

Proceedings of the



4-7 july **2000** – Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910

(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

KÓSA Emma, PAPP Z., RAFAI P., FEKETE S.

**THE PANCREATIC HYDROLASES ACTIVITY
IN PREGNANT RABBITS**

Volume C, pages 299-303

THE PANCREATIC HYDROLASES ACTIVITY IN PREGNANT RABBITS

Emma KÓSA, Z. PAPP*, P. RAFAI* and S. FEKETE

Szent István University, Faculty of Veterinary Sci.

Department of Animal Breeding, Nutrition and Laboratory Animal Sciences

and *Department of Animal Hygiene

H-1400 Budapest, P.O. Box. 2. HUNGARY

E-mail: ekosa@univet.hu

ABSTRACT

The authors have compared activity of α -amylase, lipase, trypsin and total proteolytic activity in the pancreatic tissue, small intestinal and caecum content in different days of the pregnancy (not pregnant (empty) and 14th, 21st, 24th, 26th and 28th day of pregnancy) in rabbits. The measurement of pancreatic hydrolases activity was made on the pancreas tissue homogenates supernatant, the small intestinal (duodenum) content and caecum of 5 animals from each group. The data of the present trial suggest that the pregnancy significantly alters the digestive enzyme activity.

We found the following results: *in the pancreatic tissue* - α -amylase 24,18 U/mg protein, lipase 236,74 mU/mg protein and total protease 88,90 mU/mg protein the 21st day of pregnancy significantly ($P < 0.001$) increased. The maximum trypsin activity conversely the 21st and 24th day of pregnancy was significantly increased (14,05 and 14,97 mU/mg protein, $P < 0.001$).

In the small intestinal content: - α -amylase activity the 21st day of pregnancy was significantly increased 0,81 U/mg protein ($P < 0,001$). The trypsin activity conversely the 21st, 24th and 26th day of pregnancy was significantly increased 2,91, 3,77 and 4,34 mU/mg protein ($P < 0.001$). The lipase activity the 14th and 21st day of pregnancy was significantly increased 1,84 and 1,25 mU/mg protein ($P < 0.001$). The total protease activity the 21th day of pregnancy was significantly increased 25,26 mU/mg protein ($P < 0.001$).

In the caecum content: the lipase activity the 21st day of pregnancy was significantly increased 1,68 mU/mg protein ($P < 0.001$).

The 26-28th day of pregnancy the pancreatic hydrolase activities decreased and identical with values of empty rabbits.

INTRODUCTION

However the age related changes of pH values of stomach and intestinal contents. The activities of most important digestive enzymes of domestic rabbits have thoroughly been investigated (HENSCHEL, 1973a, 1973b; CORRING et al. 1972; LEITCH, 1973). There is no

available data in the literature about the effect of different pregnancy status on the activity of pancreatic hydrolases. Since it have been shown that the different pregnancy status influence significantly the feed intake (LEBAS 1979) and the digestibility of different nutrients (LEBAS, 1975, 1979; KALUGIN, 1980; MAERTENS and De GROOTE, 1982). It is rational to hypothesize that the activity of digestive hydrolases may change according to the nutrient requirements of these animals. This is why this experiment was designed to study the effect of (not pregnant (empty) and 14, 21, 24, 26 and 28 days of pregnancy) on the activity of hydrolases in the pancreas as well as it is in the small intestinal and caecum contents.

MATERIAL AND METHODS

Since hydrolase production and secretion may be influenced by several environmental factors including ambient temperature (SZABÓ et al. 1981b). The atmospheric pressure (SZABÓ, 1981c). The composition of food consumed (SZABÓ et al., 1981a) and probably the circadian periodicity, it is rather difficult to design an experimental system in which objective comparison of the hydrolase activity changes can be made. In our experiment the following important factors were taken into considerations: optimal temperature and diet of collection. The animals (New Zealand White) in different pregnancy status by a large-scale rabbits farm. All animals were fed the optimal diet ad libitum. Drinking water was ad libitum, too.

The following experimental groups (5 rabbits/group) were selected:

Groups	Pregnant status
1.	Not pregnant (empty) rabbits
2.	14 th days of pregnancy
3.	21 st days of pregnancy
4.	24 th days of pregnancy
5.	26 th days of pregnancy
6.	28 th days of pregnancy

Rabbits were stunned and bled and pancreatic tissue, small intestinal and caecum content samples were collected between on the same day. All samples were deepfrozen until further investigation.

