

## **EFFECT OF A VEGETABLE EXTRACT (TRADE NAME: IMMUNOVET–HBM®) UPON ACTIVITY OF DIGESTIVE ENZYMES IN RABBITS**

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### **ABSTRACT**

The authors have compared activity of  $\alpha$ -amylase, lipase and trypsin in the pancreatic tissue and small intestinal content of rabbits weaned early (on day 21). Previously in the same animals the effect of a non-medicated diet and of a feed additive consisting of natural ingredients on the growth and on certain anatomical and physiological parameters of the digestive tract was examined. The aim of the study was to get more information on the developmental process of the digestive tract from birth to 42 days of age in order to decrease use of antibiotics and losses due to digestive disorders. It could be established that the early weaning of rabbits can be accomplished by the use of a non-medicated diet without any decrease in production. In the present study the measurement of pancreatic hydrolase's activity in the pancreas tissue homogenates and in the small intestinal content was examined in originated 8 animals from each group. The new-born rabbits were controlled weekly from 1 to 6 weeks of age. The data of the present trial suggest that in the early weaned significantly differs the digestive enzyme activity. The objective of present study was also to investigate the effect of IMMUNOVET–HBM® on hydrolase's activity in the 21 day-old rabbits. The results were summarized in Table 2 and 3.

**Key words:** rabbit, early weaning, non-medicated diet, hydrolase's activity, IMMUNOVET–HBM®,

### **MATERIAL AND METHODS**

The experiment was performed by fourthly dropped 15 white Pannon White does and their progeny. Does were randomly distributed into three groups and housed in flat deck cages of a separate building. The growing rabbits were kept in two-level cages in a closed air-conditioned building in which the temperature was 21-24°C.

The does were fed by three type diet (Table. 1) ad libitum till the weaning at 21 days.

One part of the does dropped at 31 day of pregnancy was treated by oxytocin (5 IE/doe).

The sucking rabbits were marked by tattooed and later weaned.

The sucking rabbits may eat diet of does till the weaning, but after 8 weeks of age kept also the basal control diet (without medicaments).

**Table 1. Analytical Compositions of Diet**

<b>Compositions</b>	<b>Control</b>	<b>Medicated</b>	<b>IMMUNOVET–HBM®</b>
Energy, MJ/kg	9.7	9.7	9.7
Crude protein, %	15.0	14.9	15
Crude fat, %	2.9	2.7	2.9
Crude fiber, %	18.3	18.7	18.3
Lysine, %	0.688	0.683	0.688
Metione, %	0.341	0.342	0.341
Met+Cist, %	0.574	0.568	0.574
Ca, %	1.399	1.401	1.399
P, %	0.733	0.733	0.733
Na,%	0.256	0.255	0.256
A-vitamin, NE/kg	15000	15000	15000
D-vitamin, NE/kg	1250	1250	1250
E-vitamin, NE/kg	50	50	50
<b>Medications</b>			
Tiamulin-hydrogen-fumarat, mg/kg	-	50	-
Dilasuril, mg/kg	-	1	-
Oxytetracilin, mg/kg	-	499	-

Eight young rabbits per groups were clinical controlled weekly till 6 weeks of age when the experimental rabbits were euthanized by overdosing of CO<sub>2</sub>, bled and measured their bodyweight. After taking out of alimentary tract the activity of trypsin,  $\alpha$ -amylase and lipase were measured of homogenized pancreas and in small intestinal content.

#### Enzyme activity assays

The frozen pancreatic tissue was cleared from fat, and was homogenized in ice cold saline, in a *Potter-Elvehjem* homogenizer. The prepared pancreas and small intestinal contents were diluted with distilled water and centrifuged (15000/min) then the supernatants were used for the enzyme tests.

Protein content of the samples was assayed by the method of LOWRY *et al.* (1961), with bovine albumin used as reference standard. The homogenate was diluted in distilled water, activated by addition of 0.02 M CaCl<sub>2</sub> solution, and tested immediately for lipase activity by the method of SHÖN *et al.* (1961). To control the activity of  $\alpha$ -amylase was used the Phadebas Amylase Test, Pharmacia Diagnostic AB, Uppsala. The proteolytic zymogens of pancreas were activated by incubation in the presence of enterokinase at 37 °C; trypsin activity was determined by Boehringer colorimetric test (SZABO *et al.* 1976). The hydrolytic products were detected with the *Folin-Ciocalteu* reagent.

