Journal of Animal and Veterinary Advances 9 (21): 2729-2737, 2010

ISSN: 1680-5593

© Medwell Journals, 2010

Light and Scanning Electron Microscopy of the Intestine of the Young Red Jungle Fowl (Gallus gallus)

¹Khalid K. Kadhim, ¹A.B.Z. Zuki, ²M.M. Noordin, ²S.M.A. Babjee and ³W. Khamas ¹Department of Veterinary Preclinical Sciences, ²Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia ³College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA 91766, USA

Abstract: Thirty males of Red Jungle Fowl (RJF) were divided into 3 equal groups, euthanized at day 1, 10 and 20 days after hatching. The morphometric analyses were performed at three different magnification levels. Different segments (duodenum, jejunum and ileum) and cecum were weighted and length of each small intestinal segments and cecum were determined at a macroscopic level. The mucosa of the intestinal segments was compared and contrasted using both, light and scanning electron microscopy. The villi height, villi surface area, crypt depth and muscularis externa were measured. The body weight was doubled at 10th day and again doubled at 20th day. The weight and length of intestinal segments were significantly higher throughout the experiment except the constant rate of the duodenal and jejunal lengths after 10th days. Relative to body weight, the organs weight and length were declined after 10th days. The duodenal villi height and surface area were greater than the jejunum followed by the ileum. The muscularis externa and crypt depth increased significantly at 10th day. However, the latter showed retardation thereafter. Day one intestinal villi appeared finger-like shape with zigzag arrangement, tongue-like and leaf-like shaped in 10 and 20th days, respectively. The villi distribution and patterns in the middle region of cecum were characteristic. The epithelial cells of the duodenal villi showed more activities and development than those on the jejunum and the ileum throughout the age. The body growth in RJF progresses very slowly while the relative intestinal segment weight and length failed to follow the body weight after 10th days. The duodenal mucosa shows better developmental features than the jejunum and the ileum.

Key words: Red jungle, intestinal morphology, villus height, surface area, decline, epithelial cell

INTRODUCTION

The wild red jungle fowl (Gallus gallus) is from Southeast Asia. This species was domesticated at least 8000 years ago for cock fighting and divination but its use for food and breeding expand during Roman times (Wood-Gush, 1959). The adult wild RJF characterized by small body weight and slow growth rate.

Many compare-offsprings of animal domesticated with their wild ancestors resulted in artificial selection on anatomical traits (Jackson and Diamond, 1995) in the fowl, (Watkings et al., 2004) and (Gille et al., 1999) in duck. The time is one of the standards used for comparison and has been a commonly accepted method to examine differences in growth (Chambers, 1990).

Other published studies have compared the gut of domestic chicken strains differing in growth rate (Dror *et al.*, 1977; Shires *et al.*, 1987; Mitchell and Smith, 1991; Nitsan *et al.*, 1991; Uni *et al.*, 1995). These studies

hypothesized that the differences in growth rates correlate with changes in the intestinal morphology. Scanning electron microscopy has added a new dimension to the study of intestinal morphology (Bayer *et al.*, 1975; Yamauchi and Isshiki, 1991; Yamauchi *et al.*, 1992, 1996; Uni *et al.*, 1998; Shamoto *et al.*, 1999; Yamauchi and Tarachai, 2000; Samanya and Yamauchi, 2001; Yamauchi, 2002)

These studies included the absorptive surface of the small intestine of chicken at different ages; the villi dimensions, epithelial cell activities, villi surface area and cell surface area. The aim of this study was to determine the growth patterns of each intestinal segment and its morphological structures in RJF at day 1, 10 and 20 days post-hatch.

MATERIALS AND METHODS

Animals: Thirty male RJF were used. The eggs of the RJF were obtained from Jenderam Hulu, Sepang. Eggs were

incubated and hatched within the University Putra Malaysia (UPM) farm. The hatched birds reared in small cages with food and water provided *ad libitum*. Standard commercial starter (0-21 days: CP, 20%) and developer (22-120 days: CP, 15%). Researchers listed eight characteristic features to differentiate between wild RJF and the domestic chicken. The eggs used for this experiment is originally from the stock chosen to have all these features mentioned by Jackson and Diamond (1995).