Enzyme Activity Assays

The frozen pancreatic tissue cleared of fat, and was homogenized in ice cold saline, in a *Potter-Elvehjem* homogenizer. The homogenate was diluted in distilled water, activated by addition of 0.02 M CaCl₂ solution, and tested immediately for lipase activity by the method of SCHÖN et al. (1961). The α -amylase activity was determined as proposed by RICK and STEGBAUER (1968). The proteolytic zymogens of pancreas were activated by incubation in the presence of enterokinase at 37°C; by Boehringer colorimetric test, and total proteolytic activity on casein substrate (SZABÓ et al. 1976) determined was trypsin activity. The hydrolytic products were detected with the *Folin-Ciocalteu* reagent. The small intestine and caecum contents were diluted with distilled water and centrifuged (15 000/min) then the supernatants were used for the enzyme tests. Protein content of the samples were assayed by the method of LOWRY et al. (1961), with bovine albumin used as reference standard.

RESULTS AND DISCUSSION

The results of experiment are summarized in *Table 1*. On the basis of these experimental data may be pointed out that the hydrolase activity of pancreatic tissue homogenate, small intestinal and caecum contents has been significantly influenced by the pregnancy of the animals.

The maximal values of enzyme activity were measured on the 21-24th day of pregnancy and later on decreased gradually to the values of empty rabbits. The phenomenon may be explained with the changing of feed intake during gestation (LEBAS et al. 1971). The effect of pregnancy on hydrolases activity is supported by those observation that the periodical variation of pancreatic and other enzyme activities in growing rabbits were not be observed (CORRING et al. 1972, FEKETE and BOKORI 1986, MAROUNEK et al. 1995). The activity the total protease and lipase in the caecum content, opposite the mentioned enzymes, earlier did not decreased till the 28th day. The reason of it was be explained by the activity of intestinal flora which is able to produce certain digestive enzyme, first of all lipase (BRUCKNER and SZABÓ, 1984).

Table 1 Changes of pancreatic hydrolases activities of does during pregnancy

Pregnant status		α -Amylase (U/mg prot.)	Trypsin (mU/mg prot.)	Lipase (mU/mg prot.)	Total Protease (mU/mg prot.)
Not pregnant (empty) rabbits (n=5)	P.	14.53±1.76	5.61±0.71	200.55±12.52	51.94±5.95
	Sic.	0.44±0.08	1.75±0.19	0.89±0.10	19.11±1.87
	Cc.	0.30±0.11	0.08±0.04	0.99±0.17	8.92±1.05
14 th day of pregnancy (n=5)	P.	18.78±0.92	10.46±1.2	207.98±10.75	75.46±4.36
	Sic.	0.76±0.10	1.92±0.22	1.84±0.19*	16.18±2.28
	Cc.	0.15±0.02	0.05±0.02	1.20±0.23	12.04±1.21
21 st day of pregnancy (n=5)	P.	24.18±2.16*	14.05±1.00*	236.74±12.22*	88.90±7.19*
	Sic.	0.59±0.09	2.91±0.46*	1.25±0.14*	25.26±2.20*
	Cc.	0.32±0.09	0.05±0.01	1.68±0.19*	11.12±1.04
24 th day of pregnancy (n=5)	P.	21.11±2.68	14.97±1.01*	207.00±9.03	69.04±6.89
	Sic.	0.81±0.12*	3.77±0.39*	0.97±0.08	21.01±1.79
	Cc.	0.15±0.04	0.06±0.01	1.22±0.15	11.14±0.95
26 th day of pregnancy (n=5)	P.	18.71±0.98	12.97±0.26	190.77±7.37	54.77±3.15
	Sic.	0.54±0.08	4.34±0.48*	1.00±0.88	21.35±2.69
	Cc.	0.11±0.03	0.05±0.03	1.62±0.19	12.19±0.98
28 th day of pregnancy (n=5)	P.	13.13±1.18	5.67±0.55	195.53±11.04	42.64±6.84
	Sic.	0.42±0.06	1.58±0.30	0.82±0.10	16.01±1.20
	Cc.	0.15±0.04	0±0	1.44±0.32	12.52±1.24

P= Pancreatic tissue supernatant; Sic= Small intestinal content; Cc= Caecum content.

