Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

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Revista Brasileira de Ciência Avícola, vol. 17, núm. 2, abril-junio, 2015, pp. 145-149
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Apr - Jun 2015 / v.17 / n.2 / 145-150
http://dx.doi.org/10.1590/1516-635x1702145-150

Determining the Best Sectioning Method and Intestinal Segment for Morphometric Analysis in Broilers

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■Keywords

Image, intestine, morphometry, poultry, villus.

Submitted: February/2014
Approved: October/2014

ABSTRACT

Brazilian poultry production is very efficient and demands maximum broiler performance. Therefore, digestive system pathologies have a relevant role. Considering it is difficult to obtain consistent information on intestinal morphometric analysis, this study aimed at establishing essential and clear criteria for the collection of intestinal segments for morphometric analysis. Fifteen 13-d-old broilers were sacrificed and three intestinal segments were collected per bird. Two 3-cm long sections were obtained from each of the intestinal segments. Samples were collected open or closed. The closed samples were transversely, hemicylindrically, or longitudinally sectioned. Samples were processed and stained with hematoxylin and eosin. The number of microscopic fields in each section was counted. Villi presenting the base clearly embedded in the submucosa, no damage or folds, and simple columnar epithelium at the tip were considered adequate for measurements. These villi were counted in each sample. The results shows that hemicylindrical sections presented the highest number of observation fields, with an average of 9.76 fields. Jejunum samples were among the three highest average villi counts, with 18.23 in longitudinal sections and 15.61 in hemicylindrical sections. The results of the present study indicate that hemicylindrical sectioning and jejunal samples were, respectively, the best sectioning method and the best intestinal segment for the morphometric analysis of the intestines of broilers.

INTRODUCTION

The poultry industry demands maximum performance from broilers. Consequently, digestive system pathologies are extremely relevant, as they may negatively affect broilers' economic potential and, according to Ferreira *et al*. (2012), increase processing losses due to lack of flock uniformity.

The intestinal mucosa consists of a layer of simple columnar epithelium (Kalil *et al*., 2000; Luna, 1968) and by villi. These projections of the lamina propria into the intestinal lumen increase nutrient digestion and absorption surface area. Despite the many studies in literature on intestinal morphometry, most do not clearly describe the methodology applied. Relevant data, such as the evaluated intestinal segment, the time between death and sample fixation, how the segments are sectioned, and criteria to measure the microstructures, are not informed (Alvarenga *et al*., 2004; Fukayama *et al*.; 2005, Okamoto *et al*., 2009 and Shiraishi *et al*., 2009). On the other hand, Caruso & Demonte (2005), studying the histomorphometry of the small intestine of rats, describe the details of the methodology applied and of the measurement criteria used. The authors describe the time between

sample collection and fixation, staining technique, the method applied to measure villi and crypt depth, and number of villi evaluated per individual.

Considering the lack of agreement among the different authors that evaluated methods of intestinal sample collection and measured structures, the objective of the present study was to establish essential and clear criteria for broiler intestinal morphometric analysis. Firstly, the best method to collect and section intestinal samples was determined, and then the most adequate combinations of intestinal segment-sectioning method were evaluated.

MATERIAL AND METHODS

Birds

Fifteen 13-day-old Cobb broilers were housed in cages and offered feed and water *ad libitum*. Birds were sacrificed by neck dislocation, and the intestinal samples were collected immediately after death was confirmed.

Sample collection

In order to obtain samples of adequate size, 3-cm long sections were collected from each intestinal segment (duodenum, jejunum, and ileum). Immediately after samples were cut, the intestinal lumen was washed with 10% buffered formalin in order to remove the lumen content. All samples were placed in individually identified flasks containing 10% buffered formalin.

Each intestinal segment was collected either closed (no exposure of the intestinal mucosa) or opened, with the mucosa exposed by longitudinal incision of the organ from the mesenteric border and then stapled on a paper card measuring 2x3 cm. Samples were stretched and stapled through the mucosa with the serosa placed in contact with the paper card.

Duodenum samples were obtained from two sections: close to the ventriculus (descending part) and close to the jejunum (ascending part). Proximal and distal fragments of the jejunum were collected, considering Meckel's diverticulum as the limit between these two fragments. Ileum samples were collected from the segment located between the ceca.

Table 1 – Sample collection and sectioning methods applied.

