PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

Session
Nutrition & Digestive Physiology

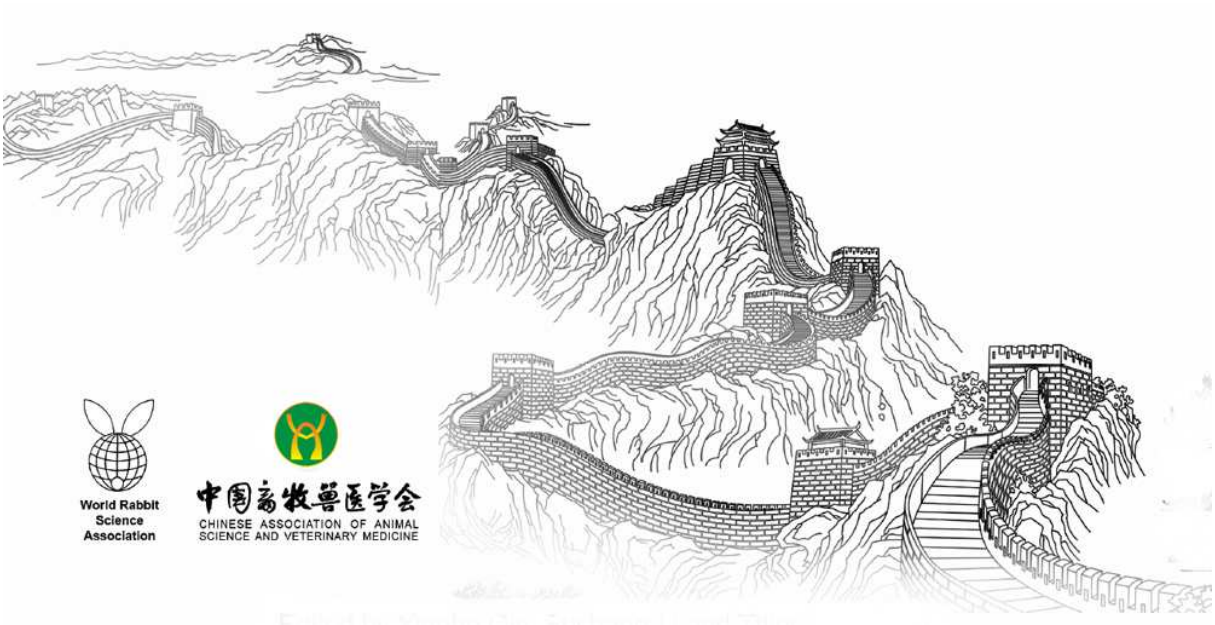
Bălăceanu R.A., Raită Șt., Tobă G.L., Stoica L., Dojană N.

SERUM DIGESTIVE ENZYME ACTIVITY BEFORE AND AFTER WEANING IN RABBITS FED ON PROTEIN, STARCH OR FIBER ENRICHED DIETS.

Full text of the communication

How to cite this paper :
Bălăceanu R.A., Raită Șt., Tobă G.L., Stoica L., Dojană N., 2016 - Serum digestive enzyme activity before and after weaning in rabbits fed on protein, starch or fiber enriched diets. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 265-268.*

.



SERUM DIGESTIVE ENZYME ACTIVITY BEFORE AND AFTER WEANING IN RABBITS FED ON PROTEIN, STARCH OR FIBER ENRICHED DIETS

Bălăceanu R.A.[1]*, Raită Șt.[1], Tobă G.L.[2], Stoica L.[3], Dojană N.[1]

[1]Department of Preclinical Sciences, Faculty of Veterinary Medicine (FVM), University of Agronomical Sciences and Veterinary Medicine of Bucharest (USAMVB), 105 Independentei str., Bucharest 050097, Romania ;
[2] Baroul Bucuresti, 3 Râureanu str., Bucharest 050047, Romania ; [3]Department of Nutrition, FMV, USAMVB, 105 Independentei str., Bucharest 050097, Romania
*Corresponding author: rosaliebalaceanu@fmvb.ro

