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Light and Scanning Electron Microscopy of the Small Intestine of Young Malaysian Village Chicken and Commercial Broiler

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ABSTRACT

The intestinal mucosa of the Malaysian village chicken (MVC) and commercial broiler breed (CBC) from day 1 to 20 post-hatch was examined by means of light and scanning electron microscopy. The results showed that the intestinal mucosa was highly developed on day 1 in CBC as compared to MVC in regard to villus height, crypt depth and thickness of tunica muscularis. At day 1 post-hatch, it was observed that the mid cecum of the CBC showed plicae with short villi, while very short villi without plicae were observed in MVC. The intestinal villi in both breeds had a finger-like shape at day-old chick and changed to plate-like and tongue-like shapes toward the end of the experiment for CBC and MVC, respectively. Unlike MVC, the surface epithelia of the intestinal villi in CBC exhibited cell activities represented by surface recesses, clear cell outline and dome-shaped cells with protuberances, while a marked corrugated surface and areas of discontinuities were dominantly seen on the intestinal villi of the MVC particularly on the ileal villi. This study revealed that the intestinal mucosa of MVC of newly hatched chick was less evolutionary as compared to CBC which showed earlier development and maturation with more active villi to provide the highest rate of absorption as the breed selected for high body weight and rapid growth rate.

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INTRODUCTION

The present Malaysian village chicken, commonly known as *ayam kampong*, are the result of cross-breeding between the

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Red jungle fowl and mixed exotic domestic breeds brought in by Europeans, mainly the British (Azahan & Zahari, 1983). Because broilers are marketed much earlier than in the past, many previous studies concentrated on the development of the gastro-intestinal tract (GIT) during the post-hatching growth period to illustrate the changes occurring in this tract during selection for fast growth (Bayer *et al.,* 1975; Yamauchi & Isshiki, 1991; Yamauchi *et al.,* 1992; Yamauchi, 2002).

The avian intestine lacks the macroscopic level of folding seen in large mammals (King & McLelland, 1979; Turk, 1982). Gussekloo (2006) established basic histological data on the intestinal villi of chickens. The mucosal surface area of the small intestine in chicken undergoes important changes during development which may determine the capacity to absorb nutrients (James *et al.,* 1988; Smith *et al.,* 1990). Mathan *et al*. (1976) and Calvert and Potheir (1990) studied the intestinal crypts at early postnatal period in rats and mice, and found that the crypts developed from the flat intervillus epithelium. At hatching, the villi of the small intestine are undeveloped while crypts in the intervillus spaces are not detectable (Geyra *et al*., 2001). The development of intestinal mucosa after hatching occurs in the duodenum and jejunum, but this is found to be lesser in the ileum of poult (Uni *et al*., 1999). The thickness of muscularis externa of the intestine is greater in heavy lines than in light lines (Ogiolda *et al.,* 1998). The development of cecal mucosa decreases from the base to apex of the caecum in chicken (Rezaian & Hamedi, 2007). The villi at the distal part of cecum represent a typical appearance accompanied with regression of the epithelium and glands (Kitagawa *et al*., 1996; Looper & Looper, 2005).

The SEM observations of the intestinal mucosa of different birds have been done by many authors, Yamauchi and Isshiki (1991) and Yamauchi (2002) who studied the mucous membrane of the intestine exhibiting regional variations and reflecting the changing functions of the digestive system. Other authors have studied the developmental aspects of intestinal villi in post-hatched chicks with the SEM (Bayer *et al.,* 1975; Yamauchi *et al*., 1992). The morphological changes in intestinal villus surface and villus tip related to intestinal function of chicken at different ages were observed by Shamoto *et al.* (1999), Samanya and Yamauchi (2001) and Maneewan and Yamauchi (2003). The tips of duodenal villi have more developed epithelial cell protuberances, cell cluster and cell exfoliation resulting in a rough surface in heavy breed compared to the smooth surface in the light breed on 1 and 10 days post-hatch (Yamauchi & Isshiki, 1991; Yamauchi, 2002). The comparisons of the epithelial cells of intestinal villi at first day and10 days post-hatched in heavy and light breed revealed that the intestinal villi of the heavy breed reached morphological and physiological maturation earlier than those in light breed (Yamauch *et al.,* 1992; Yamauchi, 2002).

In the present study, MVC, which is characterized by a slow growth rate, was compared with CBC as a breed selected for high growth rate. The experiment was undertaken to determine the alteration in the intestinal mucosa between these two breeds using light and scanning electron microscopy. The comparisons were made at 1, 10 and 20 days after hatching.

MATERIALS AND METHODS

Animals

A total of 35 birds of MVC and CBC were used in this study. The eggs of MVC were obtained from Jenderam Hulu, Sepang. The eggs were incubated and hatched in the laboratory. The day-old chicks of commercial line (Ross) selected for high body weight were supplied by a private hatchery (Linggi Poultry farm Sdn. Bhd. C-P lot 1354, Kuala Kangsar, Perak, Malaysia). The birds were reared in separate cages with food and water provided *ad libitum*.