Collection of tissue samples: Thirty birds were divided into three groups. Ten birds were euthanized by intravenous injection with sodium pentobarbitone (80 mg kg⁻¹) at days 1, 10, 20, post hatching (Mitchell and Smith, 1991). Weight of individual bird was recorded. A mid-line incision was made to expose the body cavities. The entire intestines were removed (duodenum, jejunum, ileum and cecum). The organs were thoroughly washed to remove blood and any other adhering material. The small intestine was then divided into duodenum, jejunum and ileum following Mitchell and Smith (1990) classification. Duodenum commences at the pyloric junction to the ligament of Treitz while the jejunum extends from this ligament to Meckels diverticulum. The remainder of intestine to the ileo-caecal-colonic junction regarded as the ileum. The contents of each segments gently emptied by digital pressure. The weight of each separated segment was recorded and the length of the unstretched intestine was determined.

Light microscope: Samples from each segment were taken for histological study included the midpoint of each parts of intestine (duodenum, jejunum and ileum). Specimens were fixed in 10% Neutral Buffered Formalin (NBF) for 24 h and processed using standard histological procedures. Tubular organs were embedded vertically in paraffin wax (Baddeley *et al.*, 1986). Sections of 3 µm thick were cut and stained with Hematoxyline and Eosin and Masson trichrome (Bancroft and Gamble, 2002).

Measurement of villi surface area and height: The dimensions of intestinal villi (villus height and surface area) were determined until 10 days post hatch following the procedure suggested by Iji et al. (2001). For the 20 days specimens, ten intact villi were selected from the midpoint of each intestinal segment under dissection microscope (KRUSS OOTRONIC, Germany) using fine hypodermic needles to separate the villi. Specimens were mounted on microscopic slides one drop of saline added to prevent dehydration and conformation of these villi. After that villi were flattened by the use of a coverslip and the edges of the coverslip were sealed with DPX. The villi surface areas were finally doubled, suppose each villus

have two equal surfaces (Smith *et al.*, 1990). The depth of individual crypt and the thickness of muscularis externa were measured using the regular microscopic slide. All the Slides viewed and measured using Olympus image analysis (BX 51 TF) with attached camera CC12.

Scanning electron microscope: About 1 mm slice of tissue from the middle portion of the duodenum, jejunum, ileum and cecum were fixed in 4% gluteraldyhyde. Tissue samples for SEM were processed as described previously (Yamauchi *et al.*, 1990; Maneewan and Yamauchi, 2003). The lumen was cut open under the dissection microscope to select the right orientation before mounting on the stud. 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 mL, 7 mA for 3 min before being examined with a Jeol-SEM (JSM-6400, Japan) at 8 kv.

Data analysis: All data collected were subjected to analysis of variance among the groups using one way ANOVA. Significant differences between mean values were considered at p≤0.05. The comparison done among the same intestinal segments of subsequent age groups and among the different intestinal segment in the same age.

RESULTS AND DISCUSSION

Macroscopic finding: The total body weight increased two folds from 1-10 days old and more than two folds in 20 days (Fig. 1a). The difference was statistically significant between mean values at p≤0.05. The absolute weight and length of each intestinal segment was significantly increased in both day 10 and 20. They increased 6 times in 10 days while it was <1 time at 20th day old chicks.

The relative weight of intestinal segments increased significantly at 10th days except the cecum (Fig. 1c). Thereafter, the data decreased at 20th day. While the cecum relative weight was constant throughout the experiment. The duodenum and jejunal length were stable after day 10 (Fig. 1b and d). The absolute intestinal segments lengths of the duodenum and jejunum showed no differences after 10 days while the length of ileum and cecum were persistently increased. The relative length of all intestinal segments in Fig. 1e showed no differences in 10 days except the cecum. However, the data were significantly decreased thereafter. While the relative cecum length was decreased throughout the experiment.

