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Histological, Histochemical and Immunohistochemical Investigations on the Developing Small Intestines of Broiler Embryos

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Abstract: The aim of the present study was to investigate comparatively embryonic development of the small intestine in two boiler strains by means of histological, histochemical and immunohistochemical methods. In the experiments, 125 fertile eggs from each of two commercial broiler strains were used. On the days 11, 13, 15, 18, 20 and 21 of incubation, intestinal tissue samples were taken from 10 live embryos of each strain. Rapid histological, histochemical and immunohistochemical changes occurred in the small intestine during the investigation period. Although, the intestinal villi were rudimentary and uniform on the days 11, 13 and 15, they were at different developmental stages on the days 18, 20 and 21 of incubation. Mucin containing goblet cells first appeared at 13th day of incubation. Majority of the goblet cells contained acidic mucin at 18th day of incubation. Goblet cell density increased on the days 20 and 21 of incubation. Enteroendocrine cells were first seen at 13th day of incubation. Their number was 1-4 in the majority of the villi on the day 18 of incubation and remained relatively constant thereafter. Most of the villous epithelial cells were proliferative cells. A few apoptotic cells were also observed towards the apex of the villi on the days 20 and 21 of incubation. Intestinal development patterns of both strains were quite similar. When considering the importance of embryonic development of small intestines to the chick growth and development, the data may contribute to comparative studies on the performance traits of these broiler strains.

Key words: Broiler, small intestine, apoptosis, PCNA, enteroendocrine cells, Turkey

INTRODUCTION

The embryonic development of the gastrointestinal tract is characterized by extensive structural and functional changes of the intestinal epithelium. Because of the extent of intestinal maturation varies with the duration of gestational period, significant differences are observed between the animal species at birth (Pacha, 2000). In the species with a long gestation period, the development is largely completed in utero by the end of the first trimester (Montgomery *et al*., 1999) whereas the species having a short gestation, such as rat, mature intestinal architecture is achieved later (Mathan *et al*., 1976). Uni (2006) pointed out the importance of intestinal cell dynamics in the understanding of both digestive physiology and the efficiency of animal production.

In the chicken, intestinal development definitely differs from that of the mammals because there is no suckling phase. In the hatching chick, yolk may be sole nutrient source for several days although, chicks are precocial and forage for exogenous feed. Thus, the yolk which is rich in lipids is replaced by carbohydrate and protein-rich exogenous feed, at the immediate post hatch period (Uni *et al*., 1998a; Sklan, 2001). To accommodate the rapid transition to external nutrients, the chicken small intestine undergoes morphological and enzymatic changes towards the end of incubation and post hatch period (Uni, 2006). At hatch, villi are finger-like in shape and unequal length (Sabatakou *et al*., 2003) because that villus development is asynchronous (Grey, 1972; Sabatakou *et al*., 2003; Uni *et al*., 2003a). Enterocytes are round and non-polar at hatch however, they gain more typical enterocyte morphology within 24 h post hatch (Geyra *et al*., 2001).

Although, intestinal crypts are small and rudimentary and a single crypt per villus is observed at hatch, their number increases rapidly at early post-hatch period (Uni *et al*., 2000; Geyra *et al*., 2001). In the intestinal mucosal epithelium, a continuous renewal process occurs as proliferating cells differentiate, predominantly to enterocytes which migrate to the tip of the villus until they undergo apoptosis and sloughed into the intestinal lumen from the villus tip in the mature animals (Uni, 2006). In the poultry, close to the hatch, almost all of the

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intestinal epithelial cells are proliferative with time, this pattern changes rapidly, proliferating enterocytes increasingly locate in the crypt region and also exist along the villi (Uni *et al*., 2000). Uni *et al*. (1998b) claimed that unlike mammals, the chicken enterocyte proliferation is not peculiar to the crypt region and that the site of enterocyte differentiation is not precisely localized.