Collection of Tissue Samples

Seven birds from each breed were serially euthanased at days 1, 10, and 20 post-hatch by intravenous (cutaneous ulnar vein) administration of sodium pentobarbitone (80mg/ kg) (Mitchell & Smith, 1991). The entire intestine was removed (duodenum, jejunum, ileum and cecum). The small intestine was then divided into duodenum, jejunum and ileum, following the demarcation set by Mitchell and Smith (1990) and Geyra *et al.* (2001). The organs were washed with saline solution to remove the intestinal contents.

Light Microscope Examinations

Sample from each segment was taken for histological examination which included the midpoint of each part of intestine (duodenum, jejunum, ileum and cecum). Specimens were fixed in 10% Neutral Buffered Formalin (NBF) for 24 hrs and processed according to the standard histological procedure. Sections of 3 µm thickness were cut and stained with Hematoxyline and Eosin, and Masson trichrome (Bancroft & Gamble, 2005). The sections were viewed under image analyzer (Olympus Image Analysis, BX 51 TF) provided by the CC12 camera.

Scanning Electron Microscope Examinations

One-millimetre slice of tissue from the middle portion of the duodenum, jejunum and ileum were fixed in 4% gluteraldyhyde. Tissue samples for SEM were processed as described previously (Yamauchi *et al*., 1990; Maneewan & Yamauchi, 2003). Specimens were mounted under the dissecting microscope to select the right orientation. The specimens were dried in a critical point drying apparatus (BALTEC-SPD 030) using liquid carbon dioxide as the medium, sputter-coated with gold (BALTEC-SCD 005 vacuum coater) at 100 millitorr, 7 milliamperes for 3 min, before they were examined with a Jeol- SEM (JSM-6400, Japan) at 8 kv.

RESULTS

Light Microscopic Observations

The histological observations revealed that the intestinal wall has multilayered tube containing four layers from the lumen to the external layer, the mucosa (tunica mucosa), the tunica submucosa, the tunica muscularis externa, which are represented by the inner thick circular and the outer thin longitudinal muscle fibres, and the serosa (tunica serosa). The tunica mucosa is folded into many villi. The mucosal epithelium consisted of four types of cells, chief cells, goblet cells, paneth cells and endocrine cells. These observations were similar for both breeds. However, the mucosa of small intestine of day-old chicks in CBC was found to be more developed than in MVC (see Fig.1). The differences represented by well develops villi and the crypt glands. These crypts of day-old MVC contained few cells, and invagination was not completed in all the intestinal segments particularly in the ileum. Furthermore, the thickness of tunica muscularis externa was higher in CBC than in MVC. However, the villi height, crypt depth and thickness of tunica muscularis externa were found to have increased thereafter.

The body of cecum showed similar histological layers like those of the intestine. However, in CBC, day-old chicks showed plicae (folds) with short villi, but areas without villi were also seen in the same section. A thin layer of longitudinal smooth muscle fibres represents the muscularis mucosa was also present. In MVC, very short villi without plicae were observed in the cecum of day-old chicks with additional shallow crypts that opened directly into the flat mucosal surface (Fig.2). The thin cecum wall had relatively very thin circular smooth muscle layer as compared to CBC. In 10-day-old MVC, the plicae of cecum body were observed with short villi and the crypt glands became more developed (Fig.3). The lymphoid infiltrations of the lamina propria were observed in CBC at day 10 of post-hatch, but these only appeared at day 20 of post-hatch in the MVC.

Scanning Electron Microscopic Observations

The shape of the duodenal villi of day-old chicks showed finger-like projections in both breeds (see Fig.4). The side-appearance of villi surface showed transverse recesses in CBC (Fig.5A) and with a marked corrugated surface for MVC. The corrugated surface of the villi on higher magnification showed discontinuity and disruption of the epithelial mucosa (Fig.5B). In CBC, however, it was restricted to the tips of the villi, while the remainder villus surface showed clear penta or hexagonal outlines demarcation (Fig. 5C and Fig.5D). In higher magnification, the villi tips showed cluster of dome-shaped cells and cells protuberances around the central sulcus (Fig.6). At day 10 of post-hatch, the duodenal villi appeared to have leaf-like shape with a slightly curved narrow tip in CBC without appearance of recesses on the surface as seen clearly in MVC which still showed finger–shape villi (Fig.7). The side surface of villi in both breeds appeared rough with a clear cell outline. The tips of the leaf-like shape duodenal villi for CBC