Microscopic findings: All the experimental birds had highest villi in duodenum and decreased in height toward



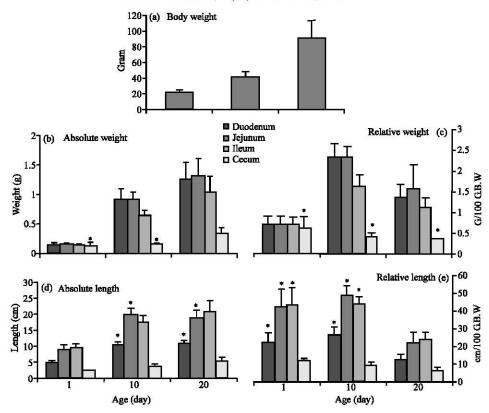


Fig. 1: Showing; a) body weight, b) absolute, c) relative intestinal segments weight (g organ/100 g) body weight, d) absolute and e) relative intestinal segments length (cm organ/100 g) body weight. At day 1st, 10th and 20th days post-hatch. The data represented as mean±SD. N = 10. *Not significant differences (p>0.05) among the same segments

the ileum (Fig. 2a). The duodenal villi height increased greatly (about two folds) at day 10, unlike the jejunum and ileum. The increases in the villus height for each intestinal segment were significantly high when compared (p≤0.05) throughout the age groups (Table 1). The villi surface areas of intestinal segments are shown in Table 1. There were statistical significant difference for each segment with the age, except the ileum when there were no differences during the first 10 days.

The data can be presented from the greatest to the lowest starting with the duodenum, jejunum and ileum (Fig. 2b). The data related to the intestinal crypt depth is shown in Table 1 showed significant increases for each segments at 10th day while after that it was significantly decreased in jejunum distributed over and between the folds and ileum and remained within the constant rate in duodenum. The duodenal crypt depth at 1 day old was significantly higher than other segments.

The jejunum and ileum at 10th day had the greatest values but they slow down again at 20th day (Fig. 2c). The tunica muscularis externa of each intestinal segment

Table 1: Micromorphometric measurement of the small intestine segments at 1st, 10th and 20th day post-hatch

Measurements	1st day	10th day	20th day
Duodenum			
Villus height (µm)	525±28°	929±81 ^b	1153±52°
Villus surface area (mm²)	0.17 ± 0.01^a	0.48 ± 0.05^{b}	$0.71\pm0.11^{\circ}$
Crypt depth (µm)	62±5ª	108±13 ^b	103±12 ^b
Muscularis externa (µm)	44±3ª	108±5 ^b	111±40°
Jejunum			
Villus height (µm)	326±32a	406±376	571±51°
Villus surface area (mm²)	0.04 ± 0.006^a	0.12 ± 0.01^{b}	$0.36\pm0.02^{\circ}$
Crypt depth (µm)	37±5ª	121±18 ^b	93±14°
Muscularis externa (µm)	44±4ª	67±16 ^b	65±23°
Ileum			
Villus height (µm)	239±15°	361±32b	405±9°
Villus surface area (mm²)	0.06 ± 0.23^a	0.07±0.01a	0.09±0.007°
Crypt depth (µm)	54±7ª	119±19 ⁶	82±14°
Muscularis externa (µm)	70±8°	111±31 ^b	126±18 ^b

Height of ten intact villi from each segment, expressed in μm , Villus surface area of ten intact villi from each segment expressed in mm^2 , Crypt depth and total thickness of muscularis externa, expressed in μm , Measurements were obtained by image analysis, Values are presented asN means±standard, deviation (n = 10). The different superscripts within, the same row, indicate significant differences at p<0.05

were shown in Table 1. The thickness of this layer increased significantly at 10th day for all segments compared with 1-day but showed no difference after that

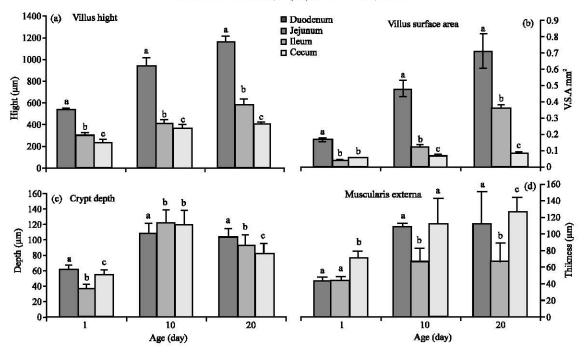


Fig. 2: Showing; a) villus height (μm), b) villus surface area (cm²), c) crypt depth (μm), d) mascularis externa (μm) of duodenum, jejunum and ileum, at 1st, 10th, 20th day post-hatch. The data represented as mean±SD. N = 10. The different superscripts within same age, indicate significant differences at p<0.05</p>