Goblet cells distribute throughout the small and large intestinal villi and are responsible for the production and maintenance of the protective mucus layer by secreting high molecular weight glycoproteins known as mucins (Specian and Oliver, 1991). Mucins are classified as neutral and acidic subtypes; the latter is further divided into sulfated or non-sulfated mucins (Sheahan and Jervis, 1976). Black and Smith (1989) reported that the rate of goblet cell differentiation significantly increases during the 3rd week of embryonic development. Uni *et al*. (2003b) observed goblet cells in the small intestine of the chicken at 18th day of incubation and they contained only acidic mucins. The proportions of acidic and neutral mucin producing goblet cells were quite similar between immediately after hatch and until 7th day of post hatch (Uni *et al*., 2003b).

Because that the gastrointestinal endocrine cells distributed through the digestive tract and synthesize various gastrointestinal hormones such as secretin, gastrin, cholecystokinin, they play a pivotal role in the physiological functions of the alimentary tract including motility and digestion (Ku *et al*., 2004). Although, they compose a small percentage of the epithelial cells, any change in their intensity is closely related to the change in the capacity of producing these hormones (De Santa Barbara *et al*., 2003; Ku *et al*., 2004). Silver impregnation techniques are the conventional methods for identifying their types. These cells give argentaffin and argyrophil reactions based on different principles when stained with two main silver impregnation techniques (Grimelius, 2004). Although, argentaffin granules have been demonstrated in the epithelial cells of the small intestine from the 6th day of incubation Baxter-Grillo (1970) and Rawdon (1984) suggested that gastrointestinal endocrine cells in chicken embryo appear late in development.

Genetic studies in the intensively concentrated on developing new commercial hybrids having desired carcass and meat performance traits (Gornowicz *et al*., 2009). Nevertheless, embryonic development and functional maturation of the intestines from different broiler strains should also been investigated because that other performance characteristics are closely related to the gastrointestinal system functions. Although, the post-hatch morphological development and functional maturation of the small intestine have been studied in detail, limited information is available on the pre-hatch period. The aim of the present study was to investigate the embryonic development of the small intestine by means of histochemical and immunohistochemical methods. Also, intestinal development patterns of the Ross 308 and Hybro broiler strains were compared since, development of small intestine has a major role in determining the developmental potential of the hatched chick.

MATERIALS AND METHODS

Incubation: In this study, 125 fertile eggs from each of the Hybro and Ross 308 strains were used. The eggs were obtained from a commercial hatchery with a maternal flock at 40th week of lay. The eggs were maintained under optimal conditions during the whole incubation period in incubator (VGS 108 Kap, Turkey).

Histological procedure and histochemical staining of goblet cells and enteroendocrine cells: On the days 11, 13, 15, 18, 20 and 21 of incubation, 10 live embryos were obtained from each strain. Tissue samples were taken from duodenum, jejunum and ileum of the embryos. The samples were fixed in 4% neutral-buffered formalin, dehydrated, cleared and embedded in paraffin blocks. The sections (6 μm thickness) taken from the paraffin blocks were placed on Poly-L-Lysine (Sigma-Aldrich P 8920) coated glass slides.

Sections were stained with Crossmon's trichrome stain (Culling *et al*., 1985) Periodic-Acid Schiff (PAS), Alcian Blue (AB) pH 2.5, Masson-Fontana's Argentaffin and Grimelius's Argyrophil silver stains (Bancroft and Cook, 1994).

Immunohistochemical demonstration of PCNA: In this study, Proliferating Cell Nuclear Antigen (PCNA) which is a nuclear protein functioning as a cofactor for DNA polymerase delta was used as a marker to characterize cell proliferation. PCNA was stained immunohistochemically using a sensitive peroxidase-labelled streptavidin-biotin detection system. The sections were dewaxed in xylene series and rehydrated. In order to unmask the antigen, the sections were placed into 10 mM citric acid buffer (pH 6.0) and heated in a microwave oven (700 W for 5 min). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 20 min. Nonspecific binding sites were blocked by incubating the sections in blocking solution (ScyTek UHP 125, USA) for 20 min. The sections were incubated with mouse anti PCNA monoclonal antibody (1:100 dilution, Genetex, GTX71945, USA) for 1 h at Room Temperature (RT). They were then

treated with a biotinylated goat anti-mouse secondary antibody (IgG) (ScyTek UHP 125, USA) for 20 min at RT following treated with Horseradish Peroxidase (HRP)-streptavidin (ScyTek UHP 125, USA) for 20 min at RT. Color reaction was developed with 3-3'-diaminobenzidine (DAB) (ScyTek ACK 125, USA). The slides were counterstained with Mayer's hematoxylin for 1 min and then mounted with synthetic resin (Entellan, Merck). In the negative control slides, the tissue sections were incubated with PBS without the primary antibodies.