ABSTRACT

Serum activities of amylase, lipase and trypsin were assayed in 22-day age suckling rabbits and in a control and three experimental rabbit groups, at 1 day after weaning (30 days of age), 8 days and 15 days from weaning. Control group was fed on a standard diet for weaned rabbits and the three experimental groups were fed on diets enriched in protein, starch and fiber, respectively. Specific amylase activity was about 3 times higher in 1-day weaned rabbits vs. 22-day old suckling rabbits. Two weeks later, they were found significant differences ($P < 0.001$) in serum amylase activity according to diet composition: high starch diet induced the highest increase (about 17 times) of this enzyme activity, while protein enriched diet produced the smallest increase. Specific activity of the lipase was maximal in suckling rabbits, and decreased thereafterregardless the diet composition. Serum trypsin activity doubled from 22 days of age to the end of the trial (45 d old), with a slightly higher increase for rabbits fed a high protein diet ($P < 0.05$ compared to control).

Key words: diets, serum digestive enzyme actvity, suckling rabbit, weaned rabbit.

INTRODUCTION

Pre and post- weaning period calls intensively digestive physiology. Change in the feed composition induced by the transition from milk to solid feeding involves intensive changing of secretor activity of all digestive glands. Most of the digestive enzyme development is age-related (Marounek *et al*., 1995; Carabaño and Piquer, 1998) but the stimulatory effect of the alimentary substrate was also demonstrated (Gutiérrez *et al*., 2002; Dojană *et al*., 2012; Wang *et al*., 2012). Taking into account the greater susceptibility of the rabbit to digestive disorders during weaning (Gidenne *et al*., 2010), the technological pattern of weaning process should take into account both the age and the composition of the diet of the weaned rabbits. The objective of our research was to find out some influences of the diet compounds on the serum digestive enzymes before and after weaning.

MATERIALS AND METHODS

Animals and experimental design

Fifty four New Zealand rabbit does and suckling litters were housed in appropriate cages (1040 mm × 520 mm × 360 mm) equipped with automatic drinkers with nipples, in a room with artificial lighting (a cycle of 12 h from 7:00 to 19:00 of light was used throughout the trial), where the temperature ranged between 22°C and 26°C. The kits were under controlled lactation until 15 days of age and afterward they were free to suckle and had free access to doe's feed, provided *ad libitum*. The doe's feed was a commercial pelleted feed based on corn, oats, wheat bran, soybean meal and alfalfa hay, containing 12.78% crude protein (CP), 16.60% starch and 13.44% crude fiber (CF). Kits were weaned at 29 days of age. After weaning, the young rabbits were randomly allocated to a control group (14 rabbits) and three experimental groups (11 rabbits each one). Weaned rabbits were housed in the same room, in individual cages (520 mm × 520 mm × 360

mm).. Water and feed were provided *ad libitum*. Four experimental pelleted diets (4 mm diameter) were calculated for weaned rabbits: a control diet based on corn, soybean meal and wheat bran, containing 12.7% CP, 16.6% starch and 12.4% CF, a high protein diet (15.9% CP by adding soybean meal), a high starch diet (22.8% starch by adding corn) and a high fiber diet (16.8% CF, by adding wheat straw), according to weaned rabbit requirements (De Blas and Mateos, 2010).

Blood sampling and chemical analyses

Blood was sampled from marginal ear vein, at 10:00 o'clock, using heparin as anticoagulant. Blood was sampled randomly from 5-6 suckling rabbits at 22 days of age and from 5-6 weaned rabbits/group at 1, 8 and 15 days after weaning. Blood serum was obtained after coagulation, centrifugation 10 min at 14,000 G and decantation. Sera were stored at 4°C until biochemical processing, but no more than 24 hours. The activity of amylase (EC 3.2.1.1), lipase (EC 3.1.1.3), and trypsin (EC 3.4.21.4) and serum protein concentration were analyzed as previously described (Dojană *et al*., 1998).

Statistical analysis

The data were statistically analyzed by ANOVA using a GLM (General Linear Model) procedure of SAS (2002). The calculated descriptive statistics were mean and standard error of mean (SEM). Statistic comparison was made between experimental fed groups and control group on one hand, and between suckling group and each experimentally fed weaned group. When any null hypothesis was rejected, Tukey's test was performed to find out the significant differences. The significance level for null hypothesis rejection was stated for $P < 0.05$.