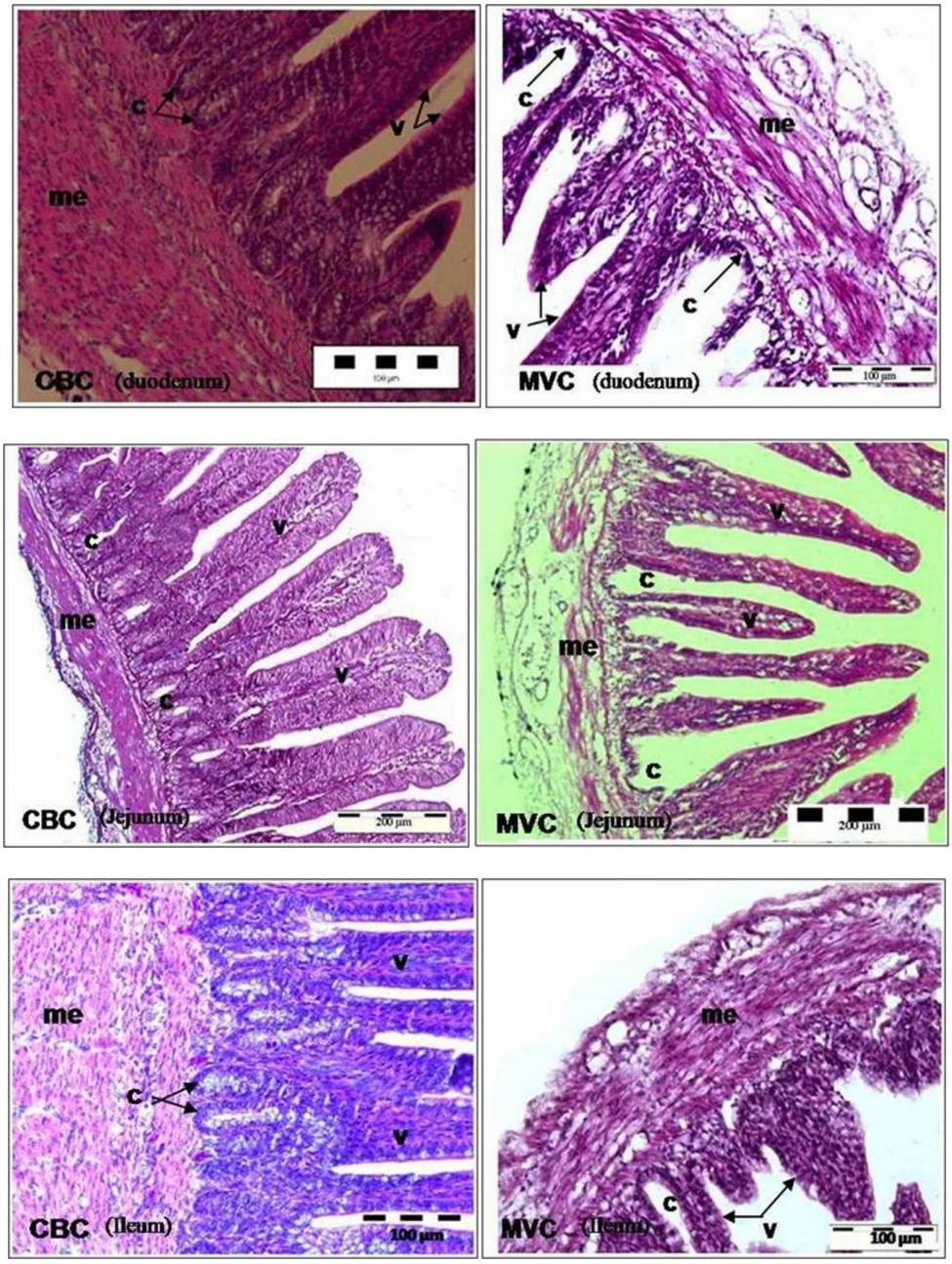

Fig.1: Microphotographs of the small intestine (Duodenum, Jejunum and Ileum) of day-old CBC and MVC shows the villi (v), the crypt (c) and muscularis externa (me) that are well developed in the CBC compared to MVC. H&E stain.

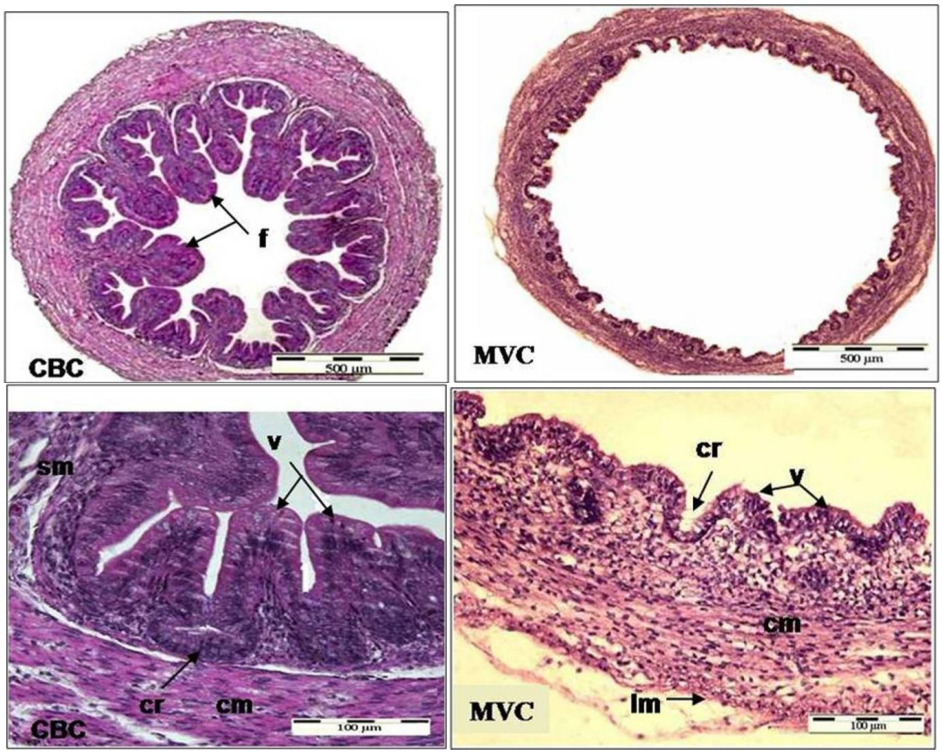

Fig.2: Microphotographs of the cross sections through cecal body of day-old CBC and MVC (upper) and similar sections with higher magnification (lower) shows the variation in hight of villus and fold, and the crypt depth between the breeds. f, fold; v, villi; cr, crypt; sm, submucosa; cm, circular muscle fibres; lm, longitudinal muscle fibre. H&E stain.

