Morphology of rectum in broiler chicken and domestic fowl: notability of retrograde peristalsis for water preservation

Rahmat Allah Fatahian Dehkordia and Masoud Shakaramb

aDepartment of Anatomical sciences, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran; bDVM student of Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran

ABSTRACT
Using light and electron microscopy, the rectum-coprodeum (R-C) segment of two species of chickens was examined. Six domestic fowls (Gallus domesticus) and 12 broiler chickens (ROSS 308) with the same ages were chosen for this study. Intestinal samples were sectioned with stereological procedures for quantitative studies. Sections were stained with Haimatoxylin and eosin (H&E) for the light microscopic study. Transmission electron microscopy (TEM) was used for R-C segment. Villi structural variables, microvillus surface area, density and absolute surface area were considered. Results showed that the mucosa surface area decreased distally from rectum towards the coprodeum in both species. Parameters of the villi and microvilli surface area, absolute surface area and density showed decrease from proximal R-C segment towards distal. There was no significant difference in all the variables of the villi and microvilli in R-C segment (P > .05) between both species. The mean villi amplification factor in the R-C segment was low, but this factor in broiler chicken was higher (P > .05) than domestic fowl. There were no significant differences in thickness of the submucosal and muscular layers between both species (P > .05). The functional consequences in microvilli population are argued in relation to retrograde peristalsis within the rectum-coprodeum segment in birds.

1. Introduction
The absorption capacity and water preservation between all the birds’ species is different. Water retention and excretion in birds is performed by production of the concentrated urine than plasma (Karavov and Diamond 1983; Sjaastad et al. 2004; Bartholomew 2010). Birds do not have an actual urinary bladder; therefore after urine is formed by the kidneys, it moves in the ureter’s tract and finally all will be entered into the cloaca (a blind pouch). Urine may travel in a reverse direction and enter into the lower intestine by retrograde peristalsis (Duke 1989; Clauss et al. 1991; Brummermann and Braun 1994; Casotti 2001). Retrograde peristalsis in lower intestine causes urine to reach the regions of microbial fermentation in ceca or upper colon. Thus, the lower intestine gets an infiltration of water from both the kidneys and the intestines (Brummermann and Braun 1994). The infiltration of water within the lower intestinal is absorbed again by its epithelium to maintain of hydration (Thomas 1982). Reabsorption of the sodium chloride and water into the lower intestine and cecum can reconstruct by the process of water associated with sodium. On the other hand, time that is spent for urine reflux varies with the animal situation, in animals under desirable condition a shorter time taken for urine reflux. Generally, time of the urine reflux into the lower intestine is low and only keeps for brief moments (Nechay et al. 1968).

It has been indicated that compositions in urine alters after entering urine into large intestine by reversible transmission (Skadhauge 1993; Laverty and Skadhauge 1999). Because the lower intestine is involved in the change of final compositions of both intestinal chyme and urine, thus the avian lower intestine has been referred as the ‘integrating segment’ (Skadhauge 1993). Clauss et al. (1991) revealed that lower intestine epithelium is clearly adapted for high-capacity transport by microvilli and mucosal surface area and also electrolyte transport pathways (Clauss et al. 1991). The practical concepts of alteration in distribution of microvilli into the lower intestine of birds can be associated with retrograde peristalsis (Casotti 2001). The small intestine (including duodenum, jejunum and ileum) is relatively simple and short but nevertheless highly efficient. Small intestine is characterized by a great morphometrical and functional variability, both between and within species. Hence, the present study was designed to provide main information on the stereological and histomorphometrical characteristics of the microvilli population between both breed domestic and broiler.