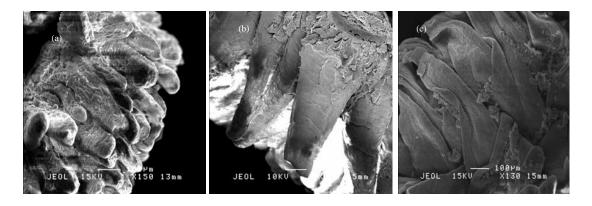


Fig. 3: SEM of duodenal villi. a) day 1, finger-shaped with transverse recesses. b) day 10, broad finger-like villi with little compressed sided, the recesses on the surface as seen clearly and c) day 20, leaf-like shape duodenal villi, the tips were slightly curved above each others

with the exception of the jejunum which were decreased. The ileum has thicker tunica muscularis externa when compared to other segments at 20th day (Fig. 2d).

Scanning electron microscopic observation: The shape of the duodenal villi at day one post hatch showed finger-like projections (Fig. 3).

The side-appearance of the villi surface showed transverse recesses with wide base, triangular on sideview. Discontinuity of epithelial surface, disruption and few cells with clear outline were observed and restricted to the tips of the villi. Goblet cells with their openings can be seen between the epithelial cells at 1 day post hatch chicks. At 10th day, the duodenal villi still have broad finger-like shape with a slightly compressed laterally and recesses on the surface may be seen clearly (Fig. 3b), curled tips and dome-shaped cells with protuberances were observed (Fig. 4 and 5a). At 20th day, the tips of leaf-like shape duodenal villi were slightly curved above each others. Exfoliated cells can be

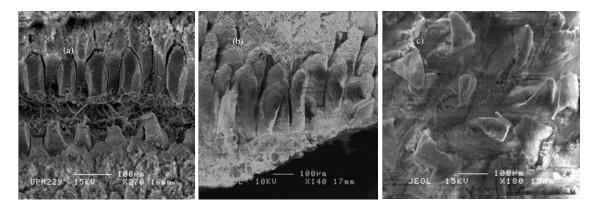


Fig. 4: SEM of jejunal villi. a) day 1, finger-shaped, b) day 10, broad finger-shaped to tongue-shaped villi and wide corrugated tips and c) day 20, the narrow tips of leaf-like shape duodenal villi were completely exfoliated

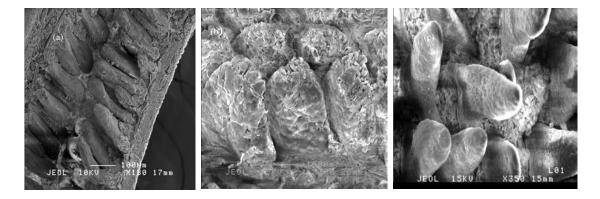


Fig. 5: SEM of ileum villi. a) day 1, finger-shaped with corrugate surface, b) day 10, the tongue-shaped villi and wide corrugated tips and c) day 20, foliate to tongue shaped villi, the proximal parts of villi appeared with epithelial cells which recently replaced the exfoliated cells

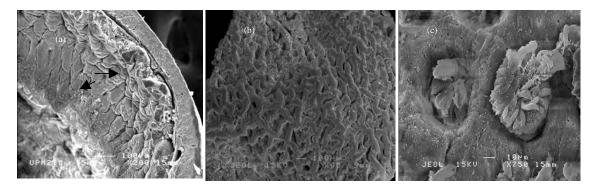


Fig. 6: SEM of mucosal surface of the Cecum. a) day 1 showed folds and lacks organized villi, b) day 10, folds and the crypt among it but lacks of villi and c) day 20 showed of developing villi (arrows) distributed over and between the folds

observed on the tips of some villi. The jejunal villi at 1st day, appeared finger-like shape with markedly curled surface (Fig. 6a). The epithelial surface showed discontinuity especially in the proximal part of villi when

viewed by high power magnification (Fig. 4b). At 10th day old chicks, the tongue-shaped jejunal villi showed recesses and wide curled tips with cell activities which represented by dome-shaped cells with protuberances

