the muscularis, and the serosa layer. This outcome was compatible with [23] in his revision of chickens, and [24] in his study of domesticus chicken, where their reading presented that the wall of (SI) is comprised of four chief tunicae.

The mucosa tunica comprised of the three sub primary tunicae, the mucosa is lined with simple columnar epithelium, lamina propria, and muscularis mucosa. This outcome is reliable with [25, 26] in their histological revision around the mucous membrane of the intestine of chicken and quail which presented that it is comprised of three sub primary tunicae.

The current histomorphometric study showed that the four layers that make up the wall of (SI) with its three parts (duodenum, jejunum, and ileum) increase in thickness with the progression of incubation until the hatching day. Also, with the progression of incubation days, the intestinal villi increases in length and width. This leads to an increase in the surface area of the intestine, this result is consistent with what was mentioned by [27] in their histological study of the development of the digestive system in broiler chickens where they found that the villi in the duodenum are longer than the villi of the jejunum and that the villi in the ileum are wider than that of the jejunum and duodenum. It is also compatible with what was mentioned by [28, 29, 30] in their histomorphometric study of the length and width of (SI) villi. They found that the villi in the ileum and jejunum are shorter and wider than the villi of the duodenum in turkey.

Also the present histomorphometric study showed that intestinal crypts appear in chickens at the age of 14th days, while in ducks at the age of 18th days of incubation, and they also increase in depth with the progression of incubation days until the day of hatching. This result is consistent with [31] in their histological study of the intestines of chicken embryos, and with what [32] mentioned in his histomorphometric study of the digestive system in birds, ducks, chickens, pigeons, geese, and turkeys, this is due to the completion of the development of all body organs in the last third of incubation so that they are fully developed at hatching.

Furthermore, the existing histochemical study showed that the epithelium lining the wall of the intestine in its three parts and the goblet cells in local chicken and duck embryos produce an acidic protein, this result is consistent with [33] in their study of broilers, where they showed that goblet cells produce glycoproteins in (SI). Where the current study showed that cells in the duodenum and ileum of 14th day incubated chickens embryo produce acidic protein of medium intensity and in the jejunum of weak intensity This result is consistent with [33] in their study of broilers, where they showed that in the ileum, goblet cells produce only acidic protein. While in ducks at the age of 18th days, a low-intensity protein is produced in the duodenum and jejunum, while in the ileum, acidic protein is produced in large quantities. As for the age of 21st days in chickens, they produce acidic protein in large quantities in the three parts of the intestine. As for ducks at the age of 28th days, they produce acidic protein in large quantities in the jejunum and little in the duodenum and ileum, and this result is consistent with what was mentioned by (34, 35, 36, 37] about

changing the secretion of the acidic protein in the intestines of chickens, and with what was mentioned by [38] in their study of the development of histochemical changes in the intestines of Muscovy duck embryos, and with what was mentioned [33] in their study of the development of goblet cells before and after hatching in (SI) of broiler chickens, where goblet cells derived from stem cells in the intestinal crypts are important for the absorption of nutrients and defense, and secrete mucins, which consist of mucins, glycoprotein, water, and the mucous layer not only acts as an immune barrier but also immunological functions that prevent pathogens from entering epithelial cells, and goblet cells were classified into cells that produce acidic protein or cells that produce neutral or mixed acid [39, 40], and with what was mentioned [1, 5, 6, 41] about the increase in differentiation of goblet cells during the third week of embryonic development and the life cycle of these cells ranges from (16-41) hours under normal conditions, and with what was mentioned [33] about the increase in the number of goblet cells located along the villi, where they are concentrated in the duodenum, jejunum and ileum, increased slightly from (18th -20th) days of incubation, and after day (20th) of incubation, the concentration of goblet cells increased significantly in the jejunum and ileum, leading to increased mucin production.