***In situ* TUNEL apoptosis detection assay:** Apoptotic cells in the small intestine were detected by enzymatic labeling of DNA strand breaks by using TUNEL Method (Terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick-end labeling). For this purpose, TdT-FragEL™ DNA Fragmentation Detection kit (Calbiochem, QIA33) was used. The sections which were dewaxed and rehydrated were incubated in permeabilization solution (20 mg mL^{-1} proteinase K) for 20 min at RT. After washing with tris-buffered saline, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 5 min. After washing, the labeling reaction was performed by using 60 μL TdT labeling solution for each slides and incubated for 1.5 h at 37°C. After washing, the slides were incubated in peroxidase streptavidin conjugate for 30 min at RT. Color development was performed by incubating the slides in DAB solution for 15 min. The slides were counterstained with Methyl-green solution for 5 min and then mounted with synthetic resin. A mixture of HL60 cells incubated with 0.5 μg mL^{-1} actinomycin D for 19 h to induce apoptosis and unstimulated HL60 cells were stained as positive-control slides.

Histological evaluation and statistical analyses: All specimens were examined under Nikon Eclipse E-400 light microscope with DS-5M digital camera head and DS-L1 camera control unit (Nikon, Japan) and the digital images of necessary-regions were recorded. Because that goblet cell numbers were very low on the days 11, 13, 15 and 18, total number of goblet cells in 10 villi was determined and mean goblet cell density were expressed as cell number per villus. Enteroendocrine cell densities were also determined and their results were also expressed in a similar manner. In the later embryonic periods, goblet cell numbers significantly increased, the results were expressed as goblet cell number in unit length (100 μm) of the villus epithelium by using a digital image analysis software (BS200 PRO, 2005). PCNA and apoptotis stained sections were evaluated qualitatively. The results were analyzed by two sample t-test using software (Minitab for Windows, Release, 9.2, 1993).

RESULTS AND DISCUSSION

Intestinal epithelium was simple columnar at all stages of investigation. Histological examinations of the segments of small intestine indicated that villus development occurred as sets which there was no synchronization in their development. However, the villi within each set developed in a synchronized manner. Rudimentary villi were seen on the days 11, 13 (Fig. 1a), and 15 however, on the days 18, 20 and 21, developing villi at various stages of the development were observed in both strains. At 18th day of incubation, two types of the developing villi, differing in length and shape (V1, V2) were seen (Fig. 1b). The larger villi were pear-shaped and the smaller ones were in a rocket-like shape. At 20th day of incubation, the developing villi elongated while preserving their shapes and third series of developing villi were also observed (V3) (Fig. 1c). At 21st day of incubation, the villi at different developmental stages continued to grow and a single rudimentary crypt was observed per villus (Fig. 1d). Histological findings of the intestinal development were quite similar in both broiler strains.

Neutral mucin containing goblet cells gave positive PAS reaction whereas acidic mucin containing goblet cells were stained with AB, at pH 2.5. Goblet cells were first detected in small numbers at 13th day of incubation (Fig. 2a). Their number slightly increased on the day 15 of incubation (Fig. 2b). At 18th day of incubation, the majority of goblet cells contained only acidic mucin. The number of both acid and neutral mucin-producing goblet cells increased in all segments of the small intestine along the villi on the days 20 and 21 of incubation (Fig. 2c and d). Goblet cells were first observed in rudimentary crypts at 21st day of incubation. Results of the goblet cell counting on PAS and AB (pH 2.5) stained specimens of the duodenal, jejunal and ileal segments are shown in Table 1. There was not any significant difference ($p>0.05$) in the number of goblet cells between Ross 308 and Hybro strains. The number of the goblet cells increased towards distal regions of the small intestine.