Collection method	Sectioning method
Close samples	Transversal (T) – Figure 1
	Longitudinal (L) – Figure 2
	Hemicylindrical (H) - Figure 4
Open samples	Open stapled (OS)– Figure 4

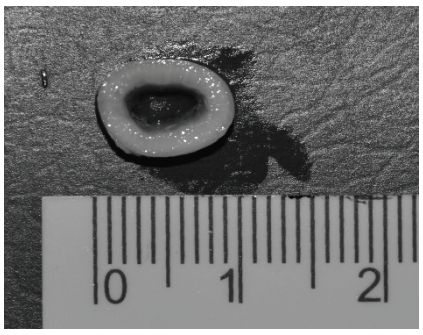

Figure 1 – Transversal section of the jejunum collected closed

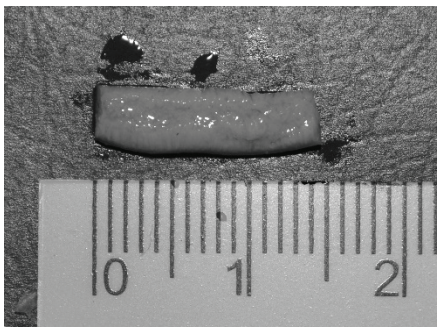

Figure 2 – Section of the jejunum collected open and stapled

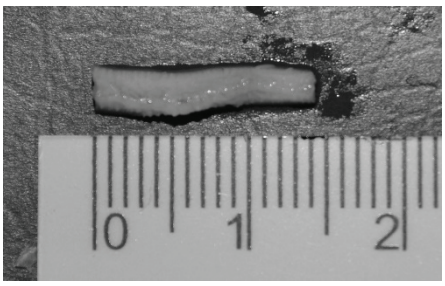

Figure 3 – longitudinal section of the jejunum collected closed

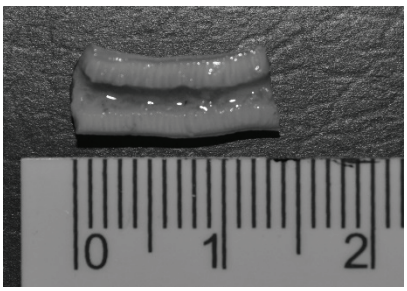

Figure 4 – Hemicylindrical section of the jejunum collected closed

Sectioning of the samples

The intestinal samples were sectioned using four different methods. All samples were approximately 1-mm thick. The longitudinal sections (hemicylindrical and longitudinal) were 15-mm long. The sections as described in Table 1.

Sample processing for histology

Samples were dehydrated in graded concentrations of alcohol, cleared in xylene, and embedded in paraffin. Samples were then cut into 4-µm thick sections and stained with hematoxylin-eosin.

Observation field count

Microscopic observation fields were counted under optical microscope (10x magnification) for each type of sample (Figure 5) of each intestinal segment, and the total number of observation fields was duly recorded.

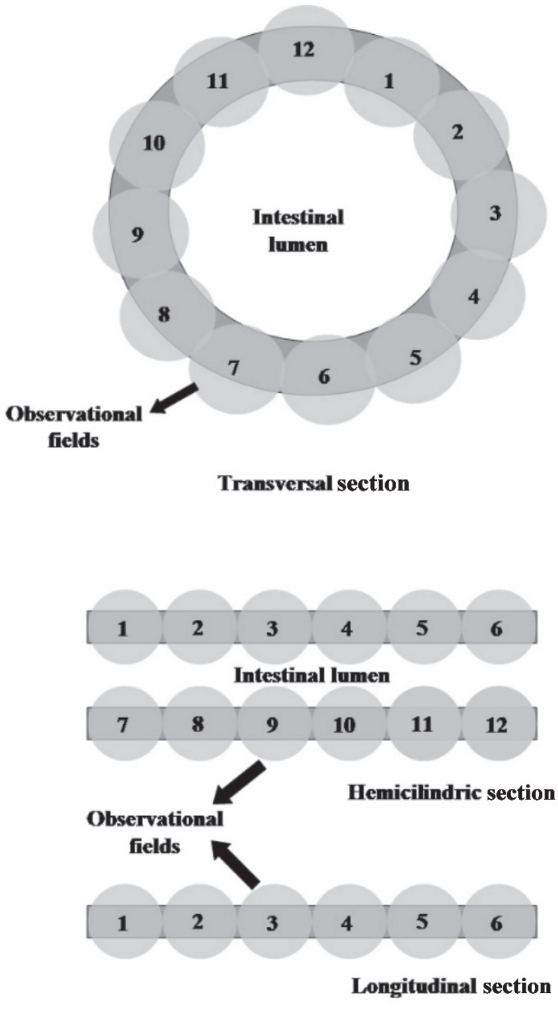

Figure 5 – Diagram of the section surface and of the observation fields of the different types of samples.

