EFFECT OF GESTATION AND LACTATION UPON DIGESTIVE HYDROLASE ACTIVITY IN RABBITS

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Abstract - The authors have compared activity of amylase, lipase, trypsine and total proteolytic activity in the pancreas, small intestinal and cecal content of the empty, the pregnant (day 14 and 28 of gestation), the mated (d 3 after mating), the lactating (d 14) as well as the simultaneously pregnant and lactating (d 28 of pregnancy and d 28 of lactation). The data of the present trial suggest that the reproductive state does not alter the digestive enzyme activity.

INTRODUCTION

However the age related changes of pH values of stomach and intestinal contents and the activity of most important digestive enzymes of domestic rabbits have thoroughly been investigated (HENSCHEL 1973a, 1973b, CORRING at all. 1972 ; LEITCH 1973 ; DAREN, 1973), there is no available data in the literature about the effect of different reproductive stages (i. e.. gestation and lactation) on the activity of pancreatic hydrolases. Since it have been shown that the different reproductive stages 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 pregnancy (20 and 28 days) and lactation together with pregnancy (3 days of second pregnancy and 15 days of lactation ; 14 days of second pregnancy and 28 days of lactation) on the activity of hydrolases in the pancreas as well as it is in the small intestinal and large intestinal contents.

MATERIAL AND METHODS

Since hydrolase production and secretion may be influenced by several environmental factors including ambient temperature (SZABO et al. 1981b), atmospheric pressure (SZABO et al. 1981c), composition of food consumed (SZABO et al. 1981a) and probably the circadian periodicity, it is rather difficult to designee 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: ambient temperature, composition of the diet and the timing of sample collection. The animals (New Zealand White x Californian) in different reproductive stages were donated by a large-scale rabbit farm. Animals were delivered to the university one week before the samples collection. The rabbits were housed in single rabbit cages and were kept in controlled climatic environment (18 ^{o}C, 70% relative humidity). All experimental animal were fed the same diet *ad libitum* (crude protein: 17%; ME: 10.5 MJ; fiber: 13.5 %). Drinking water were available *ad libitum*.
The following experimental groups (5 rabbits/group) were selected :

1. maiden does, fit for breeding
2. first pregnancy does, mated 20 d before sampling
3. first pregnancy does, mated 28 d before sampling
4. second pregnancy does, at 15th d of lactation, mated 3 d before sampling
5. second pregnancy does, at 28th d of lactation, mated 14 d before sampling

Rabbits were stunned and bled and pancreas tissue, small intestinal content and cecum content samples were collected between 9-12 am., on the same day, a week after housing the animals in climatized environment and were fed the same diet. All samples were deep frozen until further investigation.

