# WEANING AGE AND DEVELOPMENT OF THE SMALL INTESTINAL MUCOSA IN THE YOUNG RABBIT

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## ABSTRACT

The respective roles and interactions of intrinsic and extrinsic factors such as age and feed on digestive maturation of young rabbits are not yet completely identified. However, their knowledge is essential to determine the nutritional requirements of young rabbits around weaning, when they are more sensitive to digestive disorders. Twenty four litters of nine young rabbits were fed *ad libitum* from day 14 till day 49. They were weaned at either 21 (group W21, n=12) or 35 (W35 group, n=12) days of age, in order to modulate the solid feed intake. From day 14 till day 49, five young rabbits per treatment group were slaughtered weekly and pieces of duodenal, jejunal and ileal tissues were sampled and studied for villus-crypt architecture: villus height and width, as well as crypt depth were measured using a microdissection technique and a viewer analysis software system. Many changes in the intestinal mucosa occurred with increasing age irrespective of the weaning age. From day 28 to day 49, the intestinal villi heightened ($P<0.05$) at all sites (duodenum 746 µm on day 28 *vs.* 940 µm on day 49, jejunum 549 µm *vs.* 759 µm, ileum 357 µm *vs.* 570 µm (W21+ W35 together). A proximo-distal decreasing gradient in villus height was also apparent starting from day 28 ($P<0.05$). The crypts deepened from day 14 till day 49 ($P<0.05$). Villus shape, estimated by the height to width ratio, also changed with the beginning of significant solid feed intake. They became wider between day 21 and day 28 ($P<0.05$) at the duodenum (villus height to width ratio 3.7 *vs.* 2.2), jejunum (4.0 *vs.* 2.2) and ileum (4.0 *vs.* 2.4). From 21 till 35 days of age, the rabbits weaned at 21 days ate 57% more solid feed than those weaned at 35 days. However, the effect of weaning age on mucosal morphology was not significant. In conclusion, these results suggest that the maturation of the small intestinal mucosa is independent from solid feed intake level at least until day 35, but may be sensitive to the beginning of solid feed intake.

**Key words:** weaning age, villus, crypt morphology, maturation, small intestine.

## INTRODUCTION

The development of the intestinal epithelium and digestive capacity is driven by an intrinsic genetic programming but other factors, including diet and commensal flora, can

impact significantly on this development (KELLY and COUTTS 2000). In rabbits, the respective effects of age and feed intake on digestive maturation have scarcely been studied, particularly when the dry feed intake behaviour is developing. DOJANA *et al*. (1998) reported that lipase activity decreased between 15 and 43 days of age, in relation with the decrease in lipid intake (milk compared with solid feed). Fibre intake increases with age and leads to the development of the hindgut relatively to the anterior part, in relation with the development of microbial fermentation and cæcotrophy (GIDENNE and FORTUN-LAMOTHE 2002).
Despite increasing knowledge in gut morphology changes in young rabbits (CHIOU *et al.* 1994, YU and CHIOU 1997, SABATAKOU *et al*. 1999, GUTIÉRREZ *et al.* 2002), difficulties subsist to evaluate the respective roles of intrinsic factors such as age and extrinsic factors such as feed. However, the understanding of the gut maturation is essential to determine the nutritional requirements of young rabbits around weaning. Furthermore, the functional and morphological development of the intestinal mucosa is probably implicated in the sensitivity of young rabbits to digestive disorders. Indeed, the mucosa is a major site for digestion and absorption of nutrients, but also for interaction with the normal and pathogenic flora. One major factor potentially controlling this maturation is feed intake, that could be modulated through the weaning age.
The aim of this work was to investigate the impact of age and weaning age (21 *vs.* 35 days) on the architecture of the small intestine mucosa in young rabbits, using a procedure of microdissection to study villus and crypt morphometry.

