

LIPASE ACTIVITY TILL 35 DAYS OF AGE IN BROILER RABBITS

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ABSTRACT

The developmental changes of lipase in stomach, small intestine, caecum and blood till 35 days of age were followed in broiler rabbits. Two experiments were carried out on Hyplus[®] rabbits. Digesta and blood were sampled every 2 or 4 days in both experiments. In the experiment 1, the lipase activity in stomach, small intestine and caecum was determined from 14 to 35 days of age in 5 suckling rabbits at seven different periods. In the experiment 2, the lipase activity was analysed in blood by colorimetric method by Randox Laboratories Ltd. From 21 to 35 days of age in six groups of 5 suckling and weaned rabbits at each age. Gastric and small intestinal lipolytic activity, in the experiment 1, was the highest around 25 days of age (2.572 and 1.720 mmol/g/h, respectively). Caecum activity of lipase was the highest at the beginning of the experiment at 14 days of age (1.086 mmol/g/h) and at 32 days of age (1.098 mmol/g/h). In the experiment 2, the activity of lipase in blood tended to show a non-significant curvilinear response from weaning onwards.

Key words: Rabbit; Age; Lipase; Stomach; Small intestine; Caecum; Blood.

INTRODUCTION

The capacity of rabbits to digest fats is well developed from birth, as the lipids in the milk (10 to 25% on fresh basis) are their main source of energy. Suckling rabbits are capable of utilising milk fat efficiently, showing a high lipase activity (Dojana *et al.*, 1998; Debray *et al.*, 2003). Fat addition to starter diets would increase the energy intake of kits and immune system development, thus reducing weaning risk and improving resistance to illness. According to Dojana *et al.* (1998) the specific activity of the lipase is maximal at 15 days of age in the gastric mucosa and in the pancreas, and then falls until weaning (here at 42 days). On the other hand, Debray *et al.* (2002) found a linear increase of this lipasic activity in the pancreas between 25 and 52 days, and also in the intestinal lumen.

Development of enzymatic equipment of the pancreas starts around 21st or 24th day of age, regardless of the nature of the diet (Lebas *et al.*, 1971; Corring *et al.*, 1972). Indeed, total activity of amylase, lipase and chymotrypsin measured in pancreatic tissue sharply increased on 21 of age while rabbit began to ingest solid food, whereas that of trypsin remained constant from birth to weaning. Marounek *et al.* (1995) measured some enzyme activities in different digestive contents, on 28 and 90 days of age. They related a slight intestinal content lipase activity, which weakly increased with age. Debray *et al.* (2003) observed a significant increase in lipase activity of small intestinal contents in young rabbits from 25 to 32 and 42 days of age. In the same study, lipase activity in intestinal content increased as dietary fat concentration increased. Tůmová *et al.* (2006) described that rabbit weaned at 25 days of age had higher lipase activity in comparison with the rabbits weaned at 35 days. Marounek *et al.* (1995) did not report any significant change of lipase activity in the small intestine comparing rabbits at 4 weeks or 3 months of age. Dojana *et al.* (1998) observed that amylase, maltase, trypsin and chymotrypsin activities intensify after weaning, while lipase activity is already lowered.

The aim of the work was to evaluate the developmental changes of lipase activity in stomach, small intestine, caecum and blood till 35 days of age in broiler rabbits.

MATERIALS AND METHODS

Two experiments were carried out. The monitoring was realized on Hyplus[®] rabbits. The rabbits obtained from a commercial farm were slaughtered at the age of 14, 17, 21, 25, 28, 32, 35 days in the experiment 1 and in the experiment 2 at the age 21, 24, 26, 28, 31, 35 days of age. From the age 21 days the kids have access the solid feed of the does.

In the experiment 1, the lipase activity in stomach, small intestine and caecum was determined in 5 rabbits each age. After killing, the small intestine content was collected and frozen at -40°C until analyzes. The activity of lipase was analysed by the method of Marounek *et al.* (1995) which is the modified method of Bier (1955). Samples of digesta (0.1 g) were incubated with 0.0025 M Tris-HCl buffer containing 0.025 M CaCl₂ (0.8 ml), 0.2 M Na₂HPO₄ (0.1 ml) and emulsified tributyrin (1.0 ml). The emulsion was prepared from tributyrin (10 ml), Tween 80 (1 ml) and water (100 ml). The reaction mixture was incubated at physiological pH for 1h at 37°C, and then centrifuged. Liberated butyrate was determined by titration, after steam distillation in the Markham apparatus. Lipase activity was related to 1 g of the original digesta.