2. Materials and methods
2.1. Sample collection
Twelve healthy adult broiler chickens (ROSS 308) and six healthy adult domestic fowls (Gallus domesticus) with the similar ages were considered for this study. The animals were obtained from research farm came to the university of Shahrekord, Iran. The study protocol for this experiment was approved by the University Research Committee.
Birds’ weight was taken by digital scale, as the mean body weight in the domestic fowl and the broiler chicken was 1850.23 ± 150 g and 1764.65 ± 142 g respectively. After collecting and dissecting them, the birds’ abdominal cavity was exposed and their large intestine was selected for morphological and stereological studies. Base of cecum (area just distal to the attachment of the cecae on the intestine) to coprodeum (the coprodeum is the most cranial division of the cloaca) was chosen for evaluation, and was then subdivided into three segments (R-C segment).

2.2. Tissue preparation

Before sectioning tissue, the arrangement of villi within the R-C region of the intestine was evaluated by a dissecting microscope. Surveys revealed no difference in the surface architecture of villi between the two chicken species. Each R-C segment of tissue from all birds was then subdivided into five smaller segments of approximately equal length and, of these, one segment was randomly selected and processed for light microscopy. The entire samples of intestine were immersed in 10% formalin fixative solution for 48 hr to ensure optimum fixation. Tissues preparation was performed by alcohol dehydration and embedding in paraffin. Sections were stained with Haematoxylin–eosin and studied for histomorphological changes.

For transmission electron microscope (TEM), the samples of R-C segment were cut and were kept in glutaraldehyde-parafomaldehyde pre-fixing (pH 7.4) for 24 hr. They were then rinsed with cacodylate sodium buffer (0.15 M, pH 7.4) three times at 10-minute intervals and for 2 hr post-fixed in a phosphate-buffered solution of 1% osmium tetroxide at 37°C. Samples washed were stained with Haematoxylin–eosin and ready for ultrathin were observed under a transmission electron microscope.

2.3. Stereological and morphometrical findings

For stereological analysis of the R-C region, a three-level sampling scheme was used. At the light microscopic level, the surface area amplification due to villi \( S(v) \) was determined. A test grid superimposed on the light micrographs for intersection counts. Intersections between the test grid and the villi \( l(v) \) and mucosa \( l(m) \) were recorded for each segment. Amplification of the surface area was calculated using the following equation (Howard and Reed 2004):

\[
S(v) = \frac{l(v)}{l(m)},
\]

where \( S(v) \) is the amplification of the surface area due to villi, \( l(v) \) is crossing between the grid and villi, \( l(m) \) is crossing between the grid and mucosa.

Estimations of villi surface area \( (Sv) \) were performed by counting intersections between grid and the luminal side of the villi and mucosa according to (Gundersen et al. 1988, 1999):

\[
S(v) = 2 \times \sum \frac{I(v)}{P},
\]

where \( I \) is the number of intersections of the test grid with the villi and tunica mucosa separately and \( P \) is the number of test points falling on the reference.

TEM estimates of the extent to which microvilli amplify the surface area of the mucosa \( Sm(v) \) was determined by a test grid using counting the number of intersections between the test grid and microvilli \( (l(mv)) \) and villi \( (l(v)) \), calculated using the following equation (Makanya et al. 1995):

\[
S(mv) = \frac{l(mv)}{l(v)},
\]

where \( l(mv) \) is intersections between the test grid and microvilli and \( l(v) \) is intersections between the test grid and villi.

Packing density of microvilli is number of microvilli per unit surface area of apical cell membrane, was estimated by \( N(mv)/S(v) = (S(mv)/S(v))/S(mv) \) (Makanya et al. 1995), where \( S(mv) \) is the surface area of the microvilli, \( S(v) \) is surface area of villi and \( S(m) \) is surface area of an average microvillus.

2.4. Statistical analysis

Results are presented as mean ± SEM (standard error of the mean). For all data statistical significance was tested using one-way ANOVA with LSD’s post-test. When P-values were found to be less than .05 they considered statistically significant.