## MATERIAL AND METHODS

### Animals, housing and feeding

Twenty four litters with nine young rabbits each at birth were reared in cages allowing the distribution of solid feed to them independently from their mother (FORTUN-LAMOTHE *et al.* 2000). The rabbits from 12 litters were weaned at 21 days (W21 group), while the weaning of other 12 litters occurred at 35 days (W35 group). From 14 days of age onward, all the young rabbits had *ad libitum* access to the same feed, which contained 189 g/kg (as fed basis) of acid detergent fibre and 178 g/kg of crude protein. The does were fed a commercial feed. They were moved to other cages at weaning, whereas the litters remained in the same cages until the end of the experiment. The does were inseminated 25 days after the kindling to avoid interactions between gestation and milk production processes. From 14 till 49 days, the rabbits were weighed individually and solid feed intake was measured per litter, weekly, before the suckling.

### Intestinal tissue sampling and histological analysis

From 14 till 49 days, series of slaughters by cervical dislocation were performed weekly. Until the first weaning (21 days), a rabbit in one out of two litters was slaughtered. After 21 days, a rabbit per litter was sacrificed in each weaning group. This rabbit was representative of the average litter weight, not adopted and without health problems. The slaughters were carried out three hours after suckling and/or solid feed removal, depending on the group and on the age, between 10:00 and 14:30. The litter size was

maintained constant all along the experiment by replacing dead (till day 21) or sacrificed (till day 28) rabbits using youngs of the same age and similar body weight. Small intestinal tissue samples of 1 cm in length were taken from the middle of the duodenum, jejunum and ileum (respectively 1/5, 3/5 and 1/5 of the total small intestine length). They were rinsed with saline solution of NaCl (9 g/L), immersed in a solution of buffered formalin for 12 to 24 h for fixation, and then stored in 90 % ethanol (v/v).
The samples of small intestine mucosa were analysed using a microdissection procedure (GOODLAD *et al.,* 1991). After progressive rehydration, the samples were stained with the Feulgen reaction using the Schiff reagent for 30 minutes, then stored in a 45% acetic acid water solution (v/v) before analysis. Villi and crypts were carefully individualised under a dissecting microscope (magnifying power: 40). Then, the preparations were mounted between slide and strip, with addition of an aqueous agent for microscopy (Aquamount®) if non-immediate analysis (jejunum and ileum samples). Length and width of twenty villi and ten crypts of Lieberkühn from each segment of the small intestine were measured in five animals per group and per age by using an optical microscope (Nikon Eclipse E600) and a viewer analysis software (Visilog 5.2), connected with a camera (Sony XC77E). Data were analysed for variance analysis using the GLM procedure of SAS (1999), including age and weaning age as the main effects.

## RESULTS AND DISCUSSION

### Zootechnical performances

The body weights of rabbits weaned at 21 days were smaller (-9%) than those of rabbits weaned at 35 days (figure 1) between the ages of 28 and 49 days. Also, daily weight gains were significantly lower in the early-weaned rabbits until day 42 (-17%, P<0.05), but not thereafter. Solid feed intake was significantly higher from day 21 to day 35 in early-weaned rabbits (+57%, figure 1).

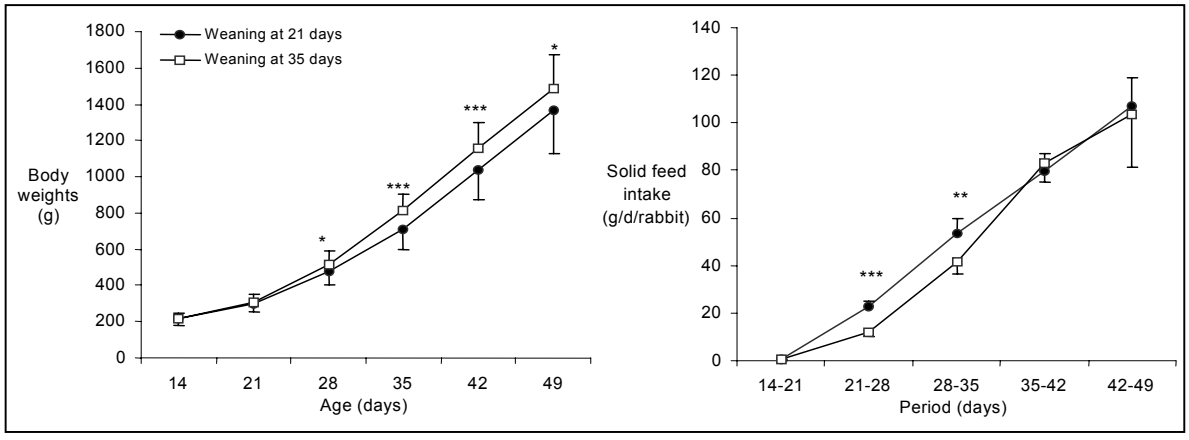


**Figure 1. Body weights and solid feed intake in young rabbits weaned at 21 or 35 days of age (*: P<0.05, **: P<0.01, ***: P<0.001)**