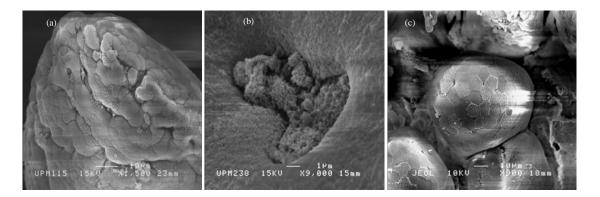


Fig. 7: SEM of a) tip of duodenum villi at day 1 post-hatch showed the wide corrugated tips, dome-shaped cells with protuberances, b) the jejunal villi at day 1 post-hatch showed discontinuity of epithelial surface and c) tip of ileal villi at day 1 post-hatch showed the clear outline of individual cells

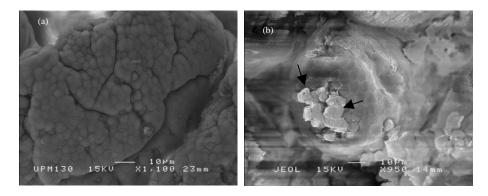


Fig. 8: SEM of a) cell cluster of epithelial cells in the tip of 10 day duodenal villi and b) ileal villi at day 10 post-hatch, exfoliated cells in the tip of villi (arrows)

were observed around a central sulcus. Some of the villi tips were showed extrusion area and replaced by new cells. However, these area were commonly observed at 20th day (Fig. 4c). The ileal villi (Fig. 7) at 1 day old chick appear to be similar to the jejunal villi of the same age but it was shorter. In addition, much smaller villi were observed among the long villi. The villi tips showed area of cells protuberances and epithelial crevices with relatively long microvilli. The epithelial cells of some villi showed clear penta or hexagonal outlines demarcation (Fig. 4c) or with clear crevices and discontinuity appeared for the others. At 10th day, the epithelial surface of the tongue-shaped ileal villi were showed very clear crevices and discontinuity. At the same time, the cell activities and exfoliated cells on the wide tips of the villi were commonly observed (Fig. 5b). At 20th day old chicks, the ileal villi were foliate to tongue shaped. The proximal parts of the villi, appeared with epithelial cells which recently replaced the exfoliated cells with no clear outline demarcation but the opening of the goblet cells were observed clearly.

The intestinal villi at 1st day showed zigzag appearance was very clear at 10th and 20th day post hatch. The epithelial surface of the cecum at 1 day old chicks lacks organized villi and has only folds and lack raised ridges (Fig. 8a). The developments were observed at 10 day which were represented by increasing the heights of villar ridges/folds, appearance of crypt area between the folds (Fig. 8b). The developments which observed at 10 day old chicks, represented by increasing the height of the folds and clear outline demarcation of the epithelial cells which covered by long microvilli in addition to the characteristic appearance of the short crypts opening among the folds.

At 20th day post-hatch, the mid segment of the cecum characterized by presence of developing villi distributed over and among the folds (Fig. 6c). The villi shape was very short cylindrical, however the shortest one appeared under the level of surface epithelia. The epithelial cells of these villi were very clear recognized with characteristic arrangements as long columnar cells, loosely attached to each other. The total body weight of the RJF increased

about four folds from 1-20 days old. While the body weight of the broilers breed increase >20 folds at this times (Smith et al., 1990) and this reflect the effects of selection for high body weight. The intestinal segments increased in weights very rapidly than the whole body weight at first 10 days. post-hatch. The absolute and relative duodenal weight markedly increased during the first 10 days post-hatch. Therefore, the development of the duodenum did not follow the slow body growth changes at this time. Uni et al. (1995) stated that the duodenum showed earlier rapid growth than either the jejunum or ileum. The marked increases in absolute weight and length of small intestine accompanying with increased growth rates are consistent with the concept of supplied organs which sustain the availability of nutrients to the demand organs as proposed by Nir et al. (1978) and Lilja (1983).