3. Results

Results obtained from stereological measurements, characteristics of microvilli and the morphometrical parameters of the R-C segment in both species are shown in Tables 1 – 3. The surface area of a single villus and mucosa decreased from proximal towards distal of the coprodeum. Segments 1 and 2 of both species showed a small change in surface area of villi, followed by a large decrease in next segment (segment 3). The mucosal surface area of segments 1 and 2 in

| Species | Domestic fowl | Broiler chicken |
|---------|---------------|----------------|
| Number  | 6             | 12             |
| Segments | S1  | S2  | S3  | S1  | S2  | S3  |
| Circumference (mm) | 11.2 ± 0.12 | 12.05 ± 0.21 | 13.4 ± 0.41 | 10.41 ± 0.32 | 11.25 ± 0.21 | 12.04 ± 0.11 |
| Villi amplification factor | 3.75 | 3.42 | 1.21 | 4.35 | 3.47 | 1.94 |
| Villi surface area (µm²) | 234 ± 9.15 | 175 ± 9.21 | 75 ± 5.22 | 212 ± 9.24 | 163 ± 8.41 | 82 ± 6.32 |
| Mucosal surface area (µm²) | 387 ± 14.41 | 384 ± 16.33 | 374 ± 12.13 | 393 ± 12.47 | 390 ± 13.36 | 381 ± 15.25 |
| Ratio of villi surface area to mucosa (%) | 60.46 | 45.69 | 38.77 | 54.49 | 42.78 | 32.36 |
domestic fowl can be compared with the broiler chicken but there was a noticeable decline in next segment for both species. Despite the changes in villi and mucosal surface area between domestic fowl and broiler chicken in segments 1 and 2, no significant difference was observed in surface area of the R-C segment in both species ($P > .05$).

For entire R-C segment, mean amplification factors for villi were 3.06 in domestic fowl and 3.32 in broiler chicken. In all birds there was gradient of intestinal morphology with smaller values of intestinal circumference in more proximal segment (segment 1) than subsequent segments (segments 2 and 3).

Surface area of microvilli (Figures 1A and B) was larger in the R-C segment of the broiler in comparison with that in the domestic fowl. The mean microvillus surface area was 32.01% larger in the broiler chicken than in domestic fowl but this increase was not significant ($P > .05$) (Table 2). Also, the mean packing density of microvilli was higher in the broiler R-C segment ($P > .05$). The absolute surface area of the microvilli into the R-C segment was increased in the broiler chicken to 17.7% ($P > .05$) (Table 2).

In broiler chicken, the mean thickness of submucosa layer was greater in the total segments when compared to domestic fowl, but these differences were no significant ($P > .05$) (Table 3). An increase in mean thickness of the muscular layer for the three segments was obtained in both species ($P > .05$) (Table 3 and Figures 2–4).

### Table 2. Characteristics of microvilli (mv) located on the luminal surface of intestine two species of chicken.

| Variable                  | Domestic fowl | Broiler chicken |
|---------------------------|---------------|-----------------|
| Surface area of mv (µm²)  | 22.85 ± 12.45 | 33.61 ± 13.62   |
| Packing density (mv/µm²)  | 4.31 ± 1.38   | 5.43 ± 1.79     |
| Absolute surface area (mm²)| 121.17 ± 28.21| 137.26 ± 31.74 |

### Table 3. The mean values (±SE) of the morphometrical parameters in the different layers of the colon between both species.

| Species     | Domestic fowl | Broiler chicken |
|-------------|---------------|-----------------|
| Number      | 6             | 12              |
| Segments    | S1 S2 S3      | S1 S2 S3        |
| Submucosa   | 13.33 ± 2.31  | 13.84 ± 2.21    |
| (µm)        | 12.45 ± 1.84  | 12.93 ± 1.96    |
| Muscular     | 354.2 ± 14.13 | 395.5 ± 12.5    |
| (µm)        | 311 ± 15.34   | 348.4 ± 13.2    |

**Figure 1.** Transmission electron micrographs showing the alteration in structure of microvilli in the level of sampling from R-C segment, that is, proximal (A) to distal (B), (Magnification = 1350, bar = 0.5 µm).