*= Significant (P<0.001)

REFERENCES

- BRUCKNER, G. and SZABÓ, J.: Nutrient absorption in gnotobiotic animals. in: Advances in Nutritional Research 1984. Vol. 6. 271-332.
- CORRING, T. - LEBAS, F. - COURTOT, D.: Controle de l'équipement enzymatique du paneréas exocrine du lapin de la naissance a 6 semaines. Annl. Biol. Anim. Biochim. Biophys., 1972. 12. 221-231.
- FEKETE, S. - BOKORI, J.: Effect of age and sex on rabbit's digestion. Állattenyésztés és takarmányozás, 1986. 35. 57-60.
- HENSCHER, M. J.: Proteolytic enzyme activity in the gut of doe-suckled and hand-reared rabbits. Br. J. Nutr., 1973b. 30. 351-359.

- HENSCHERL, M. J. Comparison of the development of proteolytic activity in the abomasum of the preruminant calf with that in the stomach of the young rabbit and guinea pig. *Br. J. Nutr.* 1973a. 30. 285-296.
- KALUGIN, Y. A.: Digestive physiology of rabbit (in Russian) Kolos, Moscow, 1980.
- LEBAS, F. - CORRING, T. - COURTOT, D.: Equipment enzymatique du pancreas exocrine chez le lapin. Mise en place et évolution de la naissance au sevrage. Relation avec la composition du régime alimentaire. *Annls. Biol. Anim. Biochim. Biophys.*, 1971. 11. 399-413.
- LEBAS, F.: Efficacité de la digestion chez la lapine adulte. Effets du niveau d'alimentation et du stade de gestation. *Annls. Biol. Anim. Biochim. Biophys.*, 1979. 19. 969-973.
- LEBAS, F.: Nutrient requirements of the rabbits. *The Commercial Rabbit*, 1975. 3. 7-10.
- LEITCH, G. J.: *Arch. Intern. Physiol. Biochem.*, 1973. 79. 279.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A.L. and RANDALL, R. J. 1961: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 1961. 193. 265-275.
- MAERTENS, L. and De GROOTE, G.: Étude de la variabilité des coefficients de la digestibilité des lapins suite aux différences de l'âge, de sexe, de race et d'origine. *Rev. Agric.*, 1982. 35. 2787-2797.
- MAROUNEK, M., VOVK, S. J. and SKRIVANOVA, V.: Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *Brit. J. Nutr.*, 1995. 73. 463-469.
- RICK, W., und STEGBAUER, H.: Zur Bestimmung der Amylaseaktivität im Harn. *Z. Anal. Chemie*, 1968. 243. 415-416.
- SCHÖN, H., RASSLER, B. und HENNING, N.: Über die Untersuchung der exkretorischen Pankreasfunktion. Methoden zur Aktivitätsbestimmung von Trypsin, Chymotrypsin, Carboxyprptidase, Lipase und Diastase. *Klin. Wschr.*, 1961. 39. 217-222.
- SZABÓ, J., RIBICZEINÉ SZABÓ, P., és RAFAI, P.: Study of the activity of some pancreas originated hydrolases in pigs kept in different environmental temperature. (in Hungarian) *Magyar Állatorvosok Lapja*, 1976. 31. 325-328.
- SZABÓ, J., RIBICZEY, P., FODOR, É. and TENK, I.: Influence of environmental factors on digestive enzymes and plasma corticosterone in rats. I. Diet. *Acta Vet. Acad. Sci. Hung.*, 1981a. 29. 399-422.
- SZABÓ, J., RIBICZEY, P., and FODOR, É.: Influence of environmental factors on digestive enzymes and plasma corticosterone in rats. II. Ambient temperature. *Acta Vet. Acad. Sci. Hung.*, 1981b. 29. 423-435.
- SZABÓ, J.: Influence of environmental factors on digestive enzymes and plasma corticosterone in rats. III. Atmospheric pressure. *Acta Vet. Acad. Sci. Hung.*, 1981c. 29. 437-440.