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Short Communication

Effect of feed restriction on organs and intestinal mucosa of growing rabbits

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ABSTRACT - This experiment was carried out to evaluate the effect of feed restriction on internal organs with respect to their weight and size, and the development of the intestinal mucosa of growing rabbits. Sixty 33-day-old New Zealand White rabbits were used in a randomized block with four treatments and five blocks. The treatments consisted of *ad libitum* feeding; feed restriction from 33 to 40 days of age; feed restriction from 54 to 61 days of age; and feed restriction from 33 to 40 and from 54 to 61 days of age. There was no effect of feed restriction on weight, length and width of internal organs, except for heart weight, which decreased when feeding was restricted from 54-61 days and from 33-40 and 54-61 days of age. There was no effect of feed restriction at the different ages on duodenal morphometry. In jejunum, the villi of rabbits fed *ad libitum* or restricted from 33 to 40 days were higher, and rabbits restricted from 33-40 days had wider villi and higher absorption surface. In the ileum, only rabbits fed *ad libitum* had higher villi. Feeding restriction reduced heart weight, but not its size, and negatively affected jejunum morphometry when performed in later stages.

Key Words: animal nutrition, compensatory growth, digestible system in rabbits

Introduction

Weaning can be a stressful period for the kits due to separation from the dam, the milk withdrawal, and the adaptation to the new solid feed. These changes can increase the susceptibility of rabbits to diseases (Gallois et al., 2008).

The quantitative feed rationing for growing rabbits is a practice in many countries, because it reduces the incidence of digestible disorders, particularly epizootic rabbit enteropathy (Laurence et al., 2003). In rabbit production, specific and nonspecific enteropathies are always a concern, leading to animal losses of approximately 30% from birth to slaughter (Gidenne & Fortun-Lamothe, 2002). This practice, in addition to the abovementioned benefits, also allows for feed economy and reduced fat content in the carcass.

Feed restriction can result in several metabolic changes that lead to lower body weight, immunodepression and modified function of the digestive system, especially the liver and small intestine. These changes affect the enzyme activity in the brush border, mucosa cell mass, protein content and mucosa integrity. Refeeding, however, can rapidly restore the morphology and functions of the intestine, repairing the intestinal atrophy and normalizing the permeability of the mucosa (Ortega et al., 1996).

This research was carried out to evaluate the effect of feed restriction on internal organs with respect to their weight and size and the intestinal mucosa in growing rabbits.

Material and Methods

The experiment was carried out in the Rabbit Sector of Centro Federal de Educação Tecnológica in Rio Verde, Goiás, Brazil, from January to August 2008, using 60 New Zealand White rabbits, initial average weight 636.76±13.28 g, weaned at 33 days and slaughtered at 81 days of age. A randomized block design was used with four treatments and five replications. The blocks were conducted at different times and each block was formed by one replication of each treatment. The treatments were as follows: free feeding (FF); feed restriction (FR) from 35 to 40 days of age (50 g/d.rabbit); FR from 54 to 61 days of age (90 g/d.rabbit); and FR from 33 and 40 days (50 g/d.rabbit) and 54 to 61 days of age (90 g/d.rabbit). The rabbits fed freely at all other times.

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The rearing system was in open air and animals were kept in groups of three (two females and one male) in cages with masonry sides, measuring 0.80 × 0.75 × 0.67 m (length × width × height), equipped with ceramic feeding trough and drinker. Water was supplied *ad libitum* and the commercial pellet feed (17% crude protein, 15% crude fiber, 2% calcium, 0.75% phosphorus, 0.94% lysine, 0.63% methionine + cysteine and 2300 kcal digestible energy/kg) was supplied according to the feeding regime of each treatment.

When the rabbits reached 81 days of age, they were feed-deprived for 12 hours and one rabbit of each replicate was slaughtered and its heart, lungs, stomach, liver, kidneys, spleen and small intestine were collected. These organs were weighed and measured for length and width.

Small intestine samples of approximately 3 cm were collected from the duodenum (10 m from the stomach), the jejunum (in the middle region of the small intestine) and the ileum (at 10 cm from the ileocecal junction). The samples were fixed in 10% formaldehyde for 24 hours and fragments were processed for blocking in paraffin. 5-micrometer seried cuts were made in each fragment stained with hematoxylin and eosin. Thirty readings were taken per fragment for villus height, perimeter and width, crypt depth, and villus height/crypt depth rate. The absorption surface (AS) was calculated by the formula:

AS (mm^2) = villus height (mm) × width at half of the villus height (mm)

The results were subjected to analysis of variance using SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1), and the Tukey test was used to compare the means, at 5% of probability.

Results and Discussion

There was no effect (P>0.05) of FR on the organ weights, except for heart weight, which was lower (P<0.05) with FR from 54 to 61 days, and from 33 to 40 and 54 to 61 days of age (Table 1). Reduction in the weight of some organs, besides the heart, over the FR period is possible; however, the weight and size of organs reached the normal values with refeeding, not differing from the animals fed *ad libitum.* According to Burrin et al. (1988), the lower protein content in the cells, followed by reduction in cell size caused by FR, could be responsible for the lower heart weight.