### 4. Discussion

The present study was prompted with a desire to compare intestinal morphology in chickens with different lifestyles. Forasmuch an achievable protocol is available, this will be the subject of future investigations. Thus far, it was detected that variations between both species of birds of present study are achieved by adaptation at several levels of structural organization. These include increases in intestinal circumference, villous amplification factor and microvillus packing density. In birds and rodents, microvillus development is part of the process of enterocyte maturation as cells migrate along the longitudinal axis (Stenling and Helander 1981; Smith and Brown 1989), and this development is a property of cell morphology during the intestine evolution (Mayhew et al. 1990).

The results of present study showed that the villi and mucosal surface area and villi amplification factor decreased with distance distally (from $S_1$ towards $S_3$) along the intestinal tract of both species chickens. Although there was no significant difference among the desired parameters between both species, disproportionate alterations were separately observed between segments 1 and 3 at each species, which is in agreement with the results obtained about those of sparrow on the lower intestine (Casotti 2001). Also, in the present study, it was shown that in both species, the microvilli absolute surface area and packing density of the R-C segment in chickens decreased from proximal towards distal. Thus, it can be inferred that the proximal segment of the rectum has the highest role in the reabsorption of urine (Casotti 2001). Also, investigations have displayed that reabsorption of water and NaCl occurs in the rectum and cecum.

The kidneys of birds cannot produce concentrated urine in comparison to those of mammals. Investigations show that...
The maximum ratio of urine concentration to plasma is 2.5 in birds, which is one-tenth of mammals (MacMillen and Lee 1967; Braun and Dantzler 1972). Hence, urine that enters into the urodeum part of the cloaca has a high water content. According to the present findings, the proximal segments of the rectum possess the greatest distribution of microvilli; therefore, based on documented reasons, it suggested that most reabsorption of urine may happen in this area. These results express that birds maintain water by retrograde peristalsis of lower intestine (Casotti 2001).

The obtained results showed that there was no significant difference in the microvilli packing density and absolute surface area in R-C segment of domestic fowl and broiler chicken. These data were inconsistent with the results that were found about difference in the number of microvilli in species that feed on the different nourishments (Makanya et al. 1997). Furthermore, findings have been shown that microvilli abundance is greater in lower intestine of birds which live under humid conditions compared to dehydrated birds. This adaptation with physiological situations shows that a noticeable amount of urine modification happens in birds with humid conditions but less seen in birds with dehydrated conditions (Goldstein et al. 1986; Goldstein and Braun 1986; Mayhew et al. 1990; Elbrønd et al. 1991).

Hence, it is expected that was no observed significant different in microvilli distribution of the R-C segment in both species of chickens. Despite the fact that was not seen significant difference, results of present study reveal that packing density of microvilli in domestic fowl is low and it followed absolute surface area than broiler chicken.

This study revealed that greater numbers from microvilli are observable in proximal segments of the rectum. It is concluded that most retention of water and sodium chloride happen in this area. On the other hand, in species that have access to the nutrition with more dilution, presumably their intestinal microvilli are higher in comparison to those in birds that feed on the less dilution diet (Casotti 2001). Although in some parameters there were not significant differences between regions and/or between both groups of birds, nevertheless results of the present study propose above occurrences about retrograde peristalsis.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was financially supported by the University of Shahrekord, Iran.

**References**

Bartholomew GA. 2010. The water economy of seed-eating birds that survive without drinking. Proceedings of the 15th International Ornithological Congress, The Hague.
Braun EJ, Dantzler WH. 1972. Function of mammalian-type and reptilian-type nephrons in kidney of desert quail. Am J Physiol. 222:617–629.