The experiment 2 was realized with 30 suckling rabbits and 105 weaned rabbits. At 21 days of age of weaning 5 suckling rabbits obtained from a commercial farm were slaughtered and other weaned rabbits at the same age were divided into groups according to age of weaning (21, 24, 26, 28, 31 and 35 days). At next time (24, 26, 28, 31 and 35 days) 5 suckling rabbits and 5 rabbits weaned at previous time were slaughtered for lipase activity in blood from *jugular cut* etc. Rabbits were placed in fattening cages (0.15 m² per rabbit). Water and feed mixture (14.8% of crude protein; 4.2% of fat; 16.3% of starch) were available *ad libitum*. Lipase activity was analysed in blood by colorimetric method by Radox Laboratories Ltd. In the analysis the chromogenic lipase substrate 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6''-methylresorufin) ester is cleaved by the catalytic action of lipase to form 1,2-o-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid and methylresorufin. The lipase activity in the specimen is proportional to the production of methylresorufin in the reaction and can be determined photometrically (Biochrom Libra S22).

Results of lipase activity were evaluated by two-way analysis of variance, age of slaughtering and age of weaning interactions using the GLM procedure of SAS (SAS Institute Inc., 2003).

RESULTS AND DISCUSSION

In the experiment 1 lipase activity was affected by days of age in different segments of the digestive tract (Figure 1). Gastric lipase increased with age and peaked at 25 days of age. Lipase activity in small intestine was higher to the 25 days of age and then decreased. Caecum activity of lipase was the highest at the beginning of the experiment and at 32 days of age. Marounek *et al.* (1995) and Dojana *et al.* (1998) described that activity of lipase decreased with age od rabbits. Suckling rabbits showing a high lipase activity. It is in agreement with Dojana *et al.* (1998) and Debray *et al.* (2003). The activity of lipase in small intestine is most important because of fat digestion proceeds.

In the experiment 2, the lipase activity was determined in blood (Table 1). The blood lipase activity in rabbits weaned at 21 days of age increased till 28 days of age. In rabbits weaned at 24, 26 days the blood activity of lipase reduced after 26th and 28th day of age. Blood lipase activity was the highest at 31st day of age in rabbits weaned at 28 days of age. Sometimes the activity of lipase in blood was higher in rabbits with solid feed in comparison with the sukling ones at the same age.

