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HISTOCHEMICAL AND BIOCHEMICAL CHARACTERISTICS OF WEANING RABBIT INTESTINE

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ABSTRACT: The aim of this research was to study rabbit digestive system at two ages during weaning by evaluating alkaline phosphatase (ALP) intestinal distribution and morphological, biochemical and haematological variables. Two groups of six New Zealand White × California rabbits, of 21 and 35 d of age respectively, were used. Significant differences between the two ages on live body weight (P<0.001), small intestine length (P<0.01) and pancreas weight (P<0.01) were noticed, being higher in 35 d rabbits. No differences were observed in the α-amylase activity of the jejunum. Nevertheless, α-amylase activity of blood serum was almost twice in 35-d rabbits compared to younger rabbits (P<0.05). Blood glucose concentration was lower in 35 d rabbits (P<0.05). The values of haematological profile were significantly higher in older rabbits for haematocrit (P<0.001), red blood cell count (P<0.05), haemoglobin concentration (P<0.001) and mean corpuscular volume (P<0.01). No differences due to age were observed for mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin. A strong ALP reaction was observed along the brush border of the villous epithelial cells of the small intestine at both ages, being this reaction more intense at 35 d. There was no ALP positive or a faint /discontinuous reaction along the brush border of the caecum at 21 d while a positive reaction was observed at 35 d. The proximal colon presented an ALP positive reaction at 21 d but stronger at 35 d of life while the colon distal was ALP negative at both ages.

Key words: Rabbit, intestine, α-amylase, alkaline phosphatase, haematological profile

INTRODUCTION

The rabbit is a monogastric herbivorous animal with a particularly well developed large intestine. As the rabbit develops from foetus to adult, marked changes of the intestine are observed related to milk feeding decrease and the onset of solid feeding and caecotrophy.

The effective intestinal adaptation of the kits during weaning is of great importance, influencing both animal health and growth performance. After weaning non-specific enteropathies arise in reared rabbits leading to a dysfunction in intestinal tract. Therefore, the period around weaning (20 to 40 days of age) is a very critical time for young rabbits (Xiccato *et al*., 2003). Up to 18-20 days, young rabbits ingest only milk while from this point onwards they begin to consume solid food as well as fermentative activity of caecum begins to develop and enzymatic digestive activities show important changes.

Many researches have been conducted concerning age-related changes in the morphology and enzymatic activity of digestive tract (Lebas *et al*., 1971 and 1972; Marounek *et al*., 1995; Dojana *et al*., 1998; Gutierrez *et al*., 2002; Debray *et al*., 2003). However, in spite of the existence of a large

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number of publications about its physiology, the domestic rabbit remains one of the species with incompletely known physiological characteristics concerning the digestive enzymatic system development.

Phosphatase enzymes are widely distributed in animal and plant tissues and works at different pH. Alkaline phospatase enzymes (ALP) have been chosen for this study because they are well connected with some broad aspects of small intestine physiology (Jervis, 1963) and the characterization of the intestinal regional differences for ALP can increase the knowledge of the intestine function in rabbits.

The aim of this research was to study the physiology of the developing intestine of rabbit at 21 and 35 d of age, corresponding to an early and a traditional weaning age, in order to extrapolate evidence of better comprehension of the physiopathology of pre- and ante-weaning digestive disorders. To this purpose, ALP intestinal distribution and morphological, biochemical and haematological variables were measured.

MATERIALS AND METHODS

Two groups of six weaning crossbreed (New Zealand × California) rabbits were used and sacrificed at 21 and 35 d respectively. The animals came from a rabbit parent stock vaccinated against haemorrhagic disease virus and they were healthy, a status that was confirmed by both *ante* and *post-mortem* examination.

The kits were under controlled lactation until 15 d of age, afterward they were free to suckle and had free access to solid food. The rabbits were fed *ad libitum* a commercial granulated feed specific for weaning rabbits containing alfalfa, soya-bean, wheat, barley, oats and a vitamin-mineral supplement. Water was also provided *ad libitum*.

Before slaughter (21 and 35 d of age), blood was sampled at 10:00 pm (two hours after feeding) from the major ear veins using heparin as anticoagulant and was analysed within 30 minutes using SYSMEX F-820 automating apparatus (Nemi, 1986; Van Kampen and Zijlstra, 1961 and 1965). Plasma was tested for the determination of α-amylase and glucose concentrations according to standard methods. The activity of α-amylase was measured using a spectrophotometer according to the instructions of the amylase kit of ELITECH diagnostics (cat. No AMYL-0030). Blood plasma glucose concentration was measured using the method of glucose oxidase (Trinder, 1969; Philips and Fuller, 1983).

The rabbits were euthanized using chloroform inhalation and then killed by cervical dislocation. The entire alimentary tract was removed. The intestine was unravelled, freed from mesentery and segments of 5 cm were taken from the middle part of the duodenum, jejunum, ileum, caecum and colon and fixed in 10% buffered formalin for 24 hours. Then, they were rinsed in tap water, dehydrated using increasing concentrations of ethanol solution and embedded in paraplast, sectioned at 4 µm of thickness and stained with a modified coupling Azo dye method for revealing the ALP presenting sites at the intestinal mucosa (Pearse, 1961).