Masson's Trichrome stain was used to investigate the type of fibers in the four layers of (SI) in each of the local chicken and duck embryos, where the connective fibers took a green color and the smooth muscle fibers a red color, while the nucleus took the black color,while the goblet cells took a green color. The thickness of the muscularis layer was thicker in the duodenal and jejunal parts, and less thick in the ileum. The collagen fibers were less too in the duck embryos at hatching day. The current study showed that the thickness of the muscularis layer was thicker in the duodenal and jejunal partsbut less thick in the ileum, and the collagen fibers were less too in the duck embryos at hatching day. This was clear through the density of the color of smooth muscle fibers and collagen fibers during using Masson's Trichrome stain and this result is consistent with what was mentioned by [17] in his study.

The current histochemical study showed also that Paneth cells in (SI) were located at the base of the villi and intestinal crypts and were characterized by acidic granular cytoplasm. This result is consistent with [4 shown by [8] that Paneth cells are located at the base of the Lieberkühn crypts and are next to goblet cells, endocrine cells, epithelium and intestinal cells, and are pyramidal shape with a nucleus located at the base of the cell, and secrete lysozyme protein into (SI) in a limited manner.

Conclusions

The present study confirms the presence of significant differences at a significance level of $p \leq 0.001$ for all the histomorphometric measurements of (SI) of local chicken and duck embryos at the last stage of incubation. The length, width, and apparent surface area of the villi presented significant differences at the 19th and 21st day of incubation in the duodenum, jejunum, and ileum. The height of epithelium and depth of the intestinal crypts presented significant differences at the 18th and 20th day of incubation for the ileum and jejunum in the duck embryo. As for the thickness of the four layers of (SI), the mucosa layer was thicker in chicken embryos on the day of hatching. The submucosal layer was thicker in ducks during the 18th day of the incubation in the duodenum, and during the 15th day of the incubation in chicken embryos in the jejunum and ileum. The muscularis layer was thicker in the duodenal and jejunal parts. less thick in the ileum and the collagen fibers were less too in the duck embryos at hatching day. The serosa layer was thicker in the duck embryos. The percentage of goblet cells was higher with a large density in duck embryos than in chicken in the three parts of the intestine at hatching day. Paneth cells were characterized by acidic granular cytoplasm. The achieved outcomes of histomorphometry and the density variances of the glycoprotein secretion are probably linked with numerous definite roles of (SI) parts in the progressions of nutrient absorption.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Table 1. The means of the Histomorphometric measurements and the standard error during the studied periods of the age of the chicken embryo.