RESULTS AND DISCUSSION

Growth, feed intake and feed conversion ranged within the classical values observed for the growing rabbit (table 1) indicating a good health status (table 1).

Table 1: Growth, intake and feed conversion ratio in rabbits, according to age and diet.

Group	Body weight evolution (g)			Weight gain (g/day)	Feed intake (g/day)	Feed conversion ratio
	1-day weaned rabbits (30-day age)	8-day weaned rabbits	15-day weaned rabbits			
Control (n = 14)	666 ± 32	856 ± 40	1060 ± 39	28.1 ± 2.5a	81.5	2.89
High protein diet fed (n = 11)	639 ± 30	845 ± 25	1109 ± 41	33.5 ± 3.0b	93.1	2.77
High starch (n = 11)	590 ± 54	835 ± 30	1085 ± 36	35.3± 2.2b	101.7	2.87
High fiber (n = 11)	625 ± 28	820 ± 26	980 ± 26	25.4 ± 2.9a	77.0	3.03
P value	NS	NS	NS	0.008	-	-

n = number of animals ; Values of body-weight and mean gain are expressed as mean ± SEM.

Amylase activity was higher in 1-day weaned rabbits (30d old) vs. 22-day suckling rabbits in all experimental groups ($P < 0.001$ for each 1-day weaned group by comparing to 22-day age suckling group, Table 2). The fact is explained, in part, by the starch content of consumed solid feed. After 22-day age, amylase activity increased in all the four rabbit groups, remarking the group fed a high starch diet (an increase of over seventeen times) compared to all other groups, those amylase activities increased only five to eight times vs. 22-day suckling rabbits, at the end of the experimental feeding. Amylase activity in the high starch diet fed group differs significantly from the control group and the other experimental groups already after the first eight days from weaning ($P = 0.003$), the differences emphasizing seven days later ($P < 0.001$).

Our results regarding the activity of serum amylase in control rabbits are in agreement (in some extent) with those obtained by Sabatakou *et al*. (2007) on New Zealand White x Californian weaned rabbits: according to these authors, despite of not significant age-differences of jejunum amylase, serum amylase doubled from 21 to 35 days of age.

Table 2: Serum amylase activity in suckling and weaned rabbits fed on different diets

Suckling rabbits (22-d old)	Diets	Age of weaned rabbits			Effect of Age (P value[1])
		1-day weaned rabbits (30 d old)	8-day weaned (38d old)	15-day weaned rabbits (45d old)	
14.6±8.5[a;b;c;d]	Control (n = 6)	35.0 ±3.2[a]	94.0 ± 6.4[†]	119.5 ± 8.0[‡]	<0.001
	High protein (n = 6)	43.0 ± 4.4[b]	85.4 ± 6.3	79.0 ± 5.0	<0.001
	High starch (n= 6)	40.7 ± 3.3[c]	181.5 ± 12.0[†]	250.0 ±22.2[‡]	<0.001
	High fiber (n= 6)	38.6 ± 4.3[d]	86.1 ± 14.3[†]	122.1 ± 10.9[‡]	<0.001
Effect of Diet [2]		*0.031*	*0.003*	*< 0.001*	

Amylase activity was expressed as mg hydrolyzed starch per mg of serum protein per 30 min, 37°C;
Values are expressed as mean ± SEM; n = number of samples; [1] P value between suckling group and weaned rabbit groups ; [2] P value between experimentally fed and control groups ; Means with same superscript letters on the same row differ significantly (Tukey test) ; Means with same superscript symbols on the same column differ significantly (Tukey test).