Small parts of each animal jejunum (middle part of it) were removed and the jejunum epithelium was homogenized using ice cold Ringer solution (pH 7) for the determination of the α-amylase activity in the epithelium according to the kinetic method (Marshall *et al*., 1977, Marshall, 1978). This method was performed by using the non-natural substrate p-nitro-phenylmaltoheptaoside and the combined action of glucoamylase and α-glucosidase.

Statistical analysis was performed using the Statgraphics Statistical Graphics System, version 4.0 (Statgraphics, Rockville, MD, USA). Normality of data distribution was tested with Kolmogorov-Smirnov test. The data normally distributed were analysed using t-test. The data not normally

distributed were analysed by the Mann-Whitney test for comparing medians. For all tests, a *P*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The rabbits showed different haematological variables at 21 and 35 d of age for haematocrit ($P<0.001$), red blood cell count ($P<0.05$), haemoglobin concentration (80.2 *vs.* 130.7 g/L; $P<0.001$) and mean corpuscular volume ($P<0.01$) (Table 1). All values were within the normal range for the referred ages and in agreement with those reported by Kaneko (1989) and Suvegova *et al*. (2004).

The α-amylase activity in jejunum was not significantly different among ages. On the contrary, the α-amylase activity in blood plasma was twice at 35 d (6.27 *vs*. 3.44×10^3 IU/L, $P<0.05$) compared to that of 21d animals. According to other researchers (Kosa *et al*., 2004; Debray *et al*., 2001), α-amylase activity coming from pancreas in the intestine level in young rabbits significantly increases with age according to the changes in the composition of food.

These results concerning amylase are in agreement with those of Marounek *et al*. (1995), Dojana *et al*. (1998), Gutiérrez *et al*. (2002) and Debray *et al*. (2003) who found that amylase is already present from birth but markedly increase after weaning from day 24 onwards suggesting that the feed greatly stimulates amylase biosynthesis.

A significant difference was also found in blood glucose concentration being higher in 21d rabbits (11.81 *vs*. 8.96 mM/L; $P<0.05$). This is in agreement with the findings of Dojana *et al*. (1998) who also referred to the values of the blood glucose concentration that were the highest in 2 weeks-old rabbits and decreased significantly in 43 day-old rabbits.

A strong ALP reaction was observed along the brush border of the villous epithelial cells of the small intestine at both ages studied but the reaction was more intense at the age of 35 d (Table 2). There was no ALP positive reaction or a faint/ discontinuous one along the brush border of the caecum at 21 d while at 35 d a positive reaction was observed. As far as the proximal colon is concerned, at 21

Table 1: Morphological, biochemical and haematological variables of rabbits at 21 and 35 d of age

		Age of rabbits		*P*-value	RSD[1]
		21 days	35 days		
Live body weight (g)		400	1284	***	57
Length of small intestine (cm)		139	179	**	9
Pancreas weight (g)		0.09	0.70	**	0.16
Amylase activity	small intestine epithelium ($\times 10^3$ IU/L)[2]	114	146	NS	43
	epithelium tissue (IU/mg)	1465	1220	NS	325
	serum ($\times 10^3$ IU/L)	3.44	6.27	*	0.98
Blood glucose concentration (mM/L)[2]		11.81	8.96	*	1.26
Haematocrit (%)		27.4	44.4	***	1.7
Red blood cell count ($\times 10^6$)		4.38	5.79	*	0.49
Haemoglobin concentration (g/L)		80.2	130.7	***	5.5
Mean corpuscular haemoglobin concentration (%)		29.8	29.5	NS	0.9
Mean corpuscular volume (L)		64.6	76.6	**	2.4
Mean corpuscular haemoglobin (pg)		19.2	22.6	NS	1.6

[1]RDS: Residual standard deviation. [2]Mann-Whitney test. NS: not significant, *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 2: Mean ALP activity in rabbit small and large intestine[1]

	Age of rabbits	
	21 days	35 days
Duodenum	3+	4+
Jejunum	3+	4+
Ileum	3+	4+
Caecum	±	2+
Proximal colon	2+	3+
Distal colon	-	-

[1]Enzyme reaction: +mild, 2+positive, 3+strong, 4+very strong, -no reaction

d the rabbits presented a ALP positive reaction and a stronger one at 35 d, whereas the distal colon showed a ALP negative reaction at both ages.

These results concerning ALP activity are in accordance with the findings of Jervis (1963), Lafont and Moretti (1970), and Sabatakou *et al*. (1999). As far as the physiological role of ALP is concerned, it was thought earlier to be involved in sugar absorption and later that it played a role in cell adhesion as well as it was suggested of being a digestive enzyme (Crane, 1968). Moreover, a dual role of ALP has been hypothesised that is that of assisting differentiation (Deren, 1968) on one hand and partaking in digestive activity on the other.

Additionally, it has been established that ALP is expressed by active and mature mucosal enterocytes and is therefore indicative of enterocyte functional activity and is considered as an enterocyte maturation marker (Uni *et al*., 1998).

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