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EFFECTS OF A ONE WEEK FEED RESTRICTION IN THE GROWING RABBIT
Part 2: DEVELOPMENT OF THE DIGESTIVE SYSTEM

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ABSTRACT

The objective of the study was to evaluate changes in growth and morphology of intestines and liver depending on feeding regime and age of growing rabbits. Hyplus rabbits (192 rabbits weaned at 35 days) were divided into 3 groups of 64. Group 1 was fed *ad libitum*, group 2 (R50) was restricted from 42 to 49 days of age (50 g per day per rabbit), and group 3 (R65) was restricted from 42 to 49 days of age (65 g). Rabbits were fed *ad libitum* from 35 to 41 days of age and from 50 days till the end of the experiment at 70 days of age. From the age 49 days, 8 rabbits from a group were slaughtered in a week interval for intestine and liver characteristic determination. Length of intestines increased from 49 to 70d old, in small intestine from 275 to 340 cm, large intestine from 98 to 139 cm and in caecum from 34 to 39 cm. Liver weight was at the age of 42 days 62 g and at 70 d 116 g. Feed restriction ($P \leq 0.01$) affected length of small intestine with the highest values in the group R65 (difference between ADL and R65 group was 21 cm). Highly significant interactions of feeding regime and age were determined in *villi* height, (the lowest values in the group R65, 448 µm at the age of 49 d and the highest in the same group but at the age of 56 d, 630 µm), crypts depth ($P \leq 0.001$; the highest and lowest depth of crypts were in the group R65 at 56 d 150 µm and 63 days of age 108 µm). Significant interactions of age and feeding regime ($P \leq 0.001$) in hepatocytes diameter revealed the smallest diameter in the ADL group and in the group R50 g, 19.2 µm at the age of 49 d and the largest in the R50 at the age of 70 d 25.2 µm.

Key words: Rabbit; feed restriction; intestine and liver morphology

INTRODUCTION

The effect of feed restriction in growing rabbit has been studied in recent years and is oriented on different aspects of the effect of duration, intensity and time of beginning on growth, meat quality and health (Gidenne *et al*., 2012). Martignon *et al.* (2010) suggested that the feed restriction is important around the weaning period, when the gut is developing and maturing and depends on nutrient intake. Gidenne *et al.* (2009) revealed that restriction of the young rabbits in three weeks after weaning is associated with a lower incidence of post weaning digestive troubles. Feed restriction in rabbits affects growth of internal organs. At the beginning of realimentation period, the stomach grew very rapidly and after 7 days of realimentation in the restricted group, all organs except kidneys had grown to the same size or higher as in the *ad libitum* fed rabbits of the same weight (Ledin, 1984). Tůmová *et al.* (2007) reported that restriction reduced weight and length of intestines but the first week after restriction found higher weight in small intestine. Mean retention time of solid phase increased by 50% for restricted animals (60% of ad-lib.) compared to the *ad libitum* fed ones (Gidenne and Feugier (2009). Therefore, it could be assume that feed restriction might modify intestine morphology, although Martignon *et al*. (2010) did not revealed the effect of feed restriction on of ileal *villi* and crypts.

The objective of the study was to evaluate changes in growth and morphology of intestines and liver depending on feeding regime and age of growing rabbits.

MATERIALS AND METHODS

The experiment used 192 Hyplus rabbits (male and female ratio 1:1) from 35 to 70 days of age, and placed in collective wire mesh cages with a floor density of 0.16 m^2 per rabbit. At weaning (35d), rabbits were divided into 3 groups of 64. Group 1 was fed *ad libitum*, group 2 (R50) was restricted from 42 to 49 days of age (50 g per day per rabbit, 29 % ad libitum), and group 3 (R65) was restricted from 42 to 49 days of age (65 g, 37 % ad libitum). In the first week of the experiment, and after restriction, *ad libitum* feeding followed up to the end of the experiment. During the experiment a commercial pelleted feed was used, composition of the diet is in the part 1 of this study. A twelve-hour photoperiod was used (8-20 h). Water was available *ad libitum*. After restriction, from the age 49 days, 8 rabbits from a group were slaughtered always at the same time, 9 a.m., in a week interval and intestine or liver characteristic were measured. Rabbits of all groups were slaughtered on similar weight. Immediately after slaughtering, rabbits were eviscerated and length of small, large intestine and caecum was measured. Small intestine was measured from duodenum to junction of ileum, caecum and colon, large intestine included proximal and distal colon, caecum was without appendix. At the same time liver was weighed. In small intestine, height of *villi* and depth of crypts were determined. Subsequently, a sample approximately one cm^3 in size was taken from the centre of the liver parenchyma and one sample was taken from each animal, from the duodenum 20 cm under the stomach. One sample was taken from each animal. The samples were fixed with a 4% Bouin solution for histological analysis. All the samples were collected within 30 minutes after slaughtering and they were fixed for one week. The samples were processed by standard histological methods. From three to five-µm-thick slices were cut from each sample, and afterwards the slices were stained with haematoxylin-eosin (HE). The prepared samples were evaluated in a light-microscopic picture using a Nikon Eclipse E600 microscope (Nikon Corporation Instruments Company, Japan). The diameter of hepatocytes and the length of *villi* and the depth of crypts were measured using the NIS-Elements version 3.0 software. The data were analysed using the SAS program (SAS Institute Inc., Cary, NC, 2003) and the ANOVA method. Two-way analysis of variance with interactions of group and age was also used.