The experimental data were evaluated by one-way analysis of variance using SPSS 9.0 programme package.

## RESULTS AND DISCUSSION

The results of laboratory findings are summarized in Table 2 and 3. On the basis of these experimental data we may point out that the hydrolase's activity of pancreatic

tissue homogenates, small intestinal contents has been significantly influenced by the weaning-time of the rabbits, at least at the 42 days periods. The enzyme activity in the small intestinal content showed higher correlation with the activity of the same hydrolase's in the pancreatic tissue. The lack of correlation between the enzyme activities of small 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 trypsin, however the flora is able to produce certain digestive enzymes i. e. lipase (BRUCKNER and SZABO 1984). In our experiment the trypsin-activity was however high in the small intestinal content. Contrary to this, in the activity of lipase was higher or at least identical in the small intestinal content than in pancreatic tissue. 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 hydrolase's. On this bases we believe, that the known changes of feed intake (LEBAS, 1979) and nutrient digestibility coefficients (MAERTENS and DEGROOTE, 1982) during the course of pregnancy and lactation (LEBAS, 1975) can not be explained on the basis of the digestive hydrolase's activity changes. The activity of pancreatic enzymes ( $\alpha$ -amylase, lipase, trypsin) increased with age of animals in all groups (Table 2).

**Table 2 Changes of pancreas tissue hydrolase's activity does during early weaned rabbits**

GROUP	THE ANIMALS AGE (days)					
	7	14	21	28	35	42
	<i>Trypsin (mU/mg ptotein)</i>					
CONTROL	35.4 ± 2.1	36.2 ± 2.0	37.0 ± 1.7 <sup>b</sup>	40.2 ± 2.0 <sup>a</sup>	42.0 ± 2.1 <sup>a</sup>	45.4 ± 2.0 <sup>a</sup>
IMMUNOVET-HBM <sup>®</sup>	<b>33.2 ± 2.0</b>	<b>36.4 ± 1.8</b>	<b>43.6 ± 1.8<sup>a</sup></b>	<b>47.3 ± 2.0<sup>b</sup></b>	<b>50.4 ± 2.1<sup>b</sup></b>	<b>57.2 ± 2.0<sup>b</sup></b>
MEDICATED	31.5 ± 2.1	35.1 ± 2.0	39.2 ± 2.3 <sup>b</sup>	44.6 ± 2.0 <sup>c</sup>	47.9 ± 2.2 <sup>c</sup>	50.3 ± 2.0 <sup>c</sup>
	<i>Lipase (mU/mg ptotein)</i>					
CONTROL	42.6 ± 1.2	45.3 ± 1.3 <sup>a</sup>	47.5 ± 1.9 <sup>a</sup>	61.4 ± 2.6 <sup>a</sup>	63.8 ± 2.5 <sup>a</sup>	69.3 ± 2.5 <sup>a</sup>
IMMUNOVET-HBM <sup>®</sup>	<b>39.6 ± 0.9</b>	<b>51.2 ± 1.9<sup>b</sup></b>	<b>57.4 ± 2.4<sup>b</sup></b>	<b>69.3 ± 2.6<sup>b</sup></b>	<b>77.2 ± 2.3<sup>b</sup></b>	<b>80.8 ± 2.6<sup>b</sup></b>
MEDICATED	41.2 ± 1.0	49.6 ± 2.3 <sup>b</sup>	52.2 ± 2.1 <sup>c</sup>	60.4 ± 3.0 <sup>a</sup>	66.0 ± 2.4 <sup>a</sup>	72.4 ± 2.2 <sup>a</sup>
	<i><math>\alpha</math>-Amylase (U/mg ptotein)</i>					
CONTROL	3.2 ± 0.5 <sup>a</sup>	3.7 ± 0.5	4.1 ± 0.7 <sup>a</sup>	6.0 ± 0.9 <sup>a</sup>	7.7 ± 1.0 <sup>a</sup>	10.3 ± 1.5 <sup>a</sup>
IMMUNOVET-HBM <sup>®</sup>	<b>3.1 ± 0.3<sup>a</sup></b>	<b>3.9 ± 0.6</b>	<b>7.9 ± 0.6<sup>b</sup></b>	<b>10.4 ± 1.6<sup>b</sup></b>	<b>12.8 ± 1.9<sup>b</sup></b>	<b>14.8 ± 1.8<sup>b</sup></b>
MEDICATED	4.0 ± 0.3 <sup>b</sup>	3.5 ± 0.7	5.8 ± 0.8 <sup>c</sup>	7.6 ± 1.0 <sup>a</sup>	9.5 ± 1.3 <sup>c</sup>	11.2 ± 1.8 <sup>a</sup>

<sup>a,b</sup> significant difference between groups (P<0,05)

The highest increase may be observed in the activity of  $\alpha$ -amylase. According to the expected the growing manifested after the second life week when the young rabbits already ate solid food, too.