According to the finding of Sell *et al.* (1991), the weight of small intestine increased more rapidly than body weight until 6 days after hatching, after that the growth seemed to be parallel with body weight. While the relationship reversed, the relative intestinal weight declined at this time (Nitsan *et al.*, 1991), the data seemed similar to the latter however, the relative intestinal segments weight were decreased after 10 days.

The result of this study related to intestinal length agreed with the finding of Mitjans *et al.* (1997) in leghorn chickens that the length of each segment increased more than two folds between 1 day post-hatch. The ileum and cecum continue to increase in their lengths.

The increased in relative segments length were failed to follow the increase of the body weight after 10 days which was reported by Siegel and Dunnington (1987) and Shires *et al.* (1987) in light breed. In this study, increased rates in the intestinal weight were persisted despite the fact of their unchanged length between 10 and 20 day for duodenum and jejunum. These changes in weight may be attributed to the increased in the two main tunics mucosa and muscularis. However, a short initial increase of relative organ weights was reported for all digestive organs for chickens (Dror *et al.*, 1977; Gille *et al.*, 1999) and in turkeys (Sell *et al.*, 1991).

The intestinal function might be determined by measuring, the villus height, villus surface area using light microscopy in addition to the morphological observations of the villus surface using scanning electron microscopy. Maneewan and Yamauchi (2003) suggested that the villus surface area, villus height and cell luminal area would be suitable parameters to assess villus function. After 10 days of age and due to the villi height which became

longer, folded and overlap each other's. These changes made it difficult to measure the dimensions of the villi by routine histological methods. Therefore, other procedure was selected for this purpose. It has been assumed that each villus consisted of two flat sheets bent to meet each other only in the region of the villus perimeter (Smith et al., 1990). In this study, the villus height of all intestinal segments increased with age. Furthermore, the duodenal villi height was greater than jejunal villi and this in turn, greater than the ileum. These results agree with the finding of Yamauchi (2002). According to the finding of Uni et al. (1998), the jujenal villi heights of broiler became greater than duodenal villi after 10 day and the ileal villi was lower throughout the small intestine. Smith et al. (1990) has attributed the smaller ileal villus to the lowest luminal concentration of nutrients that reach this intestinal segment. The villus surface area showed that the same growth pattern of villus height for all segments except the jejunum because the villus surface area depends greatly on the value of villus height more than the villus width. The width of the villi increased slightly thus, the growth in surface area tends to mirror the change in villus height (Sklan, 2001).

Furthermore, the villus height and villus surface area may be affected by the same factors (Yamauchi and Tarachai, 2000). Data collected in this study showed increased of villus surface area in jejunum which was not commensurate with the increased in villus height. These can be attributed to the increased villi width. These results are in agreement with Levin and Mitchell (1984) who have described in the broiler breed chicken, higher values of villus surface area in the jejunum than in the ileum. On the other hand, according to the finding of Watkings et al. (2004), the villus surface area of jejunum and ileum were greater than in the duodenum in duck. The crypt depth is a site of proliferating of the enterocytes along the villus thus, the crypt depth greatly increased in duodenum and lowest in the ileum which seemed differ from the findings of Uni et al. (1998) and Smith et al. (1990). These researchers have reported that the duodenal crypt depth decreased at 10 day old in broiler chicks earlier than other segments because the villus height and the villus surface area reach its maximum value earlier in the duodenum than in other segments of the intestine. The data related to the thickness of muscularis externa showed greater values in ileum, than in other segments. According to the finding of Mitchell and Smith (1991), this tunic is thicker in the jejunum and the ileum when compared with the duodenum in light breed. Lidia et al. (1998) mentioned that the tunica muscularis of the jejunum was greater than the ileum of the rat. Nevertheless, it is evident that the increased thickness of the muscle layer directed toward the distal parts of the gut where the strong force needed to push the high-dayensity intestinal contents through these regions. The results of SEM observation for the intestinal villi at 1 day showed finger like projections. Bayer et al. (1975) revealed plate-like villi at 1 day broiler chick. These are finger-like in shape in both heavy and light breeds of chicks (Yamauchi and Isshiki, 1991). The maturation features of villi surface represented by the presence of characteristic folds and numerous recesses (convoluted surface) (Bayer et al., 1975) with many active villi by the rough surface and protuberated cells (Shamoto et al., 1999). The intestinal histological alterations related to intestinal function at SEM level, represented by the dome-shaped cells, clear cell outlines, cell exfoliated, extrusion area and cell clusters were frequently observed (Samanya and Yamauchi, 2001; Maneewan and Yamauchi, 2003). The developing villi of the cecal body at 20 day RJF showed characteristic shape which it was differ in its design and arrangement than the intestinal villi however, Bemrick and Hammer (1978) reported that the villi patterns in the middle region of cecum in domestic fowl have collars and rugae.