In the sections stained with Masson-Fontana Argentaffin and Grimelius's Argyrophil silver stains, enteroendocrine cells located in both epithelium and subepithelium along the villus. These cells were pyramidal or oval in shape and brownish-black granules located at infra nuclear position. On the day 11 of incubation, enteroendocrine cells were not detected. At 13th day of incubation, only a few argyrophil cells were observed in

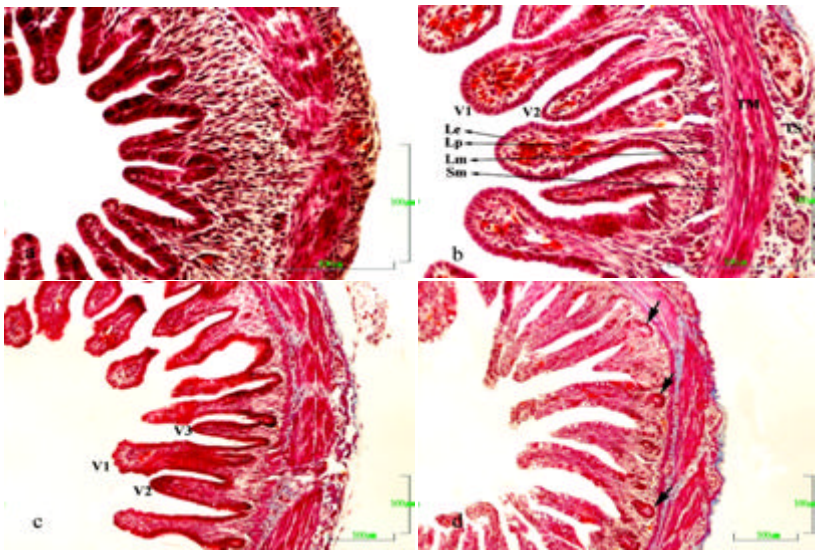

Fig. 1: Sections of small intestine are illustrated in different incubation period; a) A section prepared from jejunum of a Ross 308 chicken embryo at 13th day of incubation; b) A section prepared from duodenum of a Ross 308 chicken embryo at 18th day of incubation; c) A section prepared from ileum of a Hybro chicken embryo at 20th day of incubation; d) A section prepared from ileum of a Hybro chicken embryo at 21st day of incubation. V1: Villi in first set of development, V2: Villi in second set of development, V3: Villi in third set of development, Le: Lamina epithelialis, Lp: Lamina propria, Lm: Lamina muscularis, Sm: Submucosa, TM: Tunica Muscularis, TS: Tunica Serosa, arrows: crypts. Crossmon's trichrome stain. Bar: 100 μm