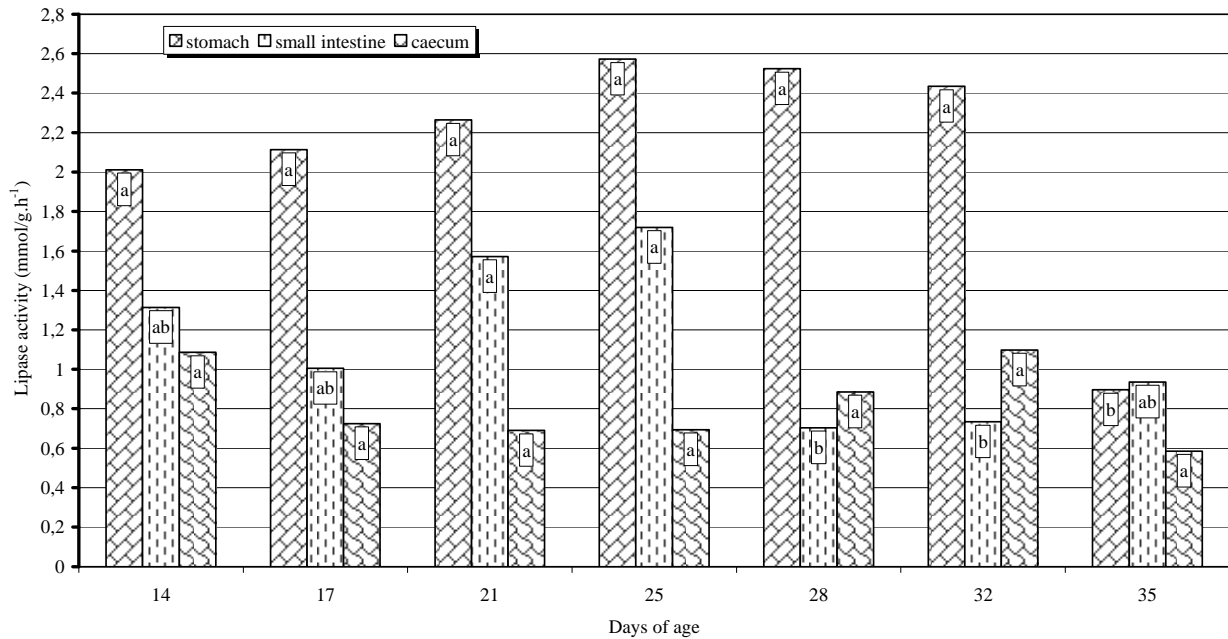


Figure 1: Experiment 1. Developmental changes in lipase activity in suckling rabbits (^{a, b}P≤0.05; a, b, in terms of age means with the same letters do not differ significantly)

Table 1: Experiment 2. Lipolytic activity in blood (U/l) in rabbits before and after weaning

Age (days)	Age of weaning (days) (n = 5)					
	21	24	26	28	31	35
21	131.98* 0.0030					
24	164.02 0.0003	164.02* 0.0003				
26	199.41 0.0001	234.80 0.0001	219.49* 0.0001			
28	238.62 0.0001	209.93 0.0001	248.19 0.0001	229.06* 0.0001		
31	203.71 0.0001	154.46 0.0006	103.29 0.0190	372.52 0.0001	230.01* 0.0001	
35	222.36 0.0001	145.85 0.0011	153.02 0.0006	152.07 0.0007	210.89 0.0001	184.11* 0.0003
Significance		age of weaning				0.2129
		age				0.1148
		age of weaning*age				0.0636
SEM						10.100

SEM = standard error mean; *rabbits obtained from a commercial farm – suckling ones

CONCLUSIONS

Lipase activity in digestive segments was influenced by weaning age. Gastric and small intestinal lipolytic activity was the highest around 25 days of age. The activity of lipase in blood was higher the 3rd day after weaning of rabbits. The colorimetric method used in the experiment 2 is available only for the quantitative *in vitro* determination of lipase in serum or plasma.

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REFERENCES

- Bier M. 1955. Lipases. In: Colowick S.P., Kaplan N.O. (EDs.). *Methods in Enzymology*. Academic Press, New York, USA, Vol. 1, 627-642
- Corring T., Lebas F., Courtot D. 1972. Contrôle de l'évolution de l'équipement enzymatique du pancréas exocrine du lapin de la naissance à 6 semaines. *Annales Biology, Animal Biochemistry Biophysics*, 12, 221-231.
- Debray L., Fortun-Lamothe L., Gidenne T. 2002. Influence of low dietary starch/fibre ratio around weaning on intake behaviour, performance and health status of young and rabbit does. *Animal Research*, 51, 63-75.
- 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*, 135A, 443-455.
- Dojana N., Costache M., Dinischiotu A. 1998. The activity of some digestive enzymes in domestic rabbits before and after weaning. *Animal Science*, 66, 501-507.
- Lebas F., Corring T., Courtot D. 1971. Equipement enzymatique du pancréas exocrine chez le lapin, mise en place et évolution de la naissance au sevrage relation avec la composition du régime alimentaire. *Annales Biology, Animal Biochemistry Biophysics*, 11, 399-414.
- 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.
- SAS Institute Inc. 2003. The SAS System for Windows. Release 9.1.3.
- Tůmová E., Zita L., Marounek M., Skřivanová V., Berladyn A. 2006. The effect of weaning age on performance, nutrient digestibility and lipase activity in broiler rabbits. In: *Proc. 3rd Rabbit Congress of the Americas, Maringá, Brasil, CD-ROM*.