CONCLUSION

If consider these facts, the results of this study showed that the epithelial cells of intestinal segments in RJF were less developed than the broiler breeds at the three selected age groups. Furthermore, the discontinuity and disruption in the epithelium were very clear with curl surface appearance of the villi especially in jejunum and ileum at 1 day of age.

The finding of Yamauchi *et al.* (1992) and Yamauchi (2002) confirmed this in light and heavy breeds. Whereas, according to the results of Yamauchi *et al.* (1996) the ileum seems to be inactive in absorptive function in leghorn breed.

REFERENCES

- Baddeley, A.J., H.J.G. Gundersen and L.M.C. Orice, 1986. Estimation of surface area from vertical sections. J. Microscopy, 142: 259-276.
- Bancroft, J.D. and M. Gamble, 2002. Theory and Practice of Histological Techniques. 5th Edn., Churchill Livingstone, London, New York and Philadelphia.
- Bayer, R.C., C.B. Chawan, F.H. Bird and S.D. Musgrave, 1975. Characteristics of the absorptive surface of the small intestine of the chicken from 1 day to 14 weeks of age. Poult. Sci., 54: 155-169.

- Bemrick, W.J. and R.F. Hammer, 1978. Scanning electro-microscopy of damage to the cecal mucosae of chickens infected with *Eimeria tenella*. Avian Dis., 22: 86-94.
- Chambers, J.P., 1990. Genetics of Growth and Meat Production in Chickens. In: Poultry Breeding and Genetics, Crawford, R.D. (Ed.). Elsevier Scientific Publishers, New York, pp. 1123.
- Dror, Y., I. Nir and Z. Nitsan, 1977. The relative growth of internal organs in light and heavy breeds. Br. Poult. Sci., 18: 493-496.
- Gille, U., F.V. Salomon and J. Ronnert, 1999. Growth of the digestive organs in ducks with considerations on their growth in birds in general. Br. Poult. Sci., 40: 194-202.
- Iji, P.A., A. Saki and D.R. Tivey, 2001. Body and intestinal growth of broiler chicks on a commercial starter diet.1. Intestinal weight and mucosal development. Br. Poult. Sci., 42: 505-513.
- Jackson, S. and J. Diamond, 1995. Metabolic and digestive responses to artificial selection in chickens. Evolution, 50: 1638-1650.
- Levin, R.J. and M.A. Mitchell, 1984. Problems involved in correlating changes of functional diffusive and anatomical surface areas of the upper and lower chicken small intestine during fasting. Br. Poult. Sci., 25: 27-31.
- Lidia, O., W. Rudiger, R. Oswald, H. Walter and W. Eckhard, 1998. Intestinal dimensions of mice divergently selected for body weight. Anat. Rec., 250: 292-299.
- Lilja, C., 1983. A comparative study of postnatal growth and organ development in some species of birds. Growth, 47: 317-339.
- Maneewan, B. and K. Yamauchi, 2003. Effects of semipurified pellet diet on the chicken intestinal villus histology. Poult. Sci., 40: 254-266.
- Mitchell, M.A. and M.W. Smith, 1990. Jejunal alanine uptake and structural adaptation in response to genetic selection for growth rate in the domestic fowl (*Gallus domesticus*). J. Physiol., 424: 7-15.
- Mitchell, M.A. and M.W. Smith, 1991. The effects of genetic selection for increased growth rate on mucosal and muscle weights in the different regions of the small intestine of the domestic fowl (*Gallus domesticus*). Comp. Biochem. Physiol., 99: 251-258.
- Mitjans, M., G. Barniol and R. Ferrer, 1997. Mucosal surface area in chicken small intestine during development. Cell Tissue Res., 290: 71-78.
- Nir, I., Z. Nitsan, Y. Dror and N. Shapira, 1978. Influence of overfeeding on growth, obesity and intestinal tract in young chicks of light and heavy breeds. Br. J. Nutr., 39: 27-35.