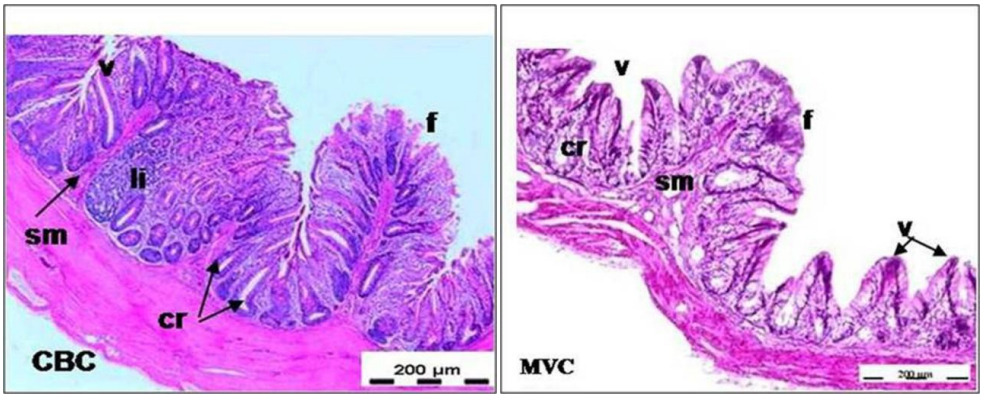

Fig.3: Microphotographs of the cecal body of 10 day-old CBC and MVC shows the long folds (f) and lymphoid infiltration (li); v, villi; sm, submucosa. H&E stain.

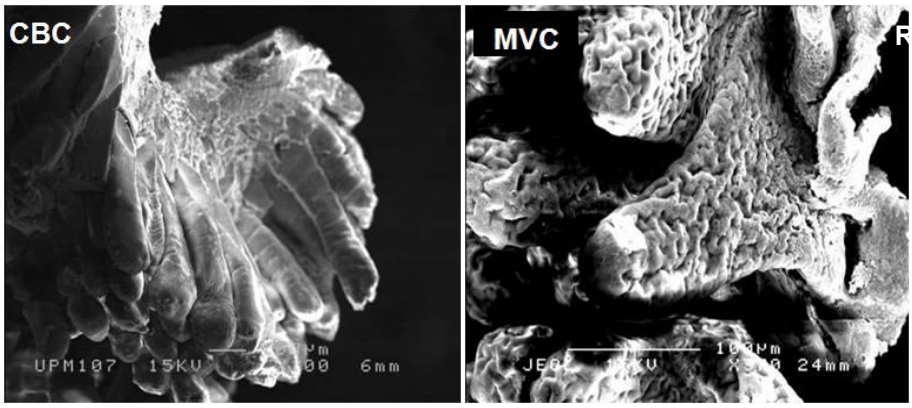

Fig.4: Scanning electron micrographs of the duodenal villi of day-old CBC and MVC shows finger-like villi, with transverse recesses in the CBC and with a marked corrugated appearance in the MVC.