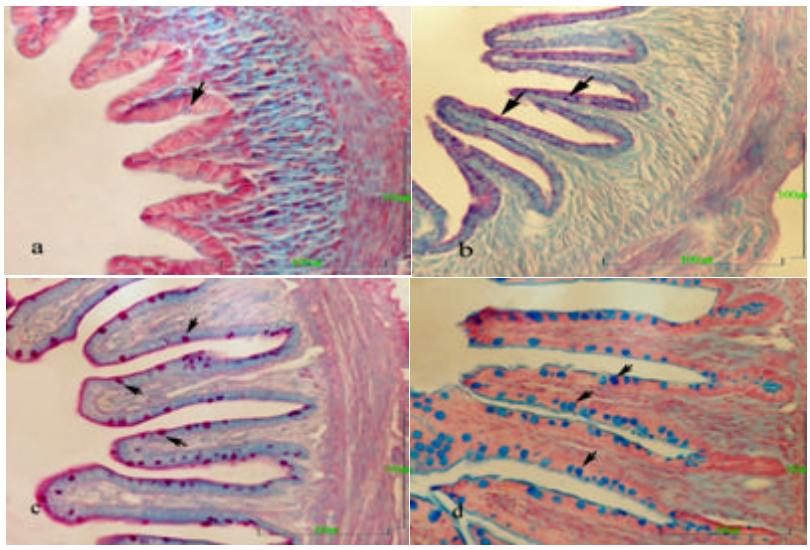

Fig. 2: Sections of small intestine stained AB (a, d) or PAS (b, c) are illustrated at different incubation periods. A section prepared from duodenum of a Hybro chicken embryo at 13th day of incubation arrow: AB (+) goblet cell; a) A section prepared from jejunum of a Ross 308 chicken embryo at 15th day of incubation arrows: PAS (+) goblet cells; b) A section prepared from jejunum of a Hybro duodenum and jejunum, except ileum on the day 15. There was no difference in the period of appearance of the chicken embryo at 20th day of incubation arrows: PAS (+) goblet cells; c) A section prepared from ileum of a Hybro chicken embryo at 21st day of incubation arrows: AB (+) goblet cells; d) Bar: 100 μm

Table 1: Goblet cell data of the strains (Goblet cell number per villus at 11th, 13th, 15th and 18th day of incubation and goblet cell number/100 μm at 20th and 21st days of incubation)

	PAS (X±SE)			AB (pH 2.5) (X±SE)		
Sampling periods	Duodenum	Jejeunum	Ileum	Duodenum	Jejeunum	Ileum
11 days of incubation						
Ross 308	ND	ND	ND	ND	ND	ND
Hybro	ND	ND	ND	ND	ND	ND
13 days of incubation						
Ross 308	0.28±0.05	0.12±0.05	0.16±0.12	ND	0.04±0.04	ND
Hybro	0.12±0.08	0.16±0.04	0.12±0.08	0.08±0.08	ND	ND
15 days of incubation						
Ross 308	0.40±0.17	0.32±0.16	0.32±0.12	0.24±0.07	0.16±0.07	0.04±0.04
Hybro	0.24±0.04	0.24±0.07	0.16±0.04	0.20±0.11	0.04±0.04	0.04±0.04
18 days of incubation						
Ross 308	1.40±0.26	0.72±0.10	1.76±0.09	4.36±0.26	2.52±0.31	2.16±0.44
Hybro	1.52±0.37	1.16±0.20	1.68±0.10	4.48±0.62	2.52±0.24	1.94±0.42
20 days of incubation						
Ross 308	5.12±0.30	6.12±0.26	7.00±0.16	4.24±0.18	5.56±0.12	5.34±0.39
Hybro	5.08±0.19	5.94±0.35	6.98±0.17	4.48±0.19	5.48±0.17	5.50±0.45
21 days of incubation						
Ross 308	6.74±0.19	7.52±0.24	8.24±0.24	6.28±0.39	7.26±0.31	7.30±0.28
Hybro	6.34±0.42	7.28±0.37	7.84±0.34	6.38±0.22	7.66±0.33	7.44±0.29

ND: Not Determined; the difference between the strains are not significant (p>0.05)