Regarding lipase activity, the 1-day weaned groups showed significantly lower activities vs. suckling rabbits ($P < 0.001$), but no significant difference ($P = 0.052$) among the 1-day weaned groups was found (Table 3). It was probably linked to the deep changes in lipid supply (high supply from milk rich in lipid and very low when fed with solid feed) that occur from 22 d of age and even more after. Logically, as the lipid supply decreased (one and two weeks after weaning) the lipase activity decreased close to null values in all the groups. With a maximal values in 22-day rabbits and a subsequent decrease, regardless the diet composition, our results are in agreement with Wang *et al*. (2012) who reported no significant influences of different dietary protein, fibre and energy levels on intestinal lipase activity, but not with Debray *et al*. (2003) who found a higher activity of lipase in small intestine content of rabbits fed on high fat diet vs. low fat diet. Our results seemed not in agreement with Zita *et al*. (2008) who reported highest gastric and lipase activity around weaning, a peak of blood lipase activity few days after weaning, and a subsequent decrease later. According to our results, lipase activity seems to be more age-related than diet-related in suckling and weaned rabbits.

Table 3: Serum lipase activity in suckling rabbits and in weaned rabbits fed by different diets

Suckling rabbits (22-day age)	Diets	**Age of weaned rabbits**			
		1-day weaned rabbits (30-day age)	8-day weaned rabbits	15-day weaned rabbits	*Age effect* *P* value[1]
654.44 ± 50.60[abcd]	Control (n = 8)	344 ±22.1[a]	43 ± 3.7	38.8 ± 4.3	< 0.001
	High protein (n = 9)	290 ± 24.1[b]	53 ± 3.5	58.8 ± 4.5	< 0.001
	High starch (n= 8)	352 ± 15.4[c]	59 ± 3.1	33.9 ± 3.9	< 0.001
	High fiber (n= 8)	303 ± 15.5[d]	29 ± 2.3	30.0 ± 3.3	< 0.001
P value[2]		0.052	0.490	0.850	

Lipase activity is expressed as mEq of liberated oleic acid per mg of serum protein per h; 37 °C; n = number of samples; Values are expressed as mean ± SEM; [1] *P* value between suckling group and weaned rabbit groups ; [2] *P* value between experimentally fed groups and control group ; [A1]Means with same letters on the same row differ significantly (Tukey test).

The activity of serum trypsin increased about 26% from 22-day age suckling rabbits to 1-day weaned rabbits (figure 1). At 8 and 15d old, the activity of serum trypsin was slightly higher for the "high protein" group compared to control ($P = 0.044$ for values at 15-day weaned rabbits). This suggest a stimulation of feed protein on the synthesis of trypsin. The general trend of increase in trypsin activity with age for all the groups could be ascribed to the rise in protein intake.

These results are in agreement with those of Debray *et al*. (2003) on rabbit pancreas and Lei *et al*. (2004) on rabbit small intestine. In contrast, Wang *et al*. (2012) reported that trypsin activity from small intestine was higher in rabbits fed on higher energy diet vs. rabbits fed on higher protein diet.

Our results should be understood taking into account that in blood plasma, trypsinogen stays inactive since appropriate conditions (alkaline pH and enterokinase activator) are missing. In duodenum lumen, these conditions are fulfilled and zymogene activates, unless the presence of exogenous inhibitors (from soybeans, for example).

According to Geller *et al*. (1990), the level of pancreatic amylase, lipase and trypsin in the blood is not able to characterize the external secretor activity of the pancreas but is reflecting the phenomenon of enzyme "deviation".