RESULTS AND DISCUSSION

Length of small intestine (Table 1) was affected by restriction feeding ($P \leq 0.01$) and increased with age ($P \leq 0.001$) from 275 cm at 49 d to 340 cm at 70 d of age without interaction. Rabbits of both restricted groups had ($P \leq 0.01$) longer small intestine (about 5% in R50 and 7 % in R65) than those fed *ad libitum*. The longest intestines were in group which received 65 g of feed at the end of the restriction (285 cm) and also in following *ad libitum* feeding period (341cm). These results are in contrast with our previous experiment (Tůmová *et al*., 2007) when we detected shorter small intestine about 7% in restricted rabbits. The interaction ($P \leq 0.001$) between effect of feeding level and age was detected for *villi* height. At the end of restriction period (49d) *villi* were the highest (506 µm) in the group with the strongest feed limitation (50 g per day and rabbit); however, after a week of realimentation the longest *villi* (630 µm) were determined in the group with medium restriction (65 g per day and rabbit). At the end of the experiment (70d), the highest *villi* were in *ad libitum* fed rabbits (614 µm). Martignon *et al.* (2010) revealed non significantly lower *villi* height in restricted rabbit than in the *ad libitum* fed ones. Result might have been affected by intensity of restriction because in our study, the group with medium restriction had the shortest *villi* and duration of restriction one week. Also in depth of crypts, the interaction between restriction and age was highly significant. In contrast with Martignon et al. (2010), who did not determined interaction of feeding and age, crypts were deeper in restricted rabbits (129 µm in R50 and 131 µm in R65) compare to *ad libitum* fed rabbits (123 µm). The lowest values of crypts depth (108 µm) were at 63 days of age and the highest (148 µm) at 70 days of age in the restricted group which received 65 g.

Significant interaction between restriction and age ($P \leq 0.05$) in length of large intestine (Table 2) resulted in the shortest large intestine in *ad libitum* fed rabbits at the end of restriction (81 cm) but the longest was at the age of 70 days in the same group (141 cm) and group which received 50 g feed (140 cm). Previously Tůmová *et al*. (2007) reported a similar trends of significant interaction of feeding and age. Length of caecum and liver weight were significantly ($P \leq 0.001$) increased with age which

Table 1. Growth and small intestine characteristics according to age and to a one week hard feed restriction

Group	Age (day)	Live weight (g)	Length of small intestine (cm)	Height of *villi* (µm)	Depth of crypts (µm)
Ad libitum	49	1624	265	466[g]	123[de]
	56	2237	308	516[f]	126[d]
	63	2526	326	578[d]	121[de]
	70	2915	338	614[ab]	121[de]
R50	49	1660	275	506[f]	120[de]
	56	2294	341	501[f]	142[b]
	63	2501	343	542[e]	117[e]
	70	2713	342	601[bc]	134[c]
R65	49	1751	285	448[g]	115[ef]
	56	2271	347	630[a]	150[a]
	63	2510	346	591[cd]	108[f]
	70	2744	341	601[bc]	148[ab]
SEM		158	8	128	49
Significance					
Group		0.135	0.007	0.001	0.001
Age		0.001	0.001	0.001	0.001
Group * age		0.151	0.604	0.001	0.001

a,b,c,d,e,f : Within a column, means having a common superscript did not differ at the level P ≤ 0.05; SEM – standard error of the mean

corresponds with our earlier findings (Tůmová *et al.*, 2007) or Ledin (1984) or Gidenne *et al.* (2012). High significant interactions were found for hepatocytes diameter, that were were larger in both restricted groups and increased with age. However, the significantly largest diameter was in the group fed by 50 g of feed at 70 days of age (25.2 µm) but the smallest in the same group and *ad libitum* fed rabbits at the end of feed restriction period (19.2 µm). These results are not comparable with literature because of the lack of data.

Table 2. Large intestine and liver characteristics in the growing rabbit according to age and to a one week hard feed restriction.

Group	Age in days	Length of large intestine (cm)	Length of caecum (cm)	Liver weight (g)	Hepatocytes diameter (µm)
Ad libitum	49	81[f]	32.8	62	19.2[e]
	56	113[d]	37.8	94	21.7[e]
	63	123[c]	36.8	100	22.4[d]
	70	141[a]	38.7	114	24.4[b]
R50	49	107[e]	34.8	58	19.2[e]
	56	126[c]	37.8	120	22.7[d]
	63	125[c]	37.5	111	23.5[c]
	70	140[a]	39.5	117	25.2[a]
R65	49	106[e]	34.9	66	19.8[de]
	56	133[b]	39.6	110	22.2[d]
	63	130[bc]	37.7	118	22.8[cd]
	70	138[ab]	38.8	116	23.9[bc]
SEM		149	2.5	17.9	4.2
Significance					
Group		0.001	0.144	0.059	0.001
Age		0.001	0.001	0.001	0.001
Group * age		0.043	0.775	0.292	0.001

a,b,c,d,e : SEM : see table 1

CONCLUSION

On the base of the results it is possible to assume that a one week hard feed restriction modify intestinal morphology of the growing rabbit. Small intestine was longer, *villi* higher and crypts deeper in restricted rabbit. Large intestine length increased during restriction and was higher till 63 days of age. Hepatocyte diameter enlarged in restricted rabbits compared to the *ad libitum* fed ones. However, some contrasted results from literature revealed that the effect of restriction is dependent on duration, intensity and time of application and further research is needed to get more detailed data.

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