Figure 1 : Proteolytic Activity of Pancreatic Tissue and Intestinal Content

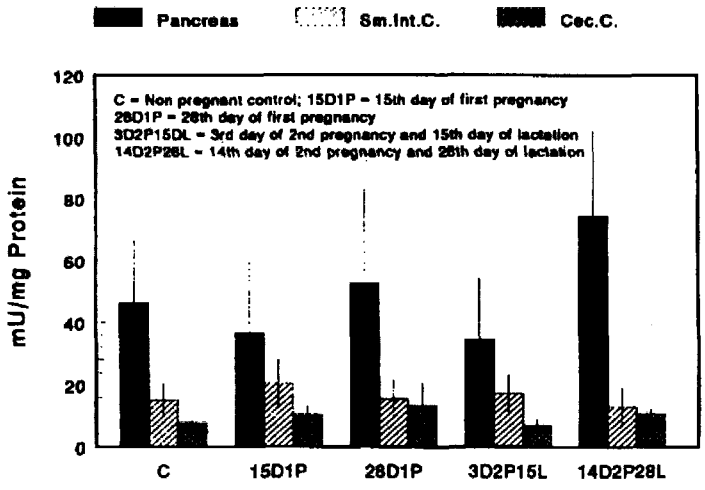


Figure 2 : Trypsin Activity of Pancreatic Tissue and Intestinal Content

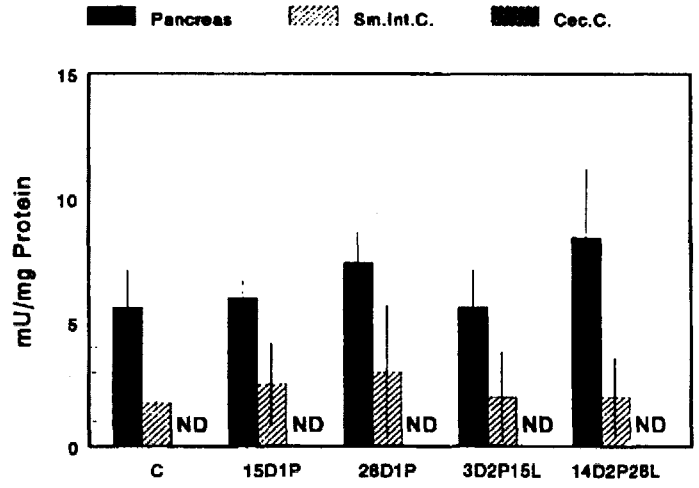
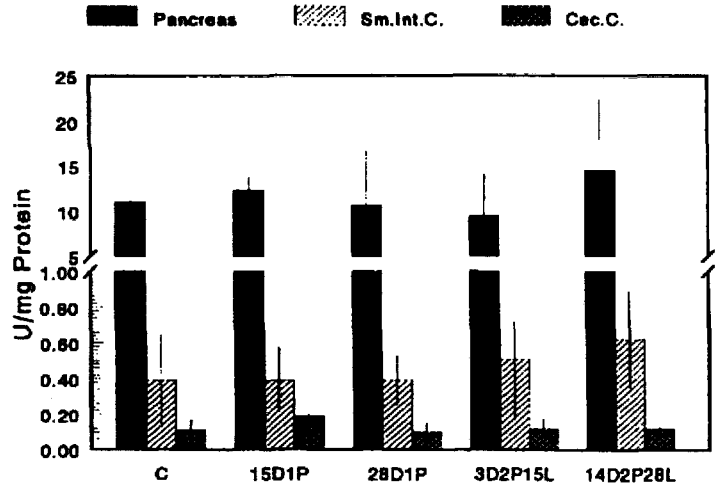