Small intestine part	age categories	Villus length M±SEM (µm)	Villus width M±SEM (µm)	virtual surface area M±SEM (µm)	Elevation of the epithelium lining the villi M±SEM (µm)	Depth of crypt M±SEM (µm)	thickness of the mucosa layer M±SEM (µm)	thickness of the submucosa layer M±SEM (µm)	thickness of the muscularis layer M±SEM (µm)	thickness of the serosa layer M±SEM (µm)	The percentage of goblet cells (%)
Duodenum	**14th day**	**61.06±1.57A**	21.73±1.66AB	**1334.71±134.30A**	**6.08±0.47A**	23.50±2.83A	**55.18±1.73A**	**18.23±1.09A**	**22.12±0.81A**	7.67±0.54A	**42%A**
	15th day	77.76±4.05A	**17.99±1.32A**	1419.44±178.71A	6.71±0.97AB	23.74±2A	78.80±9.08AB	25.41±3.28B	30.12±1.73AB	**6.06±0.50A**	35%BC
	17th day	135.95±12.88B	24.21±2.96ABC	3413.20±767.96B	8.19±0.72C	**18.92±0.79A**	122.63±11.52C	**42.18±2.59C**	44.42±2.70C	10.57±0.70B	39%AC
	19th day	130.46±14.78B	**28.19±2.42C**	3563.53±206.22B	7.63±1.02BC	22.76±3.97A	117.90±13.39BC	21.27±2.05AB	37.49±5.24BC	10.87±1.50B	28%BD
	21st day	**240.36±8.21C**	26.91±1.05BC	**6455.40±277.11C**	**13.74±0.47D**	**35.55±4.26B**	**223.34±7.14D**	27.24±1B	**70.65±2.89D**	**16.78±1.03C**	**22%D**
Jejunum	**14th day**	**58.38±3.13A**	21.75±1.17AB	1241.13±61.96A	**5.42±0.42A**	**9.84±0.70A**	**49.07±3.28A**	26.86±1.88A	**23.22±0.94A**	4.33±0.30A	37%A
	15th day	59.45±5.77A	**18.24±0.44A**	**1075.53±84.76A**	5.75±0.46AB	11.27±0.52AB	61.63±3.09AB	**30.13±1.53A**	23.95±1.13A	**4.14±0.22A**	**40%A**
	17th day	90.28±2.35B	20.90±1.03A	1889.90±115.27B	9.60±1.02C	13.12±1.36AB	82.21±1.57C	21.78±1.16B	40.61±2.75B	**16.04±1.87B**	37%A
	19th day	**102.50±6.73B**	**26.46±2C**	**2728.26±313.40C**	7.85±0.90BC	15.06±1.04B	**88.06±5.98BC**	20.78±1.68B	**45.56±5.80B**	9.56±0.97C	38%A
	21st day	98.44±5.34B	25.54±1.63BC	2513.67±224.28C	**10.04±0.62C**	**19.31±1.95C**	83.39±3.80C	**19.87±1.40B**	28.84±2.08A	11.53±0.54C	**30%B**
Ileum	**14th day**	**51.54±2.42A**	**18.76±1.12A**	**965.60±67.31A**	6.62±0.55A	**13.44±2.08A**	**44.51±3.30A**	**19.16±1.58A**	**21.83±0.94A**	7.51±0.78A	39%A
	15th day	61.49±1.57A	22.87±0.47A	1403.58±17.79B	**5.94±0.26A**	**30.58±1.79 B**	55.32±1.20A	**32.88±2.59B**	27.53±1.32AB	**7.38±0.36A**	**46%A**
	17th day	81.65±4.70B	21.02±0.95A	1715.39±122.27BC	9.13±0.67B	17.73±2.43AC	70.01±4.62B	21.43±1.69A	44.50±2.14C	**15.67±2.36B**	40%A
	19th day	99.51±4.27C	20.71±0.73A	2062.66±123.21C	9.62±0.84B	19.81±1.47AC	86.77±4.28B	23.45±1.92A	**48.50±3.62C**	12.20±1.14B	42%A
	21st day	**110.74±5.05C**	**27.35±2.42B**	**2987.09±190.78D**	**11.03±0.94B**	23.35±2.51C	**97.31±4.54B**	21.95±1.64A	31.29±2.06B	13.43±1.13B	**39%A**

M: mean, SEM: standard error of the mean; Different letters between columns indicate significant differences at P≤0.05.

Table 2. The means of the Histomorphometric measurements and the standard error during the studied periods of the age of the duck embryos.