Villus count

Only the villi considered adequate for measurements were counted. A villus was considered adequate when its base was clearly embedded in the submucosa (10x magnification; Figure 6); its body did not present any discontinuity or folds (4x magnification; Figure 7); and simple columnar epithelium was present at the tip (40x magnification; Figure 8).

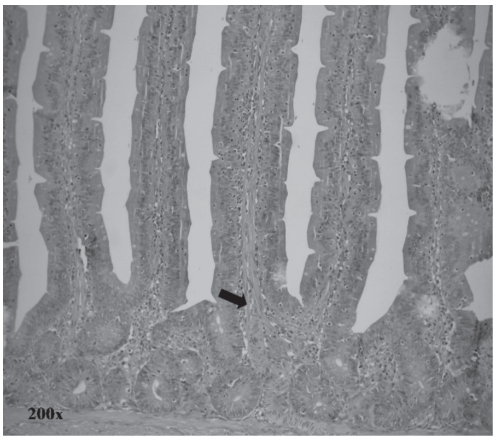

Figure 6 – Base of a villus considered adequate for measurement (arrow).

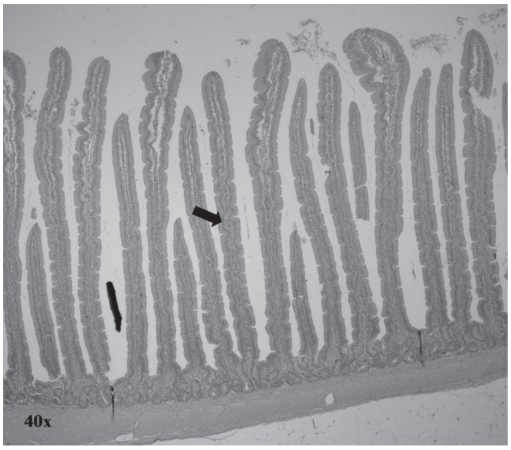

Figure 7 – Body of a villus considered adequate for measurement (arrow).

Figure 8 – Tip of a villus considered adequate for measurement, presenting simple columnar epithelium (arrow).

Statistical analysis

The collected data were submitted to one-way analysis of variance and means were compared by the test of Tukey-Kramer.

RESULTS

The results of observation field counts and number of villi considered adequate for measurement according to sample type and intestinal segment are shown in Tables 2 and 3.

Table 2 – Average number of observation fields according to sample type and intestinal segment.

Sample type	Number of samples	Number of observation fields
HCPJ	13	9.76[a]
HCAD	12	9.33[ab]
HCPI	14	9.21[ab]
TCAD	14	6.71[abc]
LCAD	14	6.28[abc]
TCPJ	10	6.20[abc]
SODI	13	6.15[abc]
TCPI	10	6.00[abc]
LCPJ	13	6.00[bc]
SODJ	13	5.69[bc]
SODD	13	5.30[c]
LCPI	9	3.66[c]

Means followed by different superscripts are significantly different atp≤0.05

LCAD	Longitudinal closed ascendant duodenum
HCAD	Hemicylindrical closed ascendant duodenum
TCAD	Transversal closed ascendant duodenum
SODD	Stapled opened descendant duodenum
SODJ	Stapled opened distal jejunum
LCPJ	Longitudinal closed proximal jejunum
HCPJ	Hemicylindrical closed proximal jejunum
TCPJ	Transversal closed proximal jejunum
SODI	Stapled open distal ileum
LCPI	Longitudinal closed proximal ileum
HCPI	Hemicylindrical closed proximal ileum
TCPI	Transversal closed proximal ileum

The results presented in Table 2 show that, independently of the intestinal segment (duodenum, jejunum, or ileum), the collection of "closed" samples and the "hemicylindrical" section provided the highest numbers of observation fields per sample.

Table 3 shows that jejunum samples provided higher average counts of villi adequate for measurement, and that samples submitted to "hemicylindrical" section presented two out of the three highest average counts of villi adequate for measurement.

Table 3 – Average number of villi considered adequate for measurement in intestinal samples of 13-d-old broilers.