According to Pálsson (1955) vital organs such as brain, lungs, kidneys, heart, esophagus, abomasum and small intestine are proportionally more developed at the birth time and, as a consequence, grow up proportionally less in the postnatal life. This different organ growth during the prenatal period modulates the effects of restriction on the organ development after the birth. Organs with precocious growth are less affected by the postnatal restriction, while the ones with belated growth are less affected by the prenatal restriction (Geraseev et al., 2008).

Table 1 - Weight and measurements of organs in growing rabbits subjected to feed restriction

Parameter	Treatments				CV (%)	P-value
	FF	R33-40	R54-61	R33-40/54-61		
Intestine weight (g)	59.00	59.00	57.50	61.25	3.67	0.9839
Intestine length (cm)	258	266	251	269	3.76	0.8112
Stomach weight (g)	17.50	19.66	18.73	18.84	2.89	0.7431
Stomach length (cm)	6.90	6.55	6.48	6.95	1.11	0.1625
Stomach diameter (cm)	3.45	3.85	3.95	3.76	3.74	0.5637
Liver weight (g)	47.48	50.04	50.52	52.96	2.68	0.9341
Liver length (cm)	10.16	10.08	10.60	10.36	1.76	0.8523
Liver diameter (cm)	8.42	9.07	9.00	9.30	1.36	0.2845
Kidney weight (g)	11.74	13.35	11.67	13.00	2.83	0.3348
Kidney length (cm)	2.99	3.01	3.07	3.00	1.54	0.8722
Kidney diameter (cm)	1.93	2.11	2.06	2.18	1.20	0.2330
Heart weight (g)	5.06a	4.76ab	4.43b	4.32b	3.39	0.0485
Heart length (cm)	3.10	2.72	2.78	2.67	2.22	0.1144
Heart diameter (cm)	1.96	1.85	1.95	1.82	2.33	0.3379
Lung weight (g)	10.89	10.21	8.92	11.19	4.16	0.3249
Lung length (cm)	4.43	4.15	3.73	4.70	2.50	0.1012
Lung diameter (cm)	5.20	4.61	5.13	5.48	1.88	0.3361
Spleen weight (g)	0.64	1.01	0.68	0.66	4.46	0.2672
Spleen length (cm)	3.70	4.43	3.96	4.70	3.11	0.1303
Spleen diameter (cm)	0.56	0.70	0.60	0.53	5.78	0.1879

FF - free feeding; R33-40 - feed restriction from 33 to 40 days of age; R54-61 - feed restriction from 54 to 61 days of age; R33-40/54-61 - feed restriction from 33 to 40 and from 54 to 61 days of age.
CV - coefficient of variation obtained with transformed means (log (X + 1).
Means followed by different letters differ by the Tukey test.

Normally, viscera show fast responses to the FR by the reduction in their sizes and metabolic activities (Lawrence & Fowler, 2002), however, in this study, there was only reduction of heart weight. It is possible that a priority is given to the internal organ maintenance in periods of feed scarcity (Tumová et al., 2006).

Tumová et al. (2003) also did not report differences in the absolute weight of the lungs, kidneys and liver in rabbits subjected to FR at different ages; however, there was a higher absolute heart weight in restricted animals. Studying the FR effects in rabbits, Tumová et al. (2007) observed that, with FR, kidney weight was reduced in restricted rabbits as compared with the rabbits fed *ad libitum*. However, the liver had its weight reduced due to FR, but its weight was similar to the rabbits fed *ad libitum* after refeeding.

Ledin (1984) reported that the internal organs in rabbits were affected by the FR of 40 and 50%, compared with the *ad libitum* feeding, and also by the refeeding. At first, in refeeding, the stomach grew rapidly and, after seven days of refeeding, all the organs, except for the kidneys, had grown up and reached the same size or were higher than in non-restricted animals. According to Tumová et al. (2007), FR reduced the intestine weight and size but, one week after refeeding, intestine weight had increased and, at the end of the experiment, there was no difference in weight among the animals, but the small intestine was longer in rabbits fed *ad libitum.*

Mazeti & Furlan (2008) verified a higher relative weight and length of small intestine in restricted animals, compared with control. The authors considered that there was a morphological adaptation to feed deprivation and the larger intestinal area should improve the intestinal absorptive capacity, allowing the animal to absorb much more available nutrients in the limited diet. Mataloun et al. (2006) subjected rabbits do FR and noted a reduction in lung volume, due to reduction of the lung alveolus.

There was a great variation in the results found in the literature, probably due to the differences in the restriction level, duration and period when FR is performed.

There was no effect (P>0.05) of FR at the different ages on duodenal morphometry (Table 2), crypt depth, villus height/crypt depth in jejunum (Table 3) and in ileum, or on villus width in ileum (Table 4). There are reports about decrease in villus width and perimeter during the FR period, but this effect was only visible in the jejunum and ileum mucosa.