Small intestine part	age categories	Villus length M±SEM (µm)	Villus width M±SEM (µm)	virtual surface area M±SEM (µm)	Elevation of the epithelium lining the villi M±SEM (µm)	Depth of crypt M±SEM (µm)	thickness of the mucosa layer M±SEM (µm)	thickness of the submucosa layer M±SEM (µm)	thickness of the muscularis layer M±SEM (µm)	thickness of the serosa layer M±SEM (µm)	The percentage of goblet cells (%)
Duodenum	**18th day**	**63.09±5.47A**	**26.09±1.78A**	1657.74±219.10A	7.17±0.52A	16.88±1.32A	**55.55±5.10A**	**37.88±2.23A**	66.73±5.75A	16.08±1.32A	**35 %A**
	20th day	71.51±5.89A	**19.49±1.16 B**	**1412.68±194A**	7.29±0.28A	16.61±1.66A	59.74±6.44AB	**19.35±1.11B**	**32.12±2.40B**	**10.14±1.24 B**	39%AB
	22nd day	76.55±6.39AB	20.53±1.19BC	1575.91±178.72A	**6.26±0.57A**	**15.46±1.02A**	68.26±8.61AB	30.78±2.46C	57.31±5.13A	13.76±0.59AB	43%BC
	25th day	88.97±2.64B	21.19±1.36BC	1894.06±163.10A	**9.92±0.56B**	23.60±1.93B	77.49±2.10B	25.53±2.02C	**68.90±2.34A**	**17.39±1.33A**	**48%C**
	28th day	**127.39±4.46C**	24.11±1.03AC	**3085.58±235.33B**	9.22±0.54B	**27.64±2.96B**	**108.81±5.41C**	28.97±2.01C	66.49±6.77A	13.66±1.33AB	42%ABC
Jejunum	**18th day**	**44.94±2.91A**	24.33±2.43AB	**1110.84±167.26A**	7.53±0.94AB	**21.11±1.57A**	**38.69±2.17A**	**27.90±2.91A**	48.07±5.02A	12.42±0.51A	**39%A**
	20th day	64.75±3.19B	**17.91±1.50A**	1174.71±146.48A	7.71±0.60AB	15.01±0.95BC	52.09±3.45AB	21.99±2.51AB	53.53±2.69A	12.04±1.08A	45%A
	22nd day	69.53±7.05B	21.48±1.91AB	1520.07±227.97A	**6.38±0.46A**	15.28±3.06BC	61.77±9.48B	26.88±3.35A	44.78±3.35A	11.38±1.30A	40%A
	25th day	61.48±2.40B	19.41±1.21A	1203.47±122.21A	8.45±0.27B	**13.56±0.99B**	48.22±2.77AB	**16.37±1.08B**	**41.15±5.08A**	**13.26±1.16A**	43%A
	28th day	**125.62±6.20C**	**27.30±3.05C**	**3436.23±450.17B**	**9.24±0.51B**	19.20±0.96AC	110.97±6C	23.57±1.95AB	**53.74±4.15A**	**11.34±0.85A**	**45%A**
Ileum	**18th day**	**43.61±4.73A**	**20.18±2.56A**	**913.08±167.97A**	6.93±0.81AB	12.58±0.94AB	**35.65±3.14A**	**31.74±5.26A**	**39.16±2.21A**	**14.48±1.38A**	**39%A**
	20th day	52.63±2.40A	19.17±1.80A	1022.74±130.33A	7.88±0.44AB	**11.70±0.78A**	43.65±1.74AB	16.68±1.16 B	33.52±1.28AB	**10.17±0.70B**	42%A
	22nd day	54.18±2.08A	23.14±1.18A	1251.89±70.92AB	7.88±0.43AB	15.52±1.84AB	46.43±1.28BC	26.83±1.59A	37.31±2.03AB	14.20±1.33A	48%AB
	25th day	64.76±3.8B	**18.31±1.41A**	1204.13±155.69AB	**6.65±0.49A**	13.24±0.93AB	55.14±3.25CD	**16.19±1.10 B**	**30.90±3.37B**	10.97±1.45AB	**53%B**
	28th day	**75.91±3.61C**	20.13±2.16A	**1545.89±205.45B**	**8.94±0.84 B**	**15.87±0.62B**	**60.07±4.67D**	17.61±1.48 B	38.88±1.05A	12.52±1.09AB	46%AB

M: mean, SEM: standard error of the mean; Different letters between columns indicate significant differences at P≤0.05

Table 3. The comparison of means of the Histomorphometric measurements and standard error during the studied periods of embryonic life between local chickens and ducks.