Sample type	Number of samples	n. of villi adequate for measurement
HCPI	14	20.57[a]
LCPJ	13	18.23[ab]
HCPJ	13	15.61[abc]
TCPJ	10	5.90[abc]
HCAD	12	5.75[abc]
SODD	13	5.69[abc]
TCPI	10	2.90[bc]
SODJ	13	2.30[c]
LCPI	9	1.88[c]
LCAD	14	1.85[c]
SODI	13	1.30[c]
TCAD	14	0.64[c]

Means followed by different superscripts are significantly different at p≤0.05.

LCAD	Longitudinal closed ascendant duodenum
HCAD	Hemicylindrical closed ascendant duodenum
TCAD	Transversal closed ascendant duodenum
SODD	Stapled opened descendant duodenum
SODJ	Stapled opened distal jejunum
LCPJ	Longitudinal closed proximal jejunum
HCPJ	Hemicylindrical closed proximal jejunum
TCPJ	Transversal closed proximal jejunum
SODI	Stapled open distal ileum
LCPI	Longitudinal closed proximal ileum
HCPI	Hemicylindrical closed proximal ileum
TCPI	Transversal closed proximal ileum

DISCUSSION

Literature reports a wide variety of methods for the collection of intestinal samples for histology. For instance, Alvarenga *et al*. (2004) collected 5-cm rings from each small intestine segment, whereas Okamoto *et al*. (2009) collected 1-cm long samples of the proximal duodenum that were opened by the mesenteric border. Shiriashi *et al*. (2009) collected ring-shaped samples of the ileum and washed them with a 0.9% NaCl solution. Fukayama *et al*. (2005) collected 1.5-cm long intestinal samples, which were immediately washed with distilled water and fixed in Bouin solution for 24 hours. Caruso & Demonte (2005) collected 1-cm samples of the small intestine of rats, fixed them in 10% formalin, refrigerated the samples

for 24 hours, and then transferred them to a medium containing 70% alcohol.

This brief literature review illustrates the diversity of methods applied in histological studies of the intestine. Perhaps the only consistent feature among them is the lack of details on the methods used for sample collection. This was the motivation of the present study, as it was not possible to reproduce the methodology described by those authors. We decided to apply part of the mentioned methodologies and to evaluate some essential aspects, such as the number of observation fields and the number of villi considered adequate for measurement, as well as to clearly define which villus characteristics that are required to determine if the villi are adequate or not. These characteristics were not described in any of the researched studies. Some intestinal samples were collected and the segments opened by the mesenteric border, as described by Okamoto *et al*. (2009), while other samples were collected without opening, as suggested by Shiriashi *et al*. (2009). All sections were 1.5cm long and were washed with a fixing solution, according to Fukuyama *et al*. (2005).

The small intestine samples that were collected and not opened and submitted to hemicylindrical section presented the highest number of observation fields, independently of intestinal segment. This result is in agreement with Kalil *et al*.(2000), who recommend that small intestine samples should always be sectioned in the longitudinal direction in order to obtain histological section which villi are not unduly increased.

The intestine is a very sensitive organ and needs to be handled with care. In the present study, strict criteria were adopted to consider that a villus was adequate for measurement. The villus body should not present any artifacts (folds, breaks, grooves, etc.), its base should be clearly embedded in the submucosa, and its tip should present simple columnar epithelium. Artifacts in the villus body may "reduce" its size, such as in the case of folds, or increase it, such as in the case of grooves or breaks. A villus base clearly embedded in the submucosa indicates that the villus belongs to the evaluated section, whereas the tip with simple columnar epithelium suggests that it was sectioned close to its sagittal line. The consideration of these characteristics provide higher measurement accuracy, as they prevent possible distortions. When these characteristics were taken into account when evaluating the villi, the samples obtained by hemicylindrical section were shown to be the most adequate for morphological evaluation, because they presented the highest number of microscopic observation fields and the highest number of villi considered adequate for measurement. In addition, because the jejunum presented the highest number of measurable villi and observation field counts, it is suggested that this intestinal segment is the most indicated for morphometric analyses.

CONCLUSIONS

Based on the results obtained in the present study, it was concluded that the samples that were collected closed and submitted to hemicylindrical section presented the highest number of observation fields, independently of intestinal segment. The jejunum is the most indicated intestinal segment for intestinal histomorphometric analysis, because it presented the highest number of villi considered adequate for measurement and due to its importance for nutrient absorption. In addition, it was observed that the structure of the intestines is very frail, requiring extreme care when handled. The present study may be used as the basis of future studies using intestinal morphometric analyses as it elucidated basic issues relative to sample collection method and processing.

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