IMMUNOHISTOCHEMICAL DETECTION OF CALBINDIN D28K AND OESTROGEN RECEPTOR IN THE SMALL INTESTINE OF PRE- AND POST- LAY HENS

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The knowledge that there is an increased requirement for dietary calcium (Ca$^{2+}$) after sexual maturation of layer hens is well established. This increase is reflected in an elevated enterocytes Calbindin D28K expression (a calcium binding protein essential for calcium transport) and an increased potential for the intestinal mucosa to absorb Ca$^{2+}$ in 25-week old hens as compared with 11 - 17 week old hens (Wu et al., 1993, 1994). However, it still remains to be clarified whether there is a direct action on the intestinal mucosa of the increased oestrogen level that is associated with sexual maturation. In order for a direct cellular action of this hormone to occur enterocytes must express oestrogen receptors. Thus, this study aimed to identify the temporal and spatial expression of Calbindin D28K and oestrogen receptors (ER) to evaluate the possibility of a direct oestrogen action on the small intestine.

Ten Isa Brown hens, 15 and 26 weeks of age (5 birds per group), were obtained from a commercial producer. Birds were killed by lethal injection (1 mL nembutal/ kg of body weight) after which the small intestine was rapidly dissected and rinsed with ice cold phosphate buffered saline. Two 1cm samples were taken from each of the duodenum, jejunum and ileum and fixed in either Carnoy’s or a 4% buffered formaldehyde for Calbindin D28K and ER localisation respectively. For immunolocalisation specific mouse monoclonal antibodies against Calbindin D28K (Sigma, Clone CI3000) and ER (Dako, Clone ID5) were used (Wu et al., 1993; Kusuahra and Tomoo, 1991). Non-specific mouse IgG was used as a negative control and all antibodies were detected using a rabbit anti-mouse IgG conjugated to horseradish peroxidase. Antibodies were used at a 1/100 dilution.

In both 15 and 26 week groups anti-Calbindin D28K immunoreactivity was detected in the enterocytes of duodenal tissue. However, in more distal regions of the small intestine the enterocyte expression of this calcium binding protein was confined to the 26 week group. In contrast, immunoreactivity against oestrogen receptors was observed in enterocytes from all tested regions of the small intestine, irrespective of the age of the bird.

In conclusion, in the distal intestine oestrogen receptor expression precedes that of Calbindin D28K thereby demonstrating that oestrogen has the potential to regulate Calbindin D28K expression in this tissue. Thus, increased oestrogen levels during sexual maturation may induce Calbindin D28K expression and hence modulate intestinal absorptive capacity for calcium.


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