The higher solid feed intake in the early-weaned rabbits seemed not sufficient to compensate for the withdrawal of milk, and thus to cover their energy requirements. SCAPINELLO *et al*. (1999) also reported that a lower milk intake in litters of 10 *vs.* 4 pups stimulated solid feed intake before weaning, but also led to lighter rabbits. However, a compensatory growth often occurred, leading to similar body weights at the end of fattening (PASCUAL *et al*. 2001, DI MEO *et al*. 2003, GIDENNE and FORTUN-LAMOTHE 2004).

**Development of intestinal villi and crypts**

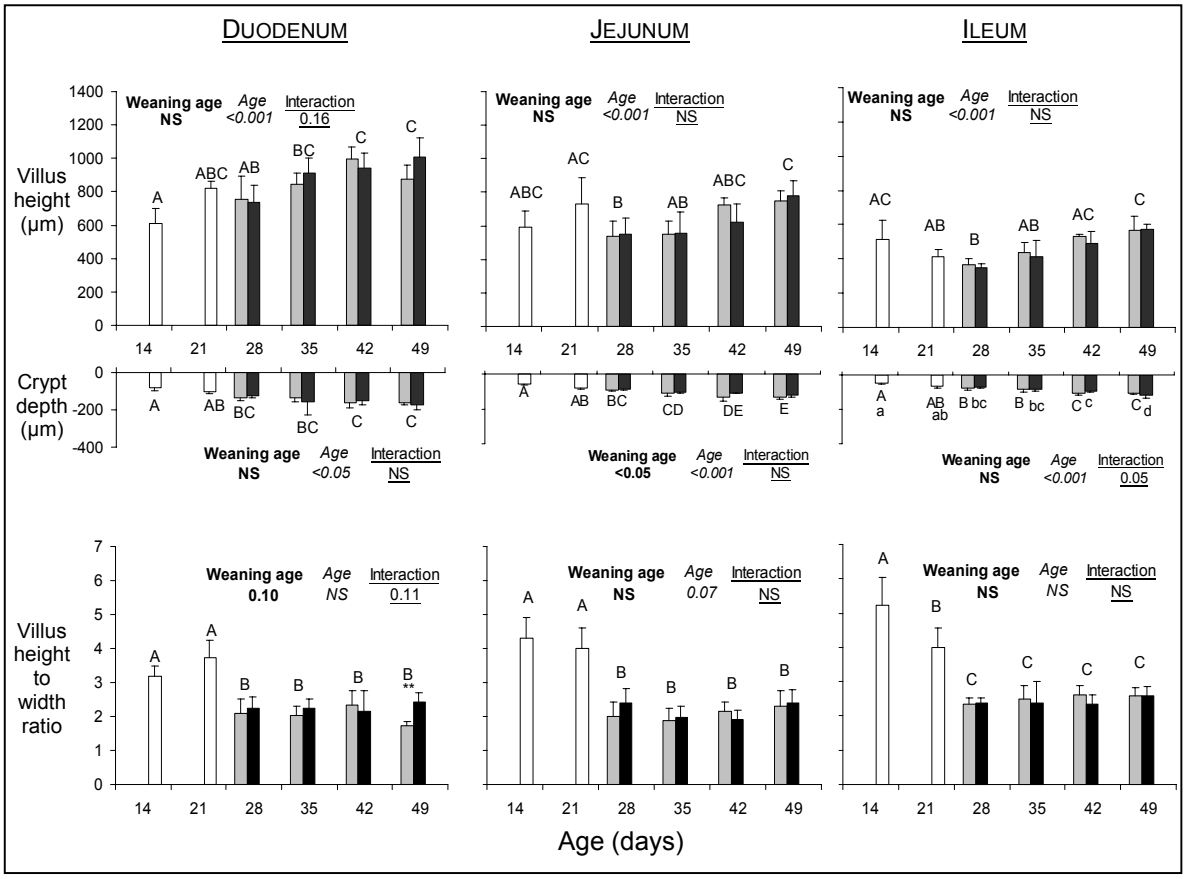


**Figure 2. Villus height, crypt depth and villus height to width ratio along the small intestine, in young rabbits weaned at 21 or 35 days of age.**

