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PREPUBERTAL AND PUBERTAL CAECAL WALL HISTOLOGY IN JAPANESE QUAILS *(COTURNIX COTURNIX JAPONICA)*

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Summary

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The present study is aimed to investigate changes in caecal wall histology of quails, during prepubertal and pubertal periods. To this end, forty quails at the ages of 10, 20, 30 (prepuberty) and 40 (puberty) days were slaughtered (10 birds in each sampling time) and histological sections from proximal, middle and distal parts of caeca were prepared and stained with H&E. It was observed that in proximal part, villus height significantly increased during prepuberty and remained constant around the puberty; moreover, the villi became wider at pubertal period. Highest width of both mucosal and muscular layers was observed on day 30. Sub mucosal layer width continued to increase with age. In middle part, the villi became significantly shorter and wider around puberty. Sub mucosal layer was thickest at puberty while the mucosal layer had its highest width. The increase in sub mucosal layer width was accompanied by expansion of lymphoid tissues in both proximal and middle parts. The villi were absent in distal part of cecum. The width of muscular layer increased with age during the prepubertal period and remained statistically constant afterwards. It can be concluded that caecal immune structures of quails in pre pubertal and pubertal ages expand by age and are more developed at puberty. The structural ability of muscular layer of caecal apex is increased at puberty which helps in returning particles back to colon when their entrance is increased due to short villi.

Key words: caecum, histology, prepuberty, puberty, quail

INTRODUCTION

Japanese quail *(Coturnix japonica)* is a small size bird with big economical profits. This species is a good "dual-purpose" bird and is now reared for meat and egg production. Quails have relatively early maturity and may be used as an experimental model due to their fast development, small size, etc. (Sedqyar *et al.,* 2012).

Avian large intestine consists of paired caeca and a short straight colon joined to ileum and cloaca. Quails, like many other galliformes, have intestinal type caeca according to Naik (1962) classification which are characterised as long structures that are histologically similar to the small intestines (Clench & Mathias, 1995). Each caecal sac consists of 3 parts: proximal

part or base, middle and distal part or apex. The mucous membrane is similar to that of the small intestine, with less goblet cell and fewer glands. The villi are well developed at the basilar part, shorter and wider in the middle part and either shorter or absent in the apex (Getty, 1975). The role of avian caeca has been investigated by many authors. There is evidence that the caeca play a role in the microbial degradation of some carbohydrates (Jorgensen *et al*., 1996; Jamroz *et al*., 2002), absorption of water (Mc Nab, 1973), microbial synthesis of vitamins (Coates *et al*. 1968), cholesterol digestion and absorption (Tortuero *et al*., 1975), and degradation of nitrogenous compounds (Goldstein, 1989). On the other hand, caecal wall contains lymphatic tissues, most in the basilar part, forming caecal tonsils which may act as an immunological surveillance against foreign micro organisms entering caeca (Getty, 1975).

As far as we know, the effect of age on the histological parameters of caecal wall of quails has not been evaluated yet. This may be important for better elucidation of the absorptive and immunologic capacity of caecum at different ages. The present study is conducted to clarify the plausible changes in caecal wall histology of quails, during pre pubertal and pubertal periods.

MATERIALS AND METHODS

Animals

Forty clinically healthy one-day old Japanese quails from both sexes were reared in similar environmental and nutritional conditions. On the ages of 10, 20, 30 (prepuberty) and 40 (puberty) days, the birds were randomly selected and slaughtered by cervical dislocation (10 birds in each sampling time) and caeca dissected from the ileocaecal valve.

Sampling and histological evaluation

Samples were prepared from proximal part or base, middle and distal part or apex of caeca and fixed in 10% buffered formalin. Routine histological laboratory methods were used and 6 μm-thick transverse sections were made by a rotary microtome. A total number of 10 sections were made from each caecal segment of each bird and stained with H&E for measuring villus height and villus basal width at the crypt-villus junction, as well as mucosal, sub mucosal and muscular width by using a linear graticule under light microscope. Arithmetic mean of 15 measurements of each parameter per section was calculated.

Statistical analysis

Data expressed as mean±SD. Data comparisons performed by one-way ANOVA method followed by Tukey's multiple comparison test and differences considered statistically significant at $P<0.05$.

All methods used in the study were in compliance with the institutional ethical guidelines for use of animals in research.

RESULTS

Proximal part or base (caecal tonsils)

As shown in Table 1, the height of villi in this part significantly increased from day 10 to 30 ($P<0.001$) (prepubertal period) and remained constant around the puberty (day 40 of age), more over the villi became significantly wider at pubertal period ($P=0.025$). Highest width of both mucosal and muscular layers was observed on day 30. The width of mucosal layer on days 20 and 40 was significantly lower than days 10 and 30 ($P<0.001$). Sub mucosal layer width continued to increase

with age and reached its highest width around puberty (P<0.001).

M-like cells were present in the epithelia of in this part of cecum at all sampling periods.

Middle part

No significant difference observed in the length and width of villi during the prepubertal period; however the villi became significantly shorter and wider around puberty (P<0.001). In this part, sub mucosal layer was thickest at puberty while the mucosal layer had its highest width. In contrast to the proximal part, the mucosal widths on days 20 and 40 were significantly higher (P<0.001) than days 10 and 30 of the birds' life. No significant change observed in muscular layer width during the experiment (Table 2).

Distal part or apex

The villi were absent in this part of the caecum. The highest width of mucosal layer observed on day 30 and then decreased (P=0.011). The sub mucosal width did not significantly change during the experiment. The width of muscular layer increased with age during prepubertal period (P<0.001) and remained statistically constant afterwards (Table 3).