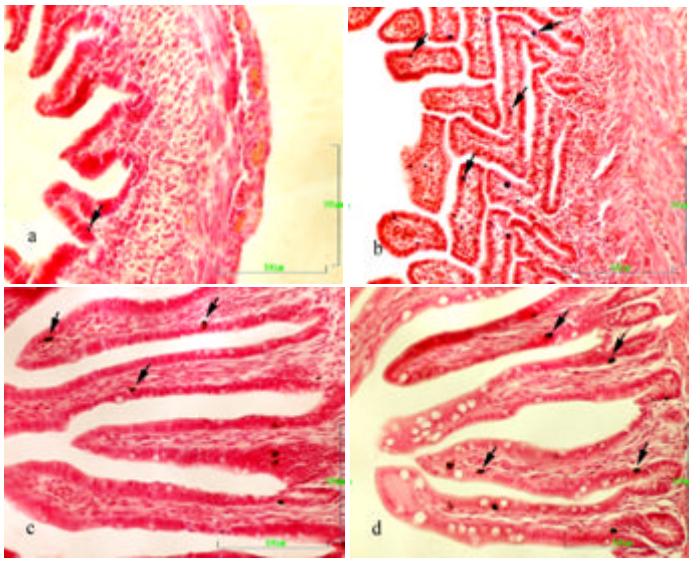

Fig. 3: a, b) Sections of small intestine stained argyrophil or c, d) argentaffin silver stain are illustrated in different incubation period. A section prepared from duodenum of a Ross 308 chicken embryo at 13th day of incubation arrow: Argyrophil cell; a) a section prepared from jejunum of a Hybro chicken embryo at 18th day of incubation arrows: Argyrophil cells; b) a section prepared from duodenum of a Hybro chicken embryo at 20th day of incubation arrows: Argentaffin cells; c) A section prepared from ileum of a Ross 308 chicken embryo at 21st day of incubation arrows: argentaffin cells; d) Bar: 100 μm

the duodenum (Fig. 3a) whereas argentaffin cells were not observed. Both cell types were demonstrated in enteroendocrine cells between two strains. There were 1-4 enteroendocrine cells in the most of the villi from 18th day of incubation (Fig. 3b). Enteroendocrine cell density did not change and displayed uniform distribution throughout intestinal segments of both strains at 20th and 21st day of incubation (Fig. 3c and d). At 21st day of incubation enteroendocrine cells were also observed in rudimentary crypts.

There was not any significant difference (p>0.05) in the number of enteroendocrine cells between Ross 308 and Hybro strains (Table 2). PCNA-positive cells were observed in the epithelium, connective tissue and

Table 2: Enteroendocrine cell data of the strains (cell number per villus)

	Argentaffin (X±SE)			Argyrophil (X±SE)		
Sampling periods	Duodenum	Jejeunum	Ileum	Duodenum	Jejeunum	Ileum
11 days of incubation						
Ross 308	ND	ND	ND	ND	ND	ND
Hybro	ND	ND	ND	ND	ND	ND
13 days of incubation						
Ross 308	ND	ND	ND	0.10±0.06	ND	ND
Hybro	ND	ND	ND	0.15±0.09	ND	ND
15 days of incubation						
Ross 308	0.44±0.12	0.60±0.14	ND	0.60±0.12	0.35±0.17	ND
Hybro	0.60±0.14	0.32±0.10	ND	0.68±0.17	0.35±0.13	ND
18 days of incubation						
Ross 308	2.48±0.15	1.72±0.10	2.00±0.13	2.20±0.14	1.84±0.26	1.64±0.10
Hybro	2.44±0.13	2.04±0.12	2.04±0.10	2.08±0.19	1.80±0.11	1.84±0.08
20 days of incubation						
Ross 308	2.48±0.15	1.96±0.15	2.04±0.08	2.48±0.10	2.24±0.17	2.08±0.21
Hybro	2.32±0.10	1.98±0.13	2.22±0.07	2.48±0.19	2.04±0.15	1.92±0.19
21 days of incubation						
Ross 308	2.60±0.09	2.56±0.20	2.52±0.14	2.60±0.14	2.44±0.16	2.36±0.13
Hybro	2.56±0.21	2.40±0.11	2.48±0.08	2.64±0.17	2.48±0.17	2.32±0.14

ND: Not Determined; the difference between the strains are not significant ($p>0.05$)

