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# Post Hatch Development of Alkaline Phosphatase Activity in the Broiler Small Intestine

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**Abstract:** This study was aimed to determine the alkaline phosphatase activity following time course (days) in the intestine of broilers. We used a sample of 54 subjects of broilers from hatching period to 56th day of age. Our finding showed a significant increase in the alkaline phosphatase activity at various levels in the different intestinal segments during the first two weeks of life and then decreased significantly with age ($p<0.05$). Moreover, alkaline phosphatase activity was higher in the duodenum and jejunum than in the ileum over the period examined. We concluded that alkaline phosphatase as a marker of enterocyte maturation, has a high enzymatic activity in the duodenum and jejunum of broilers.

**Key words:** Alkaline phosphatase, age, intestine, chicken, post hatch

## INTRODUCTION

The enzymes phosphatases are hydrolases whose substrates are phosphomonoesterase which are widely distributed in the nature and have been found in the animals (Galka *et al*., 1980; Lawrence and Vanetten, 1981), plants (Ferreira *et al*., 1999) and microorganisms (Gonzalez *et al*., 1993). One of the main groups of the family of phosphatases are alkaline phosphatases (Harrison *et al*., 1999). Alkaline phosphatase is a group of enzymes have catalytic activity for degradation of phosphate esters and separation of phosphoric acid molecules (Moog and Richardson, 1955; Knits, 2008).

In mammalians and chicken intestinal mucosal cytosol at brush border ends have considerable alkaline phosphatase activity (Japundzic *et al*., 1991). Moog (1951) reported that intestinal epithelium of chicken embryo didn't have Alkaline phosphatase earlier day 8 of embryonic life and Alkaline phosphatase became active after day 9-18 (Yoshimura *et al*., 2005; Yoshimura *et al*., 2009). Moog (1962) reports that alkaline phosphatase activity can arrive to peak at 2 or 2.5 day post-hatch.

Previous studies showed that alkaline phosphatase activity indicated maturity of intestinal cells and had a key role in long chain fatty acids and cholesterol digestion (Schussler, 1968). This study was devoted to clarify the changes of alkaline phosphatase activity in intestine of broilers chicken throughout the period of breeding.

## MATERIALS AND METHODS

**Animals and diet:** A total of 54 1-d-old Arbor-Acre broiler chickens vaccinated against infectious bronchitis were obtained from a commercial hatchery (Orac, Rouiba, Algerie). At each age, a sample of 6 subjects was taken randomly among a band of 3000 subjects of only one race, (chicken with fast growth). The animals were grown up on soil into a building with windows neither ventilated nor with air conditioned.

Animals were received water and a standard broiler diet on an *ad libitum* basis. The chickens were grown up from hatch to the eighth week under the same conditions of breeding.

**Experimental protocol and sample collection:** At each week of age, 6 chickens representative were selected (according to their weight). They were killed by slaughter. The digestive tract was removed from the beginning to the end of the intestine. The small intestine was divided into three segments: the duodenum (from gizzard to pancreo-biliary ducts), the jejunum (from pancreo-biliary ducts to Meckel's diverticulum)and the ileum (from Meckel's diverticulum to ileo-caecal junction).

To determine the intestinal alkaline phosphatase (ALP) activity, samples were taken from the middle section of each intestinal segment (duodenum, jejunum and ileum), were split longitudinally and rinsed with cold saline afterward.

**Enzyme activity assays:** The intestinal samples (duodenum, jejunum and ileum) were analyzed for enzymatic activity of alkaline phosphatase (ALP). The intestinal tissues were homogenized at a ratio of 50 mg/ml in phosphate buffer saline (Sigma, Germany) at pH 7.4 using a Moulinex Chopper (DPA 141) for 3 x 10 s and centrifuged (10 000 x g, 15 min, 4°C). The supernatants were subjected to enzyme assay immediately.

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The activity of ALP (EC 3.1.3.1) was determined by measuring spectrophotometrically (Autoanalyzar SN: 08091616 version 3.11), using p-nitrophenyl phosphate (SPINREACT, SA, Ctra. Santa Coloma « Girona » SPAIN) as a substrate. *The reaction was read at 405nm and unit of enzyme activity was defined as the amount of enzyme that released one µmol of p-nitrophenol/min, (Tietz, 1995). The values were expressed as the activity present in 1 mL of supernatant derived from the homogenate of 1 g of tissue.

**Statistical analysis:** All statistical analyses were performed using SPSS version 2.0 for Windows (SPSS Inc., Chicago, IL). Results were given as means±SE (n = 6). Data were compared by the one-way ANOVA and values were considered statistically different at $p<0.05$.

## RESULTS

The study of the evolution of alkaline phosphatase activity in the chicken according to the age during the post natal life was conducted on a sample of 54 subjects, from the first day of hatching until the age of slaughter. The results show that the activity of ALP in the three intestinal segments (duodenum, jejunum and ileum) increased significantly with age ($p<0.05$) from the first day of hatching until the 14th day and then it decreased considerably by the age to the 35th days of life chicken where it became almost unchanged until the age of slaughter in different portions of the intestine, « As shown in Fig. 1-3 ».