DISCUSSION

Caeca of avian species range in size from very long to very short, or they may be entirely absent. Unlike the case in almost all mammals, most avian caeca are paired and of approximately equal length, with separate lateral or ventrolateral openings

Table 1. Histological measurements (mean±SD; n=10) of proximal part of the caecum of quails at different ages

Age (days)	Parameters				
	Villus height (mm)	Villus width (mm)	Mucosal width (mm)	Sub mucosal width (mm)	Muscular width (mm)
10	0.23±0.02^{a}	0.09±0.01^{a}	0.40±0.06^{a}	0.09±0.01^{a}	0.06±0.01^{a}
20	0.30±0.01^{b}	0.08±0.01^{a}	0.36±0.04^{b}	0.11±0.01^{a}	0.07±0.01^{a}
30	0.35±0.02^{c}	0.09±0.01^{a}	0.45±0.04^{a}	0.12±0.02^{a}	0.12±0.01^{b}
40	0.32±0.03^{b,c}	0.11±0.01^{b}	0.37±0.02^{b}	0.45±0.04^{b}	0.09±0.01^{c}

Different letters are used to denote significant difference in a column (P<0.05).

Table 2. Histological measurements (mean±SD; n=10) of middle part of the caecum of quails at different ages

Age (days)	Parameters				
	Villus height (mm)	Villus width (mm)	Mucosal width (mm)	Sub mucosal width (mm)	Muscular width (mm)
10	0.80±0.09^{a}	0.59±0.06^{a}	0.85±0.02^{a}	0.06±0.01^{a}	0.88±0.06^{a}
20	0.81±0.07^{a}	0.66±0.05^{a}	1.03±0.02^{b}	0.06±0.02^{a}	0.84±0.03^{a}
30	0.85±0.08^{a}	0.61±0.03^{a}	0.89±0.02^{a}	0.06±0.02^{a}	0.88±0.02^{a}
40	0.19±0.01^{b}	0.92±0.01^{b}	1.31±0.02^{c}	0.15±0.02^{b}	0.91±0.07^{a}

Different letters are used to denote significant difference in a column (P<0.05).

Table 3. Histological measurements (mean±SD; n=10) of distal part of the caecum of quails at different ages

Age (days)	Parameters		
	Mucosal width (mm)	Sub mucosal width (mm)	Muscular width (mm)
10	0.05±0.01[a]	0.04±0.00[a]	0.6±0.01[a]
20	1.10±0.01[b]	0.04±0.02[a]	0.71±0.05[b]
30	1.38±0.03[c]	0.06±0.02[a]	1.27±0.01[c]
40	1.13±0.03[b]	0.04±0.00[a]	1.37±0.02[c]

Different letters are used to denote significant difference in a column ($p<0.05$).

into the colon (Clench & Mathias, 1995). Japanese quails have large caeca from the intestinal type as many other galliformes described by Naik (1962).

The caecum is exposed to continuous and constant invasion of bacterial or non-bacterial antigens of extra caecal origin, since it receives the back flowing urine from the urodeum of the cloaca through the colon. Therefore, immunological surveillance against foreign microorganisms is necessary (Kitagawa *et al*., 1998). Caecal tonsils, on which nearly half of the lymph nodules are accumulated, are major lymphoid tissue in the avian cecum. Kitagawa *et al.* (1998) found that 45.7% of lymph nodules are accumulated in caecal tonsils of 6-months old white leghorn chickens. A set of morphologically similar cells, M-like cells, are observed on chickens' caecal tonsil epithelium. These cells possess some histochemical and morphological qualifications of M cells, though their phenotype and function for absorption of foreign materials is not as developed and specialised as mammalian M cells (Jeurissen *et al*., 1989; Kato *et al*., 1992). Consistent with these findings, M-like cells were present in the epithelia of proximal part of caecum of quails at all sampling periods. We also observed that the histological feature of the proximal part of caecum changes with age where the width of sub mucosal layer increased during prepubertal period and reached its highest level at puberty. The thickest sub mucosa of middle part of caecum was also observed at puberty. These changes were accompanied by development and expansion of lymphoid tissue which was recognized as large follicles and well developed diffused lymphoid tissue in the proximal and middle parts of caecum, respectively. It may be concluded that the immune structure of the caeca of quails reaches its highest potential at puberty. Unfortunately, the fate of these changes in older quails was not investigated in the present study. It is believed that the interdigitating meshwork of villi at the caecal entrance acts as a filter, excluding large particles and allowing only fluid and fine particles to be separated and pushed from the colonic contents into the caeca by colonic anti peristalsis (Duke, 1986). On the other hand, increased villus height and area suggests an increased surface area capable of greater absorption of nutrients (Caspary, 1992; Awad *et al.,* 2006). Longer villi are associated with activated cell mitosis which can provide higher nutrient absorptive potential (Samanya & Yamauchi, 2002; Onderci *et al*., 2006). A morphometric study performed by Strong *et*

al. (1990) demonstrated that the quail caecum appears to have morphological characteristics consistent with a greater potential capacity for absorption than the fowl caecum. In the present study, the villi were widest at puberty in both proximal and middle parts of cecum. The length of villi remained constant in proximal part while it showed a significant decrease in middle part at puberty. It seems that by expansion of immune structures at puberty, the need for longer villi to filter particles has decreased and the increased width of these villi may preserve the high capacity for absorption. At the apex of the caecum, a continuous increase in the muscular layer width was observed at prepubertal period which remained statistically constant at puberty. This thick muscular layer can improve the ability of caecum to return the particles back to colon.

In conclusion, caecal immune structures of quails in prepubertal and pubertal ages expand by age and are more developed at puberty than in the prepubertal period. The structural ability of muscular layer of caecal apex is increased at puberty which may help in returning of particles back to colon when the chance for their entrance is increased due to short villi of its previous parts at this age.

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