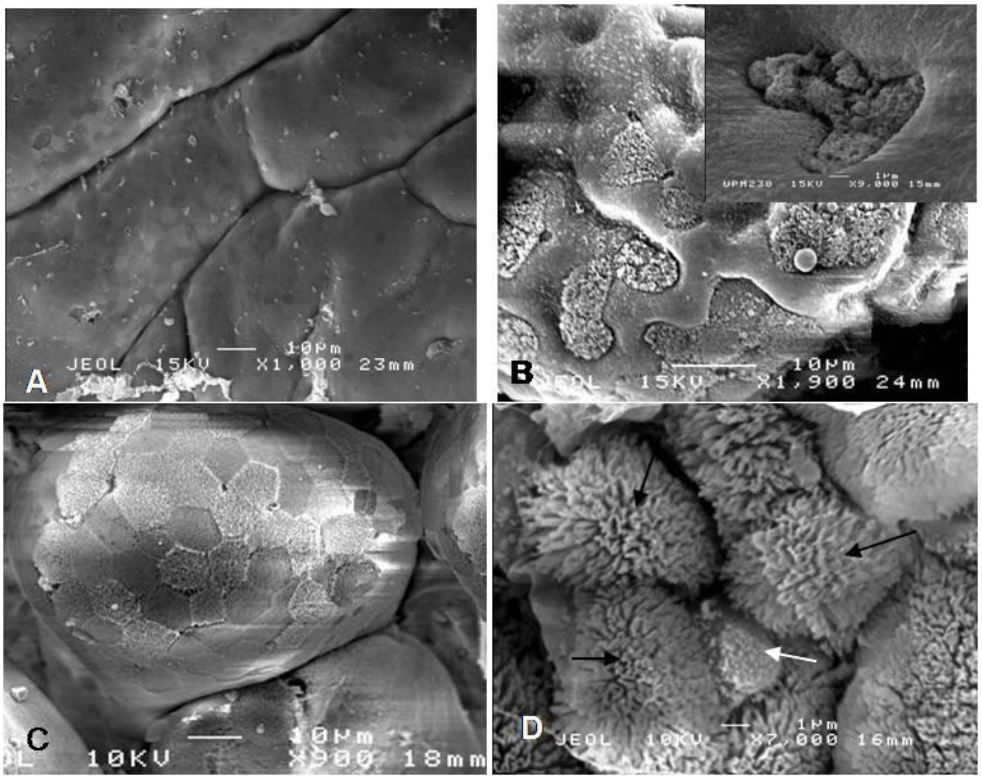

Fig.5: Scanning electron micrographs of the villi epithelium from different intestinal segments shows (A) villus surface with recesses, (B) discontinuities of the epithelial surface in the villus tip. Inset shows an enlarged view of an epithelial crevice (Bar 1 µm x 9.000), (C) villi tip with clear hexagonal or pentagonal cell outline, D) epithelial cells with long (black arrows) and short (white arrows) microvilli.

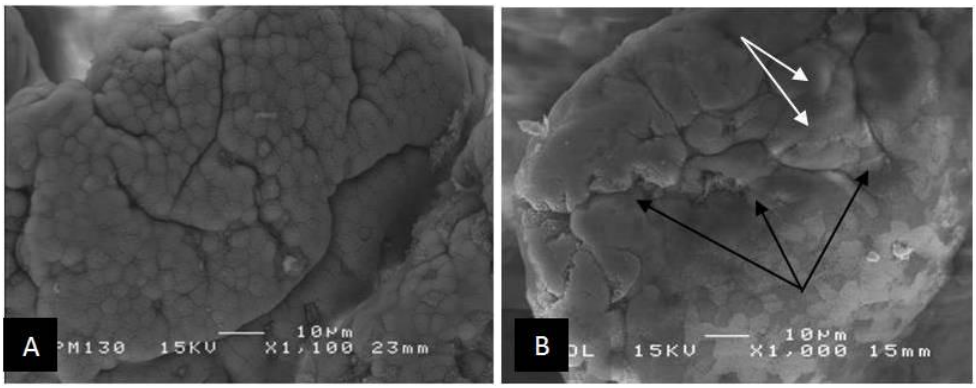

Fig.6: Scanning electron micrographs of the villi epithelium from different intestinal segments shows (A) cluster of dome-shaped cells in the villus tip, (B) rough villus tip, cells with protuberances (white arrows) around the central sulcus (black arrows).

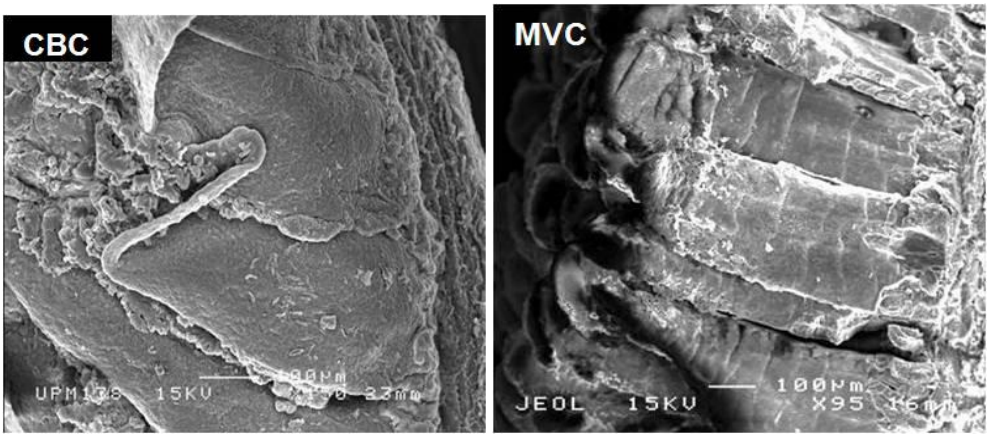

Fig.7: Scanning electron micrographs of the duodenal villi of 10 day-old CBC and MVC shows leaf-like in shape with a slightly curved narrow tip in the CBC, finger-like shape with a little compress on both sides, and appearance of the recesses on the surface in both the MVC.

were markedly curved above each other at day 20 of post-hatch (see Fig.8). The smooth surface replaced the recently exfoliated cells was commonly observed with deeper cells at these sites with short microvilli (Fig.9A). In MVC, however, the exfoliated zone (Fig.9B) was restricted on the tips of the tongue-like shape villi (Fig.8).