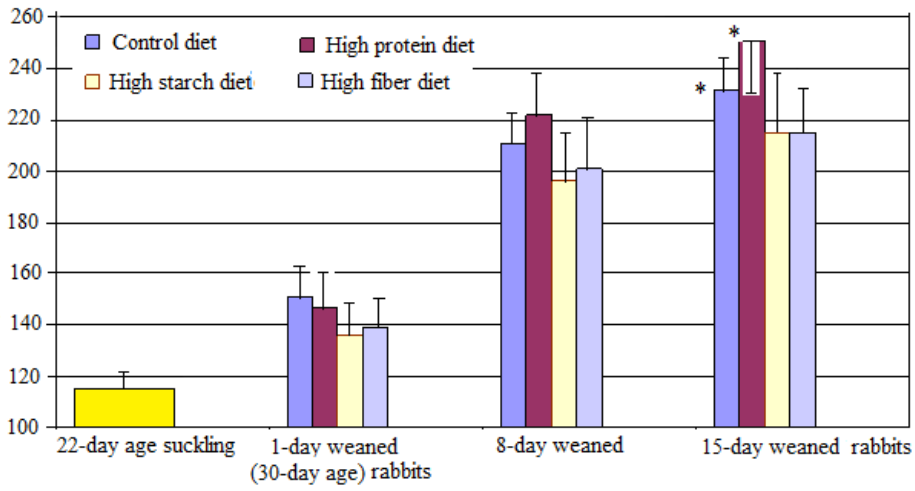


Figure 1. Evolution of serum trypsin activity in suckling and in weaned rabbits fed by different diets (in nmols BAEE / g serum protein / min; 25℃). *P = 0.021

CONCLUSION

Serum activities of amylase, lipase and trypsin vary specifically and mainly according to the intake of nutrients (protein, starch or fiber) in relation to the physiological status (suckling and weaned rabbits). Serum amylase activity strongly rises at weaning as a response to a high starch intake. Reversely the serum trypsin activity show a moderate increase as a response to a high protein diet. Serum lipase activity decreases consistently from weaning.

REFERENCES

Carabaño R., Piquer J. 1998. The digestive system of the rabbit. In: de Blas C., Wiseman J. (Eds) The Nutrition of the Rabbit, CABI Publishing, CAB International, Wallingford, Oxfordshire, UK, 1-16.

De Blas C., Mateos G.G. 2010. Feed formulation. In: de Blas C., Wiseman J. (Eds) Nutrition of the Rabbit 2nd ed., CABI Publishing, CAB International, Wallingford, Oxfordshire, UK, 222-232.

Debray L., Le Huerou-Luron I., Gidenne T., Fortun-Lamothe L. 2003. Digestive tract development in rabbit according to the dietary energetic source: correlation between whole tract digestion, pancreatic and intestinal enzymatic activities. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 135(3): 443–455.

Dojană N., Codreanu I., Orașanu A. 2012. Enzyme output capacity of the pancreas in adult rabbit according to diet composition. In Proceedings 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 651-655.

Dojană N., Costache M., Dinischiotu A. 1998. The activity of some digestive enzymes in domestic rabbits before and after weaning. Animal Science. 66:501-507.

Geller L.I., Pashko M.M. 1990. The significance of trypsin, amylase and lipase activities in the blood serum for the differential diagnosis of chronic pancreatitis and other diseases of the abdominal organs]. [in Russian] Vrach Delo, (3):86-8.

Gidenne T., Carabaño R., García J., de Blas C. 2010. Fibre digestion. In de Blas C., Wiseman J. (Eds) Nutrition of the Rabbit 2nd ed., CABI Publishing, CAB International, Wallingford, Oxfordshire, UK, 66-82.

Gutiérrez I., Espinosa A., García J., Carabaño R., de Blas C. 2002. Effect of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned rabbits. J. Anim. Sci. 80,1029–1037.

Lei, Q.X., Li, F.C., Jiao, H.C. 2004. Effects of dietary crude protein on growth performance, nutrient utilization, immunity index and protease activity in weaner to 2 month-old New Zealand rabbits. Asian-Aust. J. Anim. Sci. 17(10): 1447-1451.

Marounek M., Vovk S.J., Skřivanová V. 1995. Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. British Journal of Nutrition. 73, 463-469.

Sabatakou A.O., Xylouri M.E., Sotirakoglou A.K., Fragkiadakis G.M., Noikokyris N.P. 2007. Histochemical and biological characteristics of weaning rabbit intestine. World Rabbit Sci. 15:173 – 178.

SAS/STAT User's guide, version 2.1.3 for Windows. 2002. SAS Institute Inc. Cary, North Caroline, USA.

Wang X., Ma M., Sun L., Wang C., Zhu Y., Li F. 2012. Effects of different protein, fibre and energy levels on growth performance and the development of digestive organs in growing meat rabbit. In Proceedings 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 641- 645.

Zita L., Fučíková A., Marounek M., Tůmová E., Skřivanová V. 2008. Lipase activity till 35 days of age in broiler rabbits. In Proceedings 9th World Rabbit Congress,Verona, Italy, 243.

==========