( □ Before Weaning, ▒ Weaning at 21 days, ■ Weaning at 35 days, means ± sd)

From 28 till 49 days of age, the variance analysis model included the effects of weaning age, age and their interaction (P values, NS: P>0.20); Age effects were analysed from day 14 to day 49 for W21 and W35 groups together (capital letters only) or separately if significant interaction between age and weaning age (capital letters for W21 group and small letters for W35 group): means without common letter differed significantly (P<0.05). Within each age, significant effect of weaning age is pointed out with stars (*: P<0.05, **: P<0.01, ***: P<0.001).

The villi lengthened and the crypts deepened with age from day 28 till day 49. In the ileum, crypt deepening seemed more progressive for rabbits weaned at 35 days, while they seemed to have a disrupted growth in rabbits weaned at 21 days (age × weaning age interaction, P = 0.0518). Villus height was not affected by age at weaning, whatever the segment (figure 2).
The villus height to width ratio informs about the villus shape. Weaning age had no significant effect on this ratio, whatever the segment considered. The villi of each intestinal segment were thin before day 28. Thereafter, they became only near twice longer than wide (average of 2.2, 2.1 and 2.5 in the duodenum, jejunum and ileum, respectively, from day 28 till day 49).
Comparing the small intestine segments, we observed a proximo-distal decreasing gradient in villus height from day 28 ($P < 0.05$), this irrespective of the weaning age.
There is very little literature dealing with villus height and crypt depth in young rabbits. Contrary to the present results, GUTIÉRREZ *et al*. (2002) observed shorter villi and deeper crypts on day 35 in the jejunum of rabbits weaned at 25 days compared with suckling rabbits.
Respect to villus and crypt shapes along the small intestine, YU and CHIOU (1997) found, for 14, 28 and 56 week-old rabbits, shorter duodenal and jejunal villi and shorter or longer ileal villi than in our study, and their data did not evidence any proximo-distal gradient in villus height. In contrast, the results of CHIOU *et al*. (1994) suggested a proximo-distal decreasing gradient in the small intestine of 13 week-old rabbits. Similar conclusions were reported by KEELAN *et al.* (1985), who observed longer villi in the jejunum than in the ileum, in 6 week-old rabbits.
Changes with age in villus shape, from finger- to leaf- and tongue-shaped, has previously been reported (YU and CHIOU 1997, VAN DER HAGE 1988). Here, this change in height to width ratio appeared with the beginning of a significant solid feed intake. For piglets, a shortening of villus is typically observed at weaning (PLUSKE *et al*. 1997) and could be explained by the anorexia provoked by the weaning, or by the withdrawal of growth factors present in milk and having a positive action on villus growth (Epidermal Growth Factor, polyamines, insulin and Insulin-like Growth Factor or L-Glutamine). However, in our study, milk intake did not seem essential for villus growth. An abrasion of villi by solid feed through mechanical action, transient hypersensitivity to some components, or the presence of some antinutritional factors may be suspected (PLUSKE *et al.* 1997). In this respect, CHIOU *et al*. (1994) have previously reported that fibre supplementation would be harmful to intestinal villi in adult rabbits.

## CONCLUSIONS

These results suggest that the solid feed intake level had little impact on the development of small intestinal villi and crypts. Until day 35, the development seemed more dependant on ontogenetic factors, with probably an impact of the beginning of solid feed intake, independently of its level. Further study comparing exclusively milk-fed rabbits and early-weaned rabbits might allow to elucidate the role of the solid feed on mucosa maturation. In prospect, morphological changes in the mucosa should be completed with data on the digestive capacity of the young rabbit.

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