The jejunal villi of day-old and 10 day-old chicks of both breeds appeared similar to that of the duodenal villi of the same age. In 20 day-old chicks, the jejunal villi showed plate-like and tongue-like shape for CBC and MVC, respectively (Fig.10). However, the recesses and wide curled tips with cell activities represented by dome-shaped cells

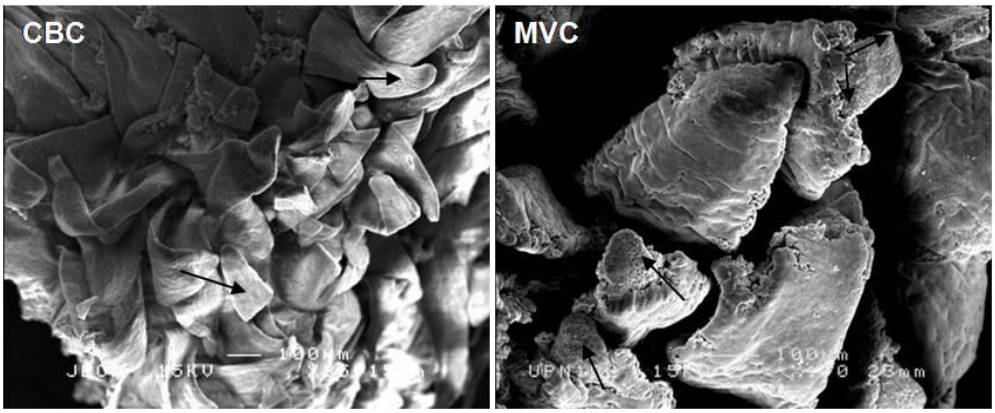

Fig.8: Scanning electron micrographs of the duodenal villi of 20 day-old CBC and MVC shows leaf-like shaped villi in CBC with curved tips above each other. Tongue-like villi with surface recesses and exfoliated area (arrows) in the MVC.

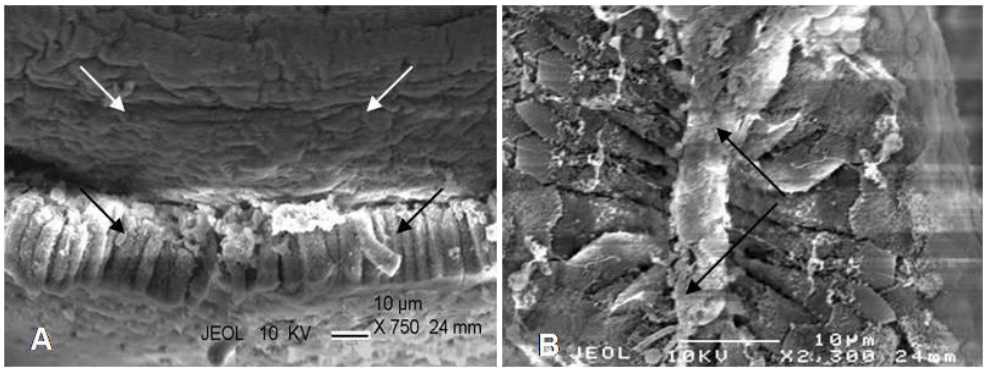

Fig.9: Scanning electron micrographs of the villi epithelium from different intestinal segments shows (A) exfoliated zone in the proximal part of villi showing the epithelial cells at this zone (black arrows) and the deeper cells (white arrows) which recently replaced the exfoliated cells, (B) exfoliated zone (arrows) restricted to the central area of the villus tip.

with cell protuberances were observed around the central sulcus particularly in MVC (Fig6).

The ileal villi of day-old chick of CBC appeared similar to the duodenal villi but without any surface recesses, while in MVC, a marked corrugated surface and areas of discontinuities were dominantly seen on the ileal villi (Fig.11). In 10-day-old chicks of MVC, the epithelial surface of finger-shaped ileal villi showed very clear crevices and discontinuity (Fig.12). The cell activities and exfoliated cells on the wide villi tips were also observed. However, these cell activities were commonly observed in the tongue-like villi of CBC at this age. In the 20-day-old chicks of CBC, the ileal villi appeared in a tongue-like shape with marked recesses and cells activities, while the villi of MVC still appeared to have finger-like shape (Fig.13).

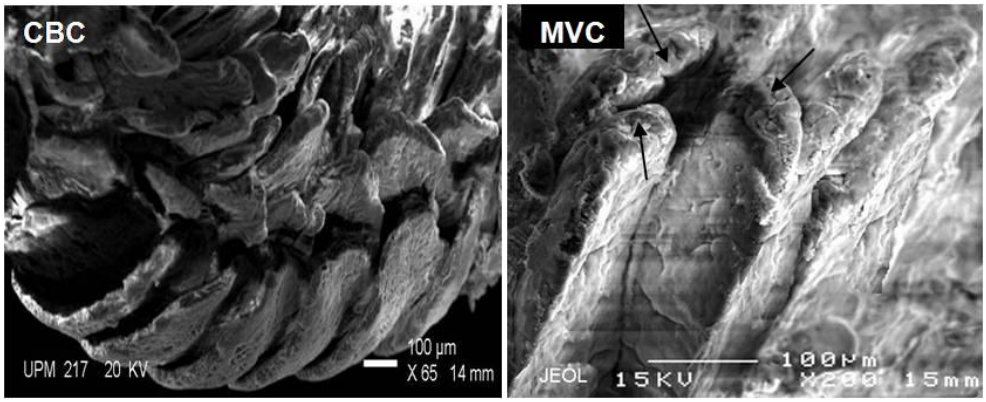

Fig.10: Scanning electron micrographs of the jejunal villi of the CBC and MVC at day 20 post-hatch shows the plate-like villi with wide tips in the CBC, tongue-like villi, thick with wide curled tips (arrows) in the MVC.