type of poultry^{s}	Small intestine part	age categories	Villus length M±SEM (µm)	Villus width M±SEM (µm)	virtual surface area M±SEM (µm)	Elevation of the epithelium lining the villi M±SEM (µm)	Depth of crypt M±SEM (µm)	thickness of the mucosa layer M±SEM (µm)	thickness of the submucosa layer M±SEM (µm)	thickness of the muscularis layer M±SEM (µm)	thickness of the serosa layer M±SEM (µm)	The percentage of goblet cells (%)
Chicken	Duodenum	14th day	61.06±1.57	21.73±1.66	1334.71±134.30	6.08±0.47	23.50±2.83	55.18±1.73	18.23±1.09	22.12±0.81	7.67±0.54	42%
Duck		18th day	63.09±5.47	26.09±1.78	1657.74±219.10	7.17±0.52	16.88±1.32	55.55±5.10	37.88±2.23***	66.73±5.75**	16.08±1.32**	35%
Chicken		15th day	77.76±4.05	17.99±1.32	1419.44±178.71	6.71±0.97	23.74±2	78.80±9.08	25.41±3.28	30.12±1.73	6.06±0.50	35%
Duck		20th day	71.51±5.89	19.49±1.16	1412.68±194	7.29±0.28*	16.61±1.66	59.74±6.44	19.35±1.11	32.12±2.40	10.14±1.24	39%
Chicken		17th day	135.95±12.88**	24.21±2.96	3413.20±767.96*	8.19±0.72*	18.92±0.79*	122.63±11.52**	42.18±2.59*	44.42±2.70	10.57±0.70	39%
Duck		22nd day	76.55±6.39	20.53±1.19	1575.91±178.72	6.26±0.57	15.46±1.02	68.26±8.61	30.78±2.46	57.31±5.13*	13.76±0.59**	43%
Chicken		19th day	130.46±14.78*	28.19±2.42*	3563.53±206.22***	7.63±1.02	22.76±3.97	117.90±13.39*	21.27±2.05	37.49±5.24	10.87±1.50	28%
Duck		25th day	88.97±2.64	21.19±1.36	1894.06±163.10	9.92±0.56*	23.60±1.93	77.49±2.10	25.53±2.02	68.90±2.34**	17.39±1.33*	48%***
Chicken		21st day	240.36±8.21***	26.91±1.05	6455.40±277.11***	13.74±0.47***	35.55±4.26	223.34±7.14***	27.24±1	70.65±2.89*	16.78±1.03	22%
Duck		28th day	127.39±4.46	24.11±1.03	3085.58±235.33	9.22±0.54	27.64±2.96	108.81±5.41	28.97±2.01	66.49±6.77	13.66±1.33	42%***
Chicken	Jejunum	14th day	58.38±3.13*	21.75±1.17	1241.13±61.96	5.42±0.42	9.84±0.70	49.07±3.28*	26.86±1.88	23.22±0.94	4.33±0.30	37%
Duck		18th day	44.94±2.91	24.33±2.43	1110.84±167.26	7.53±0.94	21.11±1.57**	38.69±2.17	27.90±2.91	48.07±5.02*	12.42±0.51***	39%
Chicken		15th day	59.45±5.77	18.24±0.44**	1075.53±84.76	5.75±0.46	11.27±0.52	61.63±3.09	30.13±1.53*	23.95±1.13	4.14±0.22	40%
Duck		20th day	64.75±3.19*	17.91±1.50	1174.71±146.48	7.71±0.60*	15.01±0.95**	52.09±3.45	21.99±2.51	53.53±2.69***	12.04±1.08***	45%**
Chicken		17th day	90.28±2.35*	20.90±1.03	1889.90±115.27	9.60±1.02*	13.12±1.36	82.21±1.57**	21.78±1.16	40.61±2.75	16.04±1.87	37%
Duck		22nd day	69.53±7.05	21.48±1.91	1520.07±227.97	6.38±0.46	15.28±3.06	61.77±9.48	26.88±3.35	44.78±3.35	11.38±1.30	40%
Chicken		19th day	102.50±6.73**	26.46±2*	2728.26±313.40**	7.85±0.90	15.06±1.04	88.06±5.98**	20.78±1.68	45.56±5.80	9.56±0.97	38%
Duck		25th day	61.48±2.40	19.41±1.21	1203.47±122.21	8.45±0.27*	13.56±0.99	48.22±2.77	16.37±1.08	41.15±5.08	13.26±1.16*	43%
Chicken		21st day	98.44±5.34	25.54±1.63	2513.67±224.28	10.04±0.62**	19.31±1.95	83.39±3.80	19.87±1.40	28.84±2.08	11.53±0.54	30%