- Nitsan, Z.G., B. Avraham, Z. Zoref and I. Nir, 1991. Organ growth and digestive enzymes levels to fifteen days of age in lines of chickens differing in body weight. Poult. Sci., 70: 2040-2048.
- Samanya, M. and K. Yamauchi, 2001. Morphological changes of the intestinal villi in chickens fed the dietary charcoal powder including wood vinegar compounds. Poult. Sci., 38: 289-301.
- Sell, J.L., C.R. Angel, F. Piquer, J. Mallarino and H.A. Al-Batshan, 1991. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poult. Sci., 70: 1200-1205.
- Shamoto, K., K. Yamauchi and H. Kamisoyama, 1999. Morphological alterations of the duodenal villi in chicks refed rice bran or grower mash after fasting. Jap. Poult. Sci., 36: 38-46.
- Shires, A., J.R. Thompson, B.V. Turner, P.M. Kennedy and Y.K. Goh, 1987. Rate of passage of corn-canola meal and corn-soybean diets through the gastrointestinal tract of broiler and white leghorn chickens. Poult. Sci., 66: 289-298.
- Siegel, P.B. and E.A. Dunnington, 1987. Selection for growth in chickens. World Poult. Sci., 1: 1-24.
- Sklan, D., 2001. Development of the digestive tract of poultry. World Poult. Sci. J., 57: 415-428.
- Smith, M.W., M.A. Mitchell and M.A. Peacock, 1990. Effects of genetic selection on growth rate and intestinal structure in the domestic fowl (*Gallus domesticus*). Comp. Biochem. Physiol. A, 97: 57-63.
- Uni, Z., S. Ganot and D. Sklan, 1998. Posthatch development of mucosal function in the broiler small intestine. Poult. Sci., 77: 75-82.

- Uni, Z., Y. Noy and D. Sklan, 1995. Posthatch changes in morphology and function of the small intestines in heavy and light strain chicks. Poult. Sci., 74: 1622-1629.
- Watkings, E.J., P.J. Butler and B.P. Kenyon, 2004. Posthatch growth of the digestive system in wild and domesticated ducks. Br. Poult. Sci., 45: 331-341.
- Wood-Gush, D.G., 1959. A history of the domestic chicken from antiquity to the 19th century. Poult. Sci., 38: 321-326.
- Yamauchi, K. and P. Tarachai, 2000. Changes in intestinal villi, cell area and intracellular autophagic vacuoles related to intestinal function in chickens. Br. Poult. Sci., 41: 416-423.
- Yamauchi, K. and Y. Isshiki, 1991. Scanning electron microscopic observations on the intestinal villi in growing white leghorn and broiler chickens from 1 to 30 days of age. Br. Poult. Sci., 32: 67-78.
- Yamauchi, K., 2002. Review on chicken intestinal villus histological alterations related with intestinal function. Poult. Sci., 39: 229-242.
- Yamauchi, K., H. Kamisoyama and Y. Isshiki, 1996. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in white leghorn hens. Br. Poult. Sci., 37: 909-921.
- Yamauchi, K., S. Iida and Y. Isshiki, 1992. Post-hatching developmental changes in the ultra-structure of the duodenal absorptive epithelia cells in 1, 10 and 60 day-old chickens, with special reference to mitochondria. Br. Poult. Sci., 33: 486-487.
- Yamauchi, K., Y. Isshiki, Z.X. Zhou and Y. Nagahiro, 1990. Scanning and transmission electron microscopic observations of bacteria adhering to ileal epithelial cells in growing broiler and White Leghorn chickens. Br. Poult. Sci., 31: 129-137.