Figure 3 : α-Amylase Activity of Pancreatic Tissue and Intestinal Content



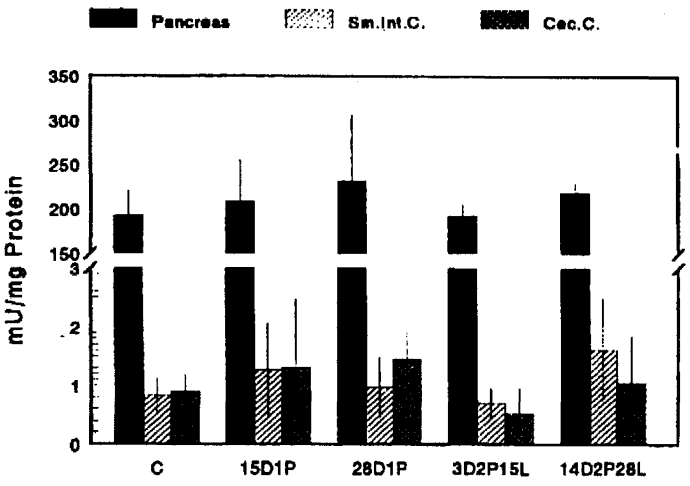
Enzyme activity assays

The frozen pancreatic gland were cleared of fat, and were homogenized in ice cold saline, in a *Potter-Elvehjem* homogenizer. The homogenate was diluted in distilled water, activated by addition of 0.02 M $CaCl_2$ solution, and tested immediately for lipase activity by the method of SHÖN 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; trypsine activity was determined by Boehringer colorimetric test, and total proteolytic activity on casein substrate (SZABO et al. 1976). The hydrolytic products were detected with the *Folin-Ciocalteu* reagent. The small and large intestinal contents was diluted with distilled water and centrifuged (15000/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 and Figure 1, 2, 3, 4. On the basis of these experimental data we may point out that the hydrolase activity of pancreatic tissue homogenates, small intestinal contents and cecum contents has not been significantly influenced by the reproductive stages of the animals, at least at the investigated time periods. The enzyme activity in the small intestinal content showed no correlation with the activity of the same hydrolases in the pancreatic tissue, moreover there have been no correlation between the enzyme activities in the small intestinal content and cecum content. The lack of correlation between the enzyme activities of small and large intestinal contents can be the consequence of the enzyme inhibitory effect of the intestinal flora. It has been shown that the intestinal flora, mainly in the large intestine, inactivates trypsine, however the flora is able to produce certain digestive enzymes i. e. lipase (BRUCKNER and SZABO 1984). In our experiment the trypsine was not detectable in the cecum content, however its activity was high in the small intestinal content.

Figure 4 : Lipase Activity of Pancreatic Tissue and Intestinal Content



Contrary to this, in the cecum content the activity of lipase was higher or at least identical with that in the small intestinal content. The experimental data clearly indicate that the lactation and/or pregnancy, contrary to that in growing rabbits (CORRING et al., 1972 ; FEKETE and BOKORI, 1986 ; MAROUNEK et al., 1995), do not influence the production (enzyme activities of pancreatic tissue) and the secretion (enzyme activity in the small intestinal content) of the measured hydrolases. On this bases we believe, that the known changes of feed intake (LEBAS, 1979) and nutrient digestibility coefficients (MAERTENS and DE GROOTE, 1982) during the course of pregnancy and lactation (LEBAS, 1975) can not be explained on the basis of the digestive hydrolase activity changes.

Table 1 : Digestive enzyme activity of does of different reproductive cycle

		α-Amylase (U/mg prot.)	Lipase (mU/mg prot.)	Trypsin (mU/mg prot.)	Total Protease (mU/mg prot.)
Maiden does fit for breeding	P.	11.00 ± 5.45	192.0 ± 29.3	5.62 ± 1.53	46.3 ± 20.7
	Sic.	0.39 ± 0.26	0.83 ± 0.29	1.75 ± 0.30	15.0 ± 5.3
	Cc.	0.11 ± 0.06	0.89 ± 0.29	ND	7.7 ± 0.8
20th d of 1st pregnancy	P.	12.40 ± 3.57	207.8 ± 51.1	6.00 ± 1.38	36.7 ± 24.3
	Sic.	0.39 ± 0.18	1.27 ± 0.78	2.48 ± 1.64	20.4 ± 7.8
	Cc.	0.19 ± 0.20	1.30 ± 1.17	ND	10.3 ± 2.6
28th d of 1st pregnancy	P.	10.67 ± 5.95	230.8 ± 76.7	7.43 ± 1.23	52.7 ± 30.8
	Sic.	0.39 ± 0.14	0.97 ± 0.51	3.00 ± 2.69	15.4 ± 6.3
	Cc.	0.10 ± 0.05	1.45 ± 0.45	ND	13.1 ± 7.4
3rd d of 2nd pregnancy, 15th d of suckling	P.	9.45 ± 4.61	191.6 ± 13.8	5.60 ± 1.52	34.5 ± 19.7
	Sic.	0.51 ± 0.34	0.70 ± 0.26	1.98 ± 1.81	17.1 ± 5.9
	Ctc	0.12 ± 0.05	0.52 ± 0.44	ND	6.9 ± 1.9
14th d of 2nd pregnancy, 28th d of suckling	P.	14.53 ± 7.85	218.0 ± 12.3	8.45 ± 2.79	74.4 ± 27.8
	Sic.	0.62 ± 0.27	1.61 ± 0.88	1.99 ± 1.58	12.8 ± 6.3
	Cc.	0.12 ± 0.03	1.04 ± 0.80	ND	10.6 ± 1.7

P= Pancraes; Sic= Small intestinal content; Cc= Cecum content.

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