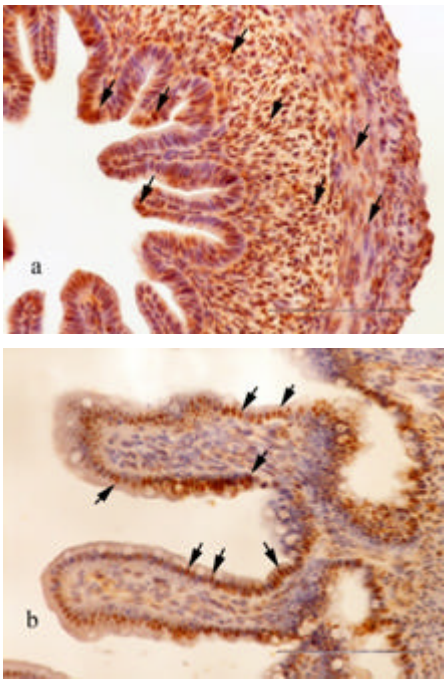

Fig. 4: Sections of small intestine stained PCNA immunohistochemical staining are illustrated in different incubation period. A section prepared from duodenum of a Ross 308 chicken embryo at 11th day of incubation; a) A section prepared from duodenum of a Ross 308 chicken embryo at 20th day of incubation; b) Arrows: PCNA (+) cells. Bar: 100 μm

muscle layers at earlier stages of incubation (Fig. 4a). PCNA-positivity of the cells tended to decrease on the day 20 and 21 of incubation in the connective and muscular tissues in investigated segments of the small intestine. Majority of the epithelial cells along the entire length of the villus were PCNA positive at 20 and 21 day of incubation (Fig. 4b). In addition, most of the cryptic epithelial cells were also PCNA positive at 21st day of incubation. The location and distribution of PCNA-positivite cells were quite similar in both strains. On the days 11, 13, 15 and 18 of incubation, apoptotic cells mainly located in mesothelium whereas they were rarely seen in the epithelium (Fig. 5a). Apoptotic cells were rare in the apices of the villi and many epithelial cells shed into the intestinal lumen in both strains on the days 20 and 21 of incubation (Fig. 5b).

Morphological findings of the present study indicated that the morphology of the small intestine of the chicken changed rapidly in late stages of incubation period. Villus development was asynchronous and followed a similar course of development to identification of earlier investigators. Similarly, Grey (1972) and Sabatakou *et al.* (2003) reported that villus formation completed in two phases: the first phase starts formation of 16 previllous ridges and completed by 13th day of incubation and the second phase begins at day 16 and completed by the 4th day after hatching. Uni *et al.* (2003a) showed that some epithelial budding formed as the primordia of the villi in late embryonic period, an additional wave of villous development started on the day 20 and these developing villi composed 30% of total villi. Third series of developing villi were also observed at 20th day of incubation in this study. Geyra *et al.* (2001) reported that crypt development is a crucial step in the intestinal maturation. The increase in the number and size of crypts provide enterocytes for increasing intestinal

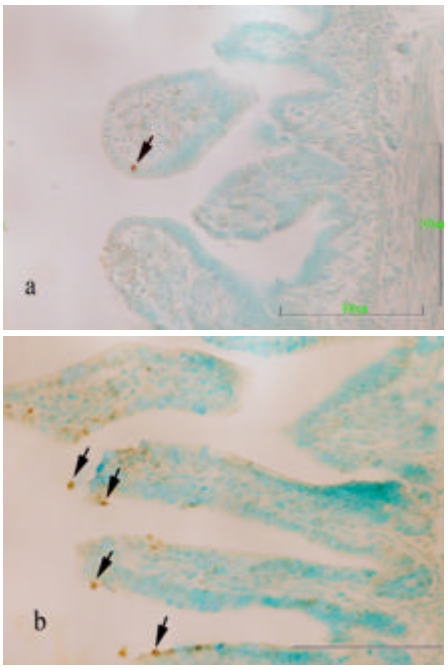

Fig. 5: Sections of small intestine stained TUNEL staining are illustrated in different incubation period. A section prepared from duodenum of a Ross 308 chicken embryo at 18th day of incubation; a) A section prepared from jejunum of a Hybro chicken embryo at 21st day of incubation b) Arrows: apoptotic cells. Bar: 100 μm

absorptive surface area as the villi grow. Crypts began to form on the day of hatch and a single rudimentary crypt per villus is as also seen in this study. As the crypts develop rapidly, they branch and increase in size, their cell numbers and cell size (Uni *et al*., 2000; Geyra *et al*., 2001).

The mucus layer is an integral structural component of the gastrointestinal tract, acting as a medium for protection, lubrication and transport between luminal content and the epithelial cells (Forstner *et al*., 1995). Thus, ontogeny of goblet cells is an important part of the studies on the intestinal development.