Duck		**28th day**	**125.62±6.20***	27.30±3.05	**3436.23±450.17***	9.24±0.51	19.20±0.96	110.97±6	23.57±1.95	**53.74±4.15****	11.34±0.85	**45%****
Chicken	**Ileum**	**14th day**	51.54±2.42	18.76±1.12	965.60±67.31	6.62±0.55	13.44±2.08	44.51±3.30	19.16±1.58	21.83±0.94	7.51±0.78	**39%***
Duck		**18th day**	43.61±4.73	20.18±2.56	913.08±167.97	6.93±0.81	12.58±0.94	35.65±3.14	31.74±5.26	**39.16±2.21****	**14.48±1.38****	39%
Chicken		**15th day**	**61.49±1.57***	**22.87±0.47***	**1403.58±17.79***	5.94±0.26	**30.58±1.79*****	**55.32±1.20****	**32.88±2.59****	27.53±1.32	7.38±0.36	**46%***
Duck		**20th day**	52.63±2.40	19.17±1.80	1022.74±130.33	**7.88±0.44****	11.70±0.78	43.65±1.74	16.68±1.16	**33.52±1.28***	**10.17±0.70***	42%
Chicken		**17th day**	**81.65±4.70****	21.02±0.95	**1715.39±122.27***	9.13±0.67	17.73±2.43	**70.01±4.62****	21.43±1.69	44.50±2.14	**15.67±2.36***	40%
Duck		**22nd day**	54.18±2.08	23.14±1.18	1251.89±70.92	7.88±0.43	15.52±1.84	46.43±1.28	**26.83±1.59***	37.31±2.03	14.20±1.33	48%
Chicken		**19th day**	**99.51±4.27*****	**20.71±0.73***	**2062.66±123.21****	**9.62±0.84***	**19.81±1.47****	**86.77±4.28*****	**23.45±1.92***	**48.50±3.62****	12.20±1.14	42%
Duck		**25th day**	64.76±3.8	18.31±1.41	1204.13±155.69	6.65±0.49	13.24±0.93	55.14±3.25	16.19±1.10	30.90±3.37	10.97±1.45	**53%***
Chicken		**21st day**	**110.74±5.05****	27.35±2.42	**2987.09±190.78****	11.03±0.94	**23.35±2.51***	**97.31±4.54*****	21.95±1.64	31.29±2.06	13.43±1.13	39%
Duck		**28th day**	75.91±3.61	20.13±2.16	1545.89±205.45	8.94±0.84	15.87±0.62	60.07±4.67	17.61±1.48	**38.88±1.05***	12.52±1.09	46%

M: mean, SEM: standard error of the mean
A single asterisk between the columns indicates a statistically significant difference at P≤0.01
The two asterisks between the columns indicate significant differences at P≤0.001
The three asterisks between the columns indicate significant differences at P≤0.0001.

Table 4. The histochemical reaction of the three intestine parts in chicken and duck embryos for PAS-AB (pH 2.5) stain.

Type of poultry	Small intestine part	Age categories		Age categories	
		14th day		21st day	
		PAS	AB 2.5	PAS	AB 2.5
Chickens	Duodenum	±	++	+	+++
	Jejunum	++	+	+++	+++
	Ileum	+	++	+++	+++
		18th day		28th day	
Ducks	Duodenum	++	+	+++	+
	Jejunum	+	+	±	+++
	Ileum	+	++	++	±

± Very weak, + Weak, ++ Average, +++ Very intense