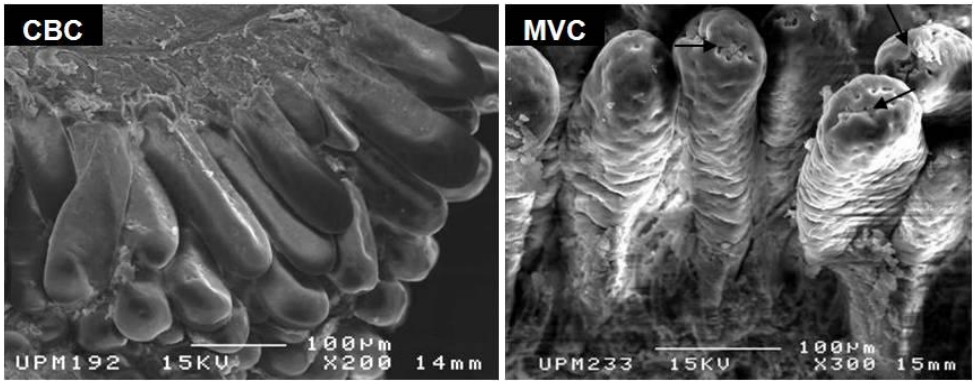

Fig.11: Scanning electron micrographs of the ileal villi of day-old CBC and MVC shows finger-like villi with smooth surface in the CBC, with a marked corrugated surface and areas of discontinuities (arrows) particularly on the villi tips in the MVC.

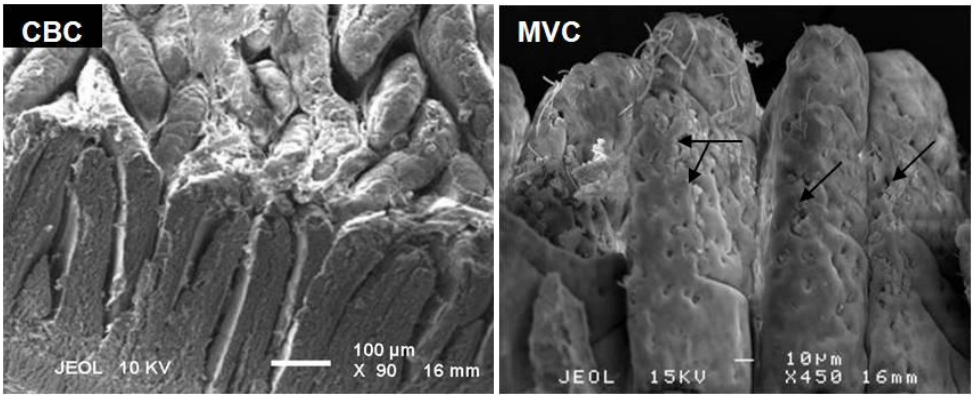

Fig.12: Scanning electron micrographs of the ileal villi of 10 day-old CBC and MVC shows the tongue-like villi in the CBC and finger to tongue-like villi in the MVC. Both breeds show curled villi tips, area of discontinuities on the epithelial surface (arrows) in the MVC.

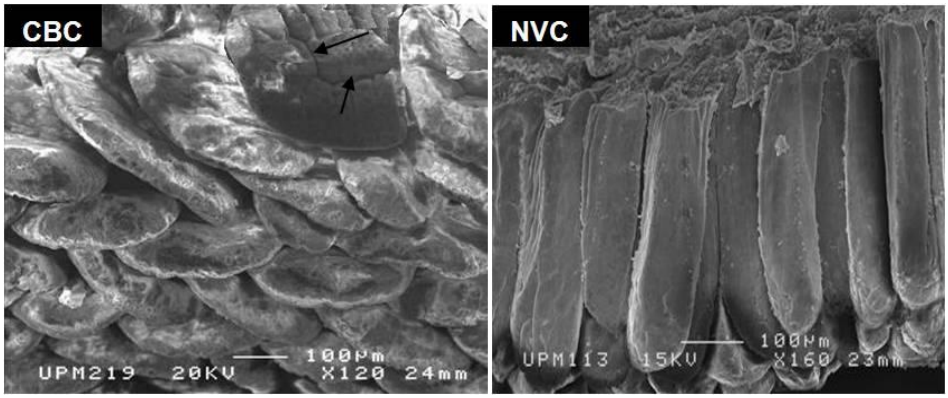

Fig.13: Scanning electron micrographs of the ileal villi of 20 day-old CBC and MVC shows tongue-like villi with marked recesses (arrows) in the CBC, finger-like with a little compress on two sides in the MVC.