Sabatakou *et al*. (2003) observed by scanning electron microscopy that goblet cell openings are visible from 16th day of incubation in the chicken embryos. In a study (Uni *et al*., 2003b) examining the ontogeny and development of goblet cells in the broiler, goblet cells were first observed in the intestine on the day 3 before hatch and at this time they contained only acidic mucin. In the present study, goblet cells were first observed earlier, at 13th day of incubation and these cells were observed more infrequently. At 18th day of incubation, the majority of goblet cells contained only acidic mucin as Uni *et al*. (2003b) have reported it. Cebra (1999) claimed that the presence of acidic mucins at early stages of the life may play a particular importance as an innate barrier because that the acquired immune system of the neonatal intestine is not fully functional. Chemical composition of the mucus glycoprotein changed on day 20 and 21 of incubation. During this period, goblet cells produced both acid and neutral mucins. Smirnov *et al*. (2006) showed that chicken intestinal mucin increased gradually from the day 17 of incubation to the day 3 post-hatch. This increase supports the increase of goblet cell number occurring in the late incubation. There was not any significant difference ($p>0.05$) between Ross 308 and Hybro broiler strains in the number of goblet cells. Western blotting analysis of chicken intestinal mucin glycoprotein revealed that mucin protein content increased throughout the small intestine especially in the distal regions (Smirnov *et al*., 2004). Similarly, goblet cell intensity increased towards distal intestinal regions in the present study.

The enteroendocrine cells have been described in the small intestine of chicken embryos using histochemistry, immunohistochemistry and electron microscopy but there are differences in the studies regarding from the point of appearance period of these cells. Although, argentaffin granules have been demonstrated (Baxter-Grillo, 1970), in the small intestine from 6th day of incubation, Rawdon (1984) suggested that only a few cells are recognized by either immunocytochemistry or electron microscopy before 16th day of incubation. Nevertheless, Alison (1989) showed that endocrine cells were present in relatively undifferentiated surface epithelium at 12th day of incubation. Penttila (1968) first observed the enterochromaffin cells at 14th day of incubation in the chicken duodenum and their number increased greatly during further development. In this study, enteroendocrine cells giving argyrophil reaction were first observed in duodenum at the 13th day of incubation. From 18th day of incubation, there was a total of 1-4 enteroendocrine cells giving both argyrophil and argentaffin reactions in the majority of the villi and their number per villus remained relatively constant in both broiler strains thereafter.

Morphological homeostasis of the small intestinal epithelium is regulated by both cell proliferation and apoptosis in mature animals (Potten and Booth, 1997). In contrast to adult mammals, proliferation of enterocytes in the chick small intestine is not confined to the crypts but also occurs along the villus (Uni *et al*., 1998b). In the newly hatched chick, all cells along the villus and the crypt are the proliferating cells (Uni *et al*., 1998b; Uni *et al*., 2000). In this study, PCNA expressing cells were commonly observed in all layers of the small

intestine at earlier stages of incubation. PCNA positivity of the connective and the muscular tissues in all segments of the small intestine tended to decrease with the embryonic age however, most of the epithelial cells along the villus expressed PCNA at 20 and 21st days of incubation in both strains. Apoptotic cells were rarely observed at the tip of villi. Epithelial cell proliferation along the villus occurring at this period of the development possibly has an important role in the rapid growth of villus which results in increase of intestinal absorptive surface.

CONCLUSION

Based on the findings of the present study, it was concluded that extensive cellular changes took place in the developing small intestine at late embryonic period, especially from the 18th of incubation to prepare for post-hatch period in which rapid transition to external nutrients occurs. There were no significant differences between Ross 308 and Hybro broiler strains in the parameters those were evaluated in this study. Chick growth and development depend on the digestion and absorption of nutrients. Because that functional and morphological development of the small intestine has a major role in determining the developmental potential of the hatched chick (Uni, 2006) morphological findings of embryonic development of the intestines may provide important clues in predicting growth and production traits.

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