The Histomorphometry of The Small Intestine on Turkey (Meleagris gallopavo) at Different Age Levels

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Abstract
This study aims to determine the histomorphometry of the small intestine of turkeys (Meleagris gallopavo) at different age levels. This study used a completely randomized design. This study used 18 male turkeys, divided into 3 groups of ages 8, 16, and 24 weeks, each group comprising 6 turkeys. The necropsied turkey's small intestine was taken into histological preparations and stained with haematoxylin-eosin (HE). The results showed that the histological picture of the small intestine layer of turkeys at different age levels consisted of four layers: tunica mucosa, tunica submucosa, tunica muscular, and tunica serosa. Histomorphometrically, there was an increase in the thickness of all layers of the small intestine from 8 and 16 weeks of age (P<0.05), and the height of the intestinal villi increased in all parts of the small intestine at 8, 16 and 24 weeks of age (P<0.05). In conclusion, with histomorphometric measurements, it can be seen that there are differences in the three age groups.

Keywords: turkey, small intestine, histomorphometry, hematoxylin-eosin

Background
The turkey (Meleagris gallopavo) is a type of fowl from Meleagridae originating from North America, which was domesticated in Europe, and is an important food source in many parts of the world (Famous et al., 2019). The growth pattern of turkeys consists of three stages: starter 0-8 weeks, grower 8-16 weeks, and finisher 16 weeks for female turkeys and 24 weeks for male turkeys. During the grower period, the increase in turkey growth increased quite sharply, whereas during the finisher period, male turkeys did not significantly increase their body weight (Prayitno et al., 2016). The different body weight growth is closely related to the digestion, absorption, and assimilation of nutrients in the intestine and the length and thickness of each layer that compose it (Wilson et al., 2018).

Histologically, the intestinal wall's morphological thickness impacts a more efficient feed digestion process and nutrient absorption. In addition to aiding in the absorption of nutrients, the small intestine acts as an internal primary line of defense against harmful microbes, toxins, and antigens (Firmansyah et al., 2019). The enterocyte cells, goblet cells, enteroendocrine cells, and Paneth cells are among the cells that make up the tunica mucosa's epithelial lamina (Khadim et al., 2012). This statement is related to Purnata et al., (2018) that the length of the villi in the small intestine is related to the ability of the small intestine to absorb food nutrients. According to Wilson et al. (2018), the length...
and width of the villi increase significantly with age.

Currently, research on the histomorphometric appearance of the small intestine of turkeys (*Meleagris gallopavo*) is very limited. Based on this background, it is necessary to conduct a study on the histomorphometric and histochemical features of the small intestine of turkeys (*Meleagris gallopavo*) at different age levels.

**Materials and method**

This research was conducted at the Laboratory of Histology and Laboratory of Research of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, since November 2020 to January 2021. This study used small intestine samples from 18 male turkeys (*Meleagris gallopavo*) obtained from turkey farms in Medan City at 4 weeks old. The turkeys were fed at the Laboratory of Experimental Animals of the Faculty of Veterinary Medicine, Universitas Syiah Kuala. Turkeys were divided into three age groups, namely 8, 16, and 24 weeks consisting of six male turkeys in each group. The number of samples was calculated based on Federer's formula (1974), cited by Kusriningrum (2010). Sampling was done by euthanizing animals to take part of their small intestine as a research sample. The Veterinary Ethics Committee of the Faculty of Veterinary Medicine Universitas Syiah Kuala has approved the ethical clearance of this research investigation.

This laboratory analysis research uses a completely randomized design. Making histological preparations using small intestine samples collected and then HE staining was carried out by adopting the Kiernan 1990 method.

**The Staining Results Observation**

The staining results were observed using a light microscope (Olympus CX31) with a magnification of 100 times and 400 times. Micrograph photos were taken with the Touview program software. Hasanzadeh and Monazzah (2011) suggested that histomorphometric measurements can be performed by measuring the thickness of the tunica serosa, tunica muscular, tunica submucosa, tunica mucosa, and the length and thickness of the villi mucosa, in the tunica mucosa measured from a single layer of columnar epithelium, lamina propria, and mucosal lamina muscular. The tunica submucosa is measured from what is under the tunica mucosa, which consists of blood vessels, cells, connective tissue fibres, and nerves. The tunica muscular is measured from under the tunica submucosa, namely the two layers of smooth muscle. Tunica serosa is a layer that exist on the visceral organs, mesothelium, and thin tissues (Bacha and Bacha, 2012).

**Data analysis**

Data from histomorphometric measurements of the small intestine layer of turkey hens at various ages were analyzed using analysis of variance (ANOVA) and continued with Duncan's test.