Brummermann M, Braun E. 1994. Physiological ecology and behavior of desert birds. In: Pleschka K, Gerstberger R, editors. Integrative and cellular aspects of autonomic functions: temperature and osmoregulation. In: Current Ornithology. Paris: John Libbey Eurotext; p. 517–525.

Casotti G. 2001. Luminal morphology of the avian lower intestine: evidence supporting the importance of retrograde peristalsis for water conservation. Anat Rec. 263:289–296.

Claus W, Dantzler V, Skadhauge E. 1991. Aldosterone modulates electrogenic Cl secretion in the colon of the hen (Gallus domesticus). Am J Physiol-Reg I. 261:R1533–R1541.

Duke GE. 1989. Relationship of cecal and colonic motility to diet, habitat, and cecal anatomy in several avian species. J Exp Zool. 252:38–47.

Elbrønd VS, Dantzer V, Mayhew T, Skadhauge E. 1991. Avian lower intestine adapts to dietary salt (NaCl) depletion by increasing transepithelial sodium transport and microvillus membrane surface area. Exp Physiol. 76:733–744.

Goldstein DL, Braun EJ. 1986. Lower intestinal modification of ureteral urine in hydrated house sparrows. Am J Physiol-Reg I. 250:R89–R95.

Goldstein DL, Hughes MR, Braun EJ. 1986. Role of the lower intestine in the adaptation of gulls (Larus glaucescens) to sea water. J Exp Biol. 123:345–357.

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A. 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. Acta Pathol Microbiol Scand. 96:379–394.

Gundersen HJG, Jensen EBV, Kieu K, Nielsen J. 1999. The efficiency of systematic sampling in stereology — reconsidered. J Micr. 193:199–211.

Howard V, Reed M. 2004. Unbiased stereology: three-dimensional measurement in microscopy. Oxford: Garland Science.

Karasov WH, Diamond JM. 1983. Adaptive regulation of sugar and amino acid transport by vertebrate intestine. Am J Physiol-Gastr L. 245:G443–G462.

Laverty G, Skadhauge E. 1999. Physiological roles and regulation of transport activities in the avian lower intestine. J Exp Zool. 283:480–494.

MacMillen RE, Lee AK. 1967. Australian desert mice: independence of exogenous water. Sci. 158:383–385.

Macanya A, Maina J, Mayhew T, Tschanz S, Burri P. 1997. A stereological comparison of villous and microvillus surfaces in small intestines of frugivorous and entomophagous bats: species, inter-individual and cranio-caudal differences. J Exp Biol. 200:2415–2423.

Macanya AN, Mayhew TM, Maina JN. 1995. Stereological methods for estimating the functional surfaces of the chiropteran small intestine. J Anat. 187:361–368.

Mayhew T, Dantzler V, Elbrønd V, Skadhauge E. 1990. A sampling scheme intended for tandem measurements of sodium transport and microvillus surface area in the coprodaeal epithelium of hens on high- and low-salt diets. J Anat. 173:19–31.

Nechay BR, Boyarsky S, Catacutan-Labay P. 1968. Rapid migration of urine into intestine of chickens. Comp Biochem Physiol. 26:369–370.

Sjaastad OV, Hove K, Sand O. 2004. Physiology of domestic animals. Oslo: Scandinavian Veterinary Press.

Skadhauge E. 1993. Basic characteristics and hormonal regulation of ion transport in avian hindguts. In: Ion Transport in Vertebrate Colon. America: Springer; p. 67–93.

Smith M, Brown D. 1989. Dual control over microvillus elongation during enterocyte development. Comp Biochem Phys A. 93:383–389.

Stenling R, Helander HF. 1981. Stereological studies on the small intestinal epithelium of the rat. Cell Tissue Res. 217:11–21.

Thomas DH. 1982. Salt and water excretion by birds: the lower intestine as an integrator of renal and intestinal excretion. Comp Biochem Phys A. 71:527–535.