Rabbits fed *ad libitum* or restricted from 33 to 40 days of age showed higher (P<0.05) villus height and perimeter and rabbits restricted from 33 to 40 days had larger villus and higher absorption surface (P<0.01) than the non-restricted rabbits or the ones restricted from 54 to 61 days

Table 2 - Duodenal morphometry of growing rabbits subjected to feed restriction

Parameter	Treatments				CV (%)	P-value
	FF	R33-40	R54-61	R33-40/54-61		
Villus height (µm)	3141	3059	2916	3209	1.15	0.3241
Villus perimeter (µm)	6244	6468	6158	6338	0.87	0.2418
Villus width (µm)	362	339	364	357	4.33	0.1713
Crypt depth (µm)	180	207	187	192	3.60	0.2133
Villus height/crypt depth	17.44	14.71	16.04	17.41	7.42	0.2814
Absorption surface (mm^2)	0.113	0.104	0.106	0.115	8.02	0.1029

FF - free feeding; R33-40 - feed restriction from 33 to 40 days of age; R54-61 = feed restriction from 54 to 61 days of age; R33-40/54-61 - feed restriction from 33 to 40 and from 54 to 61 days of age.
CV - coefficient of variation obtained with transformed means (log (X + 1).

Table 3 - Jejunum morphometry of growing rabbits submitted to feed restriction

Parameter	Treatments				CV (%)	P-value
	FF	R33-40	R54-61	R33-40/54-61		
Villus height (µm)	2262a	2633a	1711b	2016b	2.19	0.0438
Villus perimeter (µm)	4662a	5686a	3877b	3973b	2.02	0.0387
Villus width (µm)	282b	358a	265bc	216c	2.44	0.0022
Crypt depth (µm)	192	172	128	130	5.68	0.1913
Villus height/crypt depth	12.03	16.74	13.35	15.98	9.75	0.2368
Absorption surface (mm^2)	0.063b	0.095a	0.045c	0.044bc	9.91	0.0013

FF = free feeding; R33-40 = feed restriction from 33 to 40 days of age; R54-61 = feed restriction from 54 to 61 days of age and R33-40/54-61 = feed restriction from 33 to 40 and from 54 to 61 days of age.
CV = coefficient of variation obtained with transformed means (log (X + 1).
Means followed by different letters differ by Tukey test.

Table 4 - Ileum morphometry of growing rabbits subjected to feed restriction

Parameter	Treatments				CV (%)	P-value
	FF	R33-40	R54-61	R33-40/54-61		
Villus height (μm)	2109a	1661b	1581b	1883b	2.81	0.0038
Villus perimeter (μm)	4521a	3590b	3273b	3979b	2.34	0.0028
Villus width (μm)	218	248	188	222	2.31	0.3418
Crypt depth (μm)	178	152	158	194	2.97	0.2300
Villus height/crypt depth	12.95	10.95	10.08	9.96	9.11	0.1389
Absorption surface (mm^2)	0.046	0.041	0.030	0.042	8.42	0.0894

FF - free feeding; R33-40 - feed restriction from 33 to 40 days of age; R54-61 - feed restriction from 54 to 61 days of age; R33-40/54-61 - feed restriction from 33 to 40 and from 54 to 61 days of age.
CV - coefficient of variation obtained with transformed means (log (X + 1).
Means followed by different letters differ by Tukey test.

or from 33 to 40 and 54 to 61 days of age in the jejunum mucosa (Table 3).

In ileum, rabbits fed *ad libitum* showed higher (P<0.04) villus height and perimeter compared to the animals of the other treatments (Table 4).

Feed restriction can result in lower villus perimeter and lower crypt depth, in addition to decreasing the enzymatic activity in the enterocytes. It is possible that in duodenum, refeeding contributed to the recovery of the mucosa hypoplasia, and since this segment is responsible for most of the digestion and absorption processes, its importance is so great that, in birds, duodenum weight is positively associated with body weight (Wijtten et al., 2010).

Several authors have suggested that the nutrient digestibility is increased during the FR period (Di Meo et al., 2007; Tumová et al., 2008). According to Gilbert et al. (2008), nutrient digestibility is improved because there is an increase in the number of peptides, amino acids and glucose transporters and also in enzyme production caused by FR. It is likely that, due to the higher nutrient digestibility in the initial part of the small intestine, demand for tissues in jejunum and ileum were lower.

In addition, young animals have higher nutrient requirements and their intestinal mucosa recovers faster in terms of villus and crypt development during the refeeding period. Since older animals have a lower nutritional requirement, there is no reason for the intestinal mucosa to increase with refeeding, since the nutrients absorbed by the mucosa with a smaller villus would meet the animal needs.

Conclusions

Feeding restriction at both ages caused reduction in ileal villus and, when performed later (54 to 61 days of age) decreased heart weight and villus size and absorptive surface of the jejunum.

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