**Results and Discussion**

**Turkey Small Intestine Histomorphometry**

Turkey Small Intestine Layer

Measurements results on the lining of the small intestine of turkeys at different age levels are presented in Table 1. Based on Table 1, the mean ± SD of the small intestine layer of turkeys in the age group of 8, 16, and 24 weeks consisted of the tunica mucosa, tunica submucosa, tunica muscular, and tunica serosa. According to the results of statistical tests, the thickness of the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa in the duodenum, jejunum, and ileum of turkeys increased in each age group. This result is related to the report by Rana et al. (2016), who suggested that the digestive tract undergoes rapid structural changes after hatching with age. The increase in the thickness of the tunica mucosa at various age levels is thought to be due to an increase in the number of intestinal glands. The intestinal glands are in the form of Liberkuhn's crypts which will continue to grow until the turkey reaches a certain age. The intensity of crypt growth in the duodenum, jejunum, and ileum increases...
more rapidly during periods of intensive turkey growth (Yovchev et al., 2019). The number of Liberkuhn crypts in each bird has differences which are influenced by the type of feed and softening process (Zainuddin et al., 2016). The increase in the tunica mucosa of the small intestine of turkeys in the duodenum and jejunum was significantly different at 8-16 weeks of age compared to 16-24 weeks of age. It is suspected that at 24 weeks of age, the male turkey is already at the finisher stage, so the small intestine has reached its optimum development stage.

Table 1. The histomorphometric mean of the lining of the small turkey intestine (µm)

<table>
<thead>
<tr>
<th>Intestine tenue</th>
<th>Group of ages</th>
<th>Week 8th</th>
<th>Week 16th</th>
<th>Week 24th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMA</td>
<td>1281.46±58.12a</td>
<td>1561.27±30.68b</td>
<td>1602.56±14.23b</td>
<td></td>
</tr>
<tr>
<td>TSM</td>
<td>33.77±9.13a</td>
<td>50.33±8.83b</td>
<td>54.57±3.46b</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>234.14±10.34a</td>
<td>289.87±8.9b</td>
<td>302.52±11.13c</td>
<td></td>
</tr>
<tr>
<td>TSA</td>
<td>83.29±4.10a</td>
<td>104.53±2.70b</td>
<td>112.58±8.70c</td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMA</td>
<td>1017.33±104.23a</td>
<td>1209.11±37.88b</td>
<td>1294.03±58.75b</td>
<td></td>
</tr>
<tr>
<td>TSM</td>
<td>29.51±4.50a</td>
<td>42.09±8.29b</td>
<td>44.44±4.47b</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>212.30±9.79a</td>
<td>269.08±5.57b</td>
<td>297.02±17.28c</td>
<td></td>
</tr>
<tr>
<td>TSA</td>
<td>62.36±4.42a</td>
<td>87.55±7.13b</td>
<td>95.37±3.62c</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMA</td>
<td>835.03±37.11a</td>
<td>1028.20±26.39b</td>
<td>1118.70±67.13c</td>
<td></td>
</tr>
<tr>
<td>TSM</td>
<td>24.92±3.14a</td>
<td>35.11±1.57b</td>
<td>39.08±4.20c</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>136.44±8.32a</td>
<td>199.53±10.66b</td>
<td>220.85±10.03c</td>
<td></td>
</tr>
<tr>
<td>TSA</td>
<td>50.80±6.1a</td>
<td>76.35±4.13b</td>
<td>80.28±7.32b</td>
<td></td>
</tr>
</tbody>
</table>

abcDifferent superscripts in the same column show significant differences (P<0.05). Tunica mucosa (TMA), tunica submucosa (TSM), tunica muscularis (TMS), and tunica serosa (TSA).

The tunica muscular of the small intestine of turkeys in the duodenum, jejunum, and ileum at various ages has increased in thickness. The thick muscular wall allows more mixing or mechanical digestion of the feed and speeds up digestion, thus minimizing the digestive load (Godwin et al., 2016). Based on observations of the tunica muscular of the duodenum, jejunum, and ileum, an increase was significantly different in each age group (P<0.05). This result is presumably because muscle bundles are added in the circular and longitudinal muscles during the turkeys' growth period. Muscle bundles have experienced development during the incubation period, as revealed by Halawa (2013), that there is cell growth and development during the incubation period. The results of the tunica serosa
measurements at various ages of the turkey small intestine showed significantly different increases (P<0.05). This result happens because of an increase in the number of cells and the thicknesses. In addition, there is also an increase in the size of blood vessels and fat cells in the tunica serosa.