Additionally, our results showed, that the activity of alkaline phosphatase increased most dramatically form the first day of hatching till the 14th in the duodenum, compared with the jejunum and ileum, where the ALP activity in the ileum was lower, as compared with the duodenum and jejunum throughout the life period.

## DISCUSSION

The occurred variations in the functional development of the small intestine have been studied in various animals. In the present study, intestinal ALP activity was examined from 1 to 56 days post hatch at 3 sites in the broiler small intestine. ALP activity per gram of intestine was slightly changed with age in all 3 segments. The ALP activity in the ileum was lower as compared with the duodenum and jejunum during 56 d.

It has been confirmed that ALP is expressed in mature mucosal enterocytes and has been established as an enterocyte maturation marker (Weiser, 1973; Traber *et al*., 1991). During their migration and differentiation, enterocytes of numerous species increase expression of a variety of enzymes, including disaccharidase, alkaline phosphatase, hydrolase, amino-peptidase N and maltase (King *et al*., 1983; Semenza, 1986).

In our study, although the bird has not ingested feed at the time of hatch, intestinal alkaline phosphatase activity

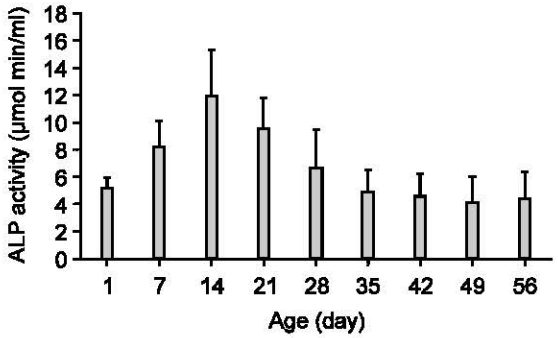


Fig. 1: Activity of Alkaline Phosphatase in 1 mL of supernatant derived from the homogenate of 1 g of tissue, in the duodenum of broiler with age ($p<0.05$)

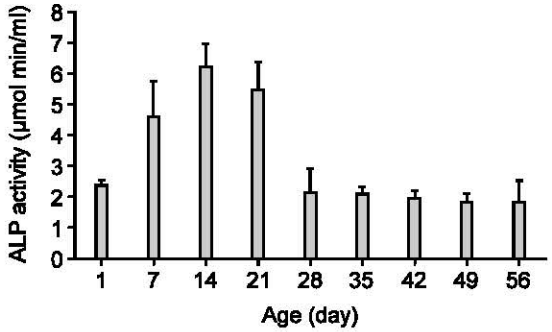


Fig. 2: Activity of Alkaline Phosphatase in 1 mL of supernatant derived from the homogenate of 1 g of tissue, in the jejunum of broiler with age ($p<0.05$)

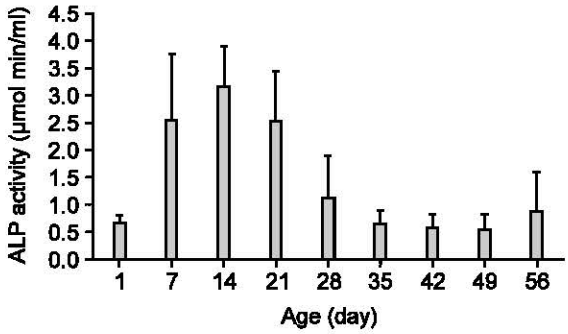


Fig. 3: Activity of Alkaline Phosphatase in 1 mL of supernatant derived from the homogenate of 1 g of tissue, in the ileum of broiler with age ($p<0.05$)

was present. Buddington (1992) reported the same data. The age of the chicken had also impacts on the gastrointestinal structure, dynamics and function. A tremendous amount of cellular differentiation and maturation occurs the first several days after hatching.

In addition the rate of epithelial cell migration along the villus also varies as a function of age. It appears that the migration rate is up to twice as fast in new hatchlings. (Imondi and Bird, 1966; Menge, 1983), reflecting in our study by the rapid increase in alkaline phosphatase activity during the first two weeks of life. Previous study showed that the general direction of development is anterior to posterior with the foregut being the most differentiated at the time of hatch (Romanoff, 1960). Moreover, in the immediate post hatch period, duodenal enterocytes may be more mature than enterocytes of the distal segments Uni *et al*. (1998). Thus, Jejunal and ileal enterocyte density was initially lower than that of duodenal.

While its results explain the changes of alkaline phosphatase activity observed in different segment of the intestine during our experimentation. Nir *et al*. (1978), Biviano *et al*. (1993), Jackson and Diamond, (1996) and Uni *et al*. (1999) have reported the same data in poultry.

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