DISCUSSION

In our experiment, the interior surface of the intestine of both breeds consisted of villi and lacked folding as seen in large mammals, and this observation is line with that of King and McLelland (1979) and Turk (1982). The results of the present study are also similar to the finding of Gussekloo (2006) who recognized four types of cells that constituted the epithelial mucosa. Through the histological slides, the intestinal villi showed more development in CBC than that of MVC. James *et al*. (1988) and smith *et al*. (1990) reported that in line selected for high growth rate, modification of villus structures occur toward increases in villus size and finally increases the intestinal absorptive surfaces. These in role are related directly to metabolic requirements for rapid body growth (Mayhew & Middleton, 1985).

From this study, the intestinal villi and crypts of day-old chi cks were less developed in MVC than in CBC, especially in the ileum. Similar result was also reported by Uni *et al*. (1999) in poult where the ileum show less development feature than other segments at first day of post-hatch. In rats and mice, crypts develop during the early postnatal period from the flat intervillus epithelium (Mathan *et al.*, 1976; Calvert & Potheir, 1990) but crypts in hatching chicks are not fully defined until 24 h post-hatch (Geyra *et al*., 2001). Smith *et al*. (1990) reported that between the selected breeds for high body weight and unselected one, there are differences in the crypt size of the small intestine, which are also reported in this experiment. Meanwhile, Yamauchi *et al.* (1996) reported that the ileum seems to be inactive in absorptive function in leghorn breed.

Our data are in line with those of Ogiolda *et al.* (1998) who reported that the thickness of the muscularis externa of the intestine is greater in heavy lines than in light lines. Karasova and Diamond (1983) and Watkins *et al*. (2004) explained that

broiler chickens and commercial ducks increases their nutrient transporter capacities by increasing the mass of the entire small intestine as a kind of adaptation to the large volume of food eaten. However, their tunica muscularis and serosa do not seem to be different from those of other birds (Chikilian & De Speroni, 1996; Gussekloo, 2006).

Our observations are similar to those of Rezaian and Hamedi (2007) who showed shorter villi and fewer glands found within the mucous membrane of the middle part of the cecum. In the present study, the cecum body showed a thin wall and poorly developed villi and crypt depth, particularly in MVC. The findings of Kitagawa *et al*. (1996) and Looper and Looper (2005) revealed that the distal two thirds of the cecum undergo regression and involve the atrophy of the epithelium and glands, while the villi never represent any typical appearance.

As for the SEM observation, the results of this study agree well with the findings of Yamauchi and Isshiki (1991) who reported that in a day-old chick, the intestinal villi showed finger-like projections in both heavy and light breeds of chicks. Bayer *et al*. (1975) revealed plate-like villi in day-old broiler chick. The villi, particularly in the duodenum, showed more matured surface epithelial cells in CBC than in MVC even at day 1 of post hatch. These observations seem similar to the findings of Yamauchi *et al.* (1992) and Yamauchi (2002), who showed that at day-old post hatch, the maturation of epithelial cells in light line is involved in the process of cell maturation but epithelial cells of villi in heavy line are almost matured at hatching. The histological alterations related well to the intestinal function at SEM level, represented by characteristic folds and numerous recesses (convoluted surface) (Bayer *et al*., 1975), however, the presence of rough surface, protuberated cells, dome-shaped cells, clear cell outlines, cell exfoliated, extrusion area, and cell clusters were frequently observed (Shamoto *et al*., 1999; Samanya & Yamauchi, 2001; Maneewan & Yamauchi, 2003). Furthermore, the results are also in agreement with the report by Yamauchi and Isshiki (1991) that the broiler breed showed more developed epithelial cell protrusions over the whole apical surface of the intestinal villi. In light breed, the protrusions were not so apparent and located only in the central area of the villus tip. The results of the present study, unlike CBC the villi of MVC at day-old chicks, showed marked corrugated appearance. Bayer *et al.* (1975) demonstrated that during early development stage, the villi show sub-epithelial crevices and discontinuity which is seen among some epithelial cells. The data of the present study, nonetheless, disagreed with the reports of Yamauchi and Isshiki (1991) that the intestinal villi is plate-like at day 10 of post hatch in heavy and light lines breeds. However, as in the finding of Yamauchi (2002) for day 10 broilers, the total proximal part of jejunal villi in the current work showed a complete replacement by deeper cells at the sites of exfoliated cells with unclear cell outline. Meanwhile, the villi of MVC showed cell

activities were observed around the central sulcus.

In our results, no much further development takes place in regards to villi shape at day 20 as compared to day 10 chicks for broiler breed. According to reports of Yamauchi and Isshiki (1991) in heavy and light lines, villi develop a plate-like shape in the duodenum, a wave-like shape in the jejunum and a tongue-like shape in the ileum for day 30 chicks. However, they stated that the fundamental villous shape and arrangement seem to be accomplished by day 10 of post hatch.

CONCLUSION

From the light and SEM observations, it was obvious that the intestinal mucosa of the CBC showed earlier development and more active villi than in MVC. Meanwhile, the intestinal mucosa of the newly hatched chick of the latter breed was in a process of development and maturation. The epithelial cells of the intestinal mucosa in CBC were more active for digestive and absorptive functions and they were also found to have reached morphological maturation earlier and faster than those in MVC, in which these alterations may have a great importance in the short life of the broiler breed associated with rapid growth.

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