Turkey Small Intestinal Villi

In addition of the turkey’s small intestine layer, histomorphometry was also performed on the height and width of the villi, as reported by Hasanzadeh and Monazzah (2011) and Yovchev et al. (2019). The results of measuring the height and width of the villi of the small intestine of turkeys at various ages can be seen in Table 2 and Table 3.

Table 2. Average histomorphometric height of turkey small intestine villi (µm)

<table>
<thead>
<tr>
<th>Intestine tenue</th>
<th>Group of ages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 8th</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1098.15±49.34a</td>
</tr>
<tr>
<td>Jejunum</td>
<td>859.82±29.30a</td>
</tr>
<tr>
<td>Ileum</td>
<td>659.49±21.76a</td>
</tr>
</tbody>
</table>

abc Different superscripts in the same column show significant differences (P<0.05).

Table 3. Histomorphometric mean villi width of turkey small intestine (µm)

<table>
<thead>
<tr>
<th>Intestine tenue</th>
<th>Group of ages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 8th</td>
</tr>
<tr>
<td>Duodenum</td>
<td>191.88±32.21a</td>
</tr>
<tr>
<td>Jejunum</td>
<td>185.48±46.11a</td>
</tr>
<tr>
<td>Ileum</td>
<td>168.98±57.22a</td>
</tr>
</tbody>
</table>

abc Different superscripts in the same column show significant differences (P<0.05).

Table 2 shows the mean ± SD villi height of the small intestine of turkeys at various age levels. Statistical tests show that the height of the small intestinal villi from the duodenum, jejunum, and ileum experienced significantly different increases at different age levels (P<0.05). In Table 3, the width of the duodenal villi at 8-16 weeks has increased. Based on statistical tests, it has increased significantly (P <0.05). At the age of 16 weeks, there was an increase in the width of the villi in the duodenum compared to the age of 8 weeks. This result is in line with the research of Yovchev et al. (2019) that the height of the villi at the age of 56 days in the duodenum, jejunum and ileum was 1417.71 µm; 976.54 µm; and 800.12 µm. However, the height of turkey small intestinal villi in the duodenum at 16 weeks was higher than native chickens at 20 weeks of age, namely 863.03 ± 367.95 µm (Lisnahan et al., 2019). Differences influence the difference in villi height in poultry species, feed, and environment. The difference in villi height will affect the extent of absorption in the small intestine. Laudadio et al. (2012) suggested that maximum digestive tract absorption greatly affects chickens' growth and reproduction. Digestion of feed and absorption of nutrients is more in the gut with a thicker
Villi in the small intestine gradually decrease in size from the duodenum to the ileum (Zaher et al., 2012). Based on the villi measurements, the duodenum villi is higher than the jejunum and ileum. These results are related to the previous research reported by Bacha and Bacha (2012), Zaher et al. (2012) and Hamdi et al. (2013) that the villi in the duodenum are higher than those in the jejunum and ileum. Contrary to the findings of Godwin et al. (2016), the highest villi were found in the jejunum, followed by the duodenum and the fewest in the ileum. Such variations may be due to differences in phylogenetic signals. Villi in the duodenum are higher related to the function of the digestive tract. Apriyani et al. (2016) stated that the absorption process in the duodenum is optimized by having villi that protrude along the surface of the lumen.

The height of the intestinal villi in the duodenum increased 3.4 times, while those in the jejunum and ileum increased 5.7 times and 6.0 times, respectively. Lishana et al. (2019) suggested that in free-range chickens, the high villi of the small intestine may have reached a maximum in the final grower phase. At the age of villi, height growth does not affect broiler chickens for more than 28 days. Villi in chickens are more developed at the time of hatching, but the growth after hatching in the intestine is not affected by the age of the chicken.

**Conclusion and recommendation**

The present findings confirm that the thickness of the small intestine layer of turkeys increased at 8 and 16 weeks of age (P<0.05), while the height of the villi increased at 8, 16 and 24 weeks of age (P<0.05). On histochemical staining, Goblet cells and Liberkuhn's glands showed strong AB staining intensity (+++) on all parts of the small intestine of turkeys at all ages. PAS staining results showed strong intensity (+++) only at 8 and 16 weeks of age. Meanwhile, turkeys aged 24 weeks showed medium staining intensity (+) on the duodenum and jejunum and strong staining intensity (+++) on the ileum.

**Funding**

This study received no external funding.

**Acknowledgments**

The authors gratefully acknowledge the cooperation of staff in the Laboratory of Histology, Laboratory of Research, and Laboratory of Experimental Animals of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, as well as who participated in the study.

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