The increase hydrolase's of enzyme activity in group IMMUNOVET-HBM<sup>®</sup> which was sudden and remained remarkably higher until the end of experiment.

In the present experiment significant increase in the activity of  $\alpha$ -amylase may be observed in group IMMUNOVET–HBM<sup>®</sup> after 21 days of age, especially following the weaning (42 days of age).

Significant activity of lipase may measured in new-born rabbits which is based also the high fat content of does-milk.

The notable increase in activity of lipase may be observed after 28 days of age and mainly following the weaning (42 days of age) first of all in the group IMMUNOVET–HBM<sup>®</sup>.

The difference in activity of trypsin though was less, but mainly at 42 days of age could be measured significant deviation amongst the three groups.

The activity change of same enzymes originated from content of small intestine in tendency were similar to enzymes were measured in homogenized pancreas (Table 3).

**Table 3 Changes of small intestinal content hydrolase's activity does during early weaned rabbits**

GROUP	THE ANIMALS AGE (days)					
	7	14	21	28	35	42
	<i>Trypsin (mU/mg ptotein)</i>					
CONTROL	36.9 ± 3.0	38.6 ± 1.0	40.5 ± 2.0	41.8 ± 1.5	43.6 ± 1.7	44.1 ± 2.1 <sup>a</sup>
IMMUNOVET–HBM <sup>®</sup>	<b>36.6 ± 2.3</b>	<b>39.5 ± 2.2</b>	<b>42.9 ± 3.1</b>	<b>44.9 ± 2.3</b>	<b>46.8 ± 1.3</b>	<b>48.7 ± 2.3<sup>b</sup></b>
MEDICATED	36.0 ± 0.9	38.0 ± 1.3	41.6 ± 1.3	42.7 ± 1.4	45.5 ± 2.4	47.1 ± 1.6 <sup>a</sup>
	<i>Lipase (mU/mg ptotein)</i>					
CONTROL	57.9 ± 7.2	58.4 ± 8.4	61.1 ± 9.1	62.0±10.6	64.6 ± 8.5	66.9±11.3 <sup>a</sup>
IMMUNOVET–HBM <sup>®</sup>	<b>55.7 ± 8.1</b>	<b>58.2 ± 9.3</b>	<b>66.3 ± 8.4</b>	<b>71.1 ± 9.7</b>	<b>77.5 ± 9.9</b>	<b>81.1±11.4<sup>b</sup></b>
MEDICATED	56.6 ± 7.6	56.8 ± 10.3	63.3 ± 9.6	66.5±10.1	69.7±10.6	71.8±12.4 <sup>a</sup>
	<i><math>\alpha</math>-Amylase (U/mg ptotein)</i>					
CONTROL	0.47 ± 0.07	0.49 ± 0.09	0.52 ± 0.11	0.57 ± 0.07	0.61 ± 0.12	0.69± 0.14 <sup>a</sup>
IMMUNOVET–HBM <sup>®</sup>	<b>0.42 ± 0.06</b>	<b>0.47 ± 0.09</b>	<b>0.56 ± 0.07</b>	<b>0.64 ± 0.08</b>	<b>0.71 ± 0.09</b>	<b>0.82± 0.09<sup>b</sup></b>
MEDICATED	0.44 ± 0.09	0.48 ± 0.07	0.54 ± 0.08	0.59 ± 0.09	0.63 ± 0.08	0.7 ± 0.09 <sup>a</sup>

<sup>a,b</sup> significant difference between groups (P<0,05)

Although the differences were significant the highest hydrolase's activity of three enzymes may be established in IMMUNOVET–HBM<sup>®</sup> group.

### CONCLUSION

Summarizing the results can be established that the activity of enzymes ( $\alpha$ -amylase, lipase, trypsin) originated from the hydrolase's activity increased after feeding medicated (IMMUNOVET–HBM<sup>®</sup>) and conventional diet.

The results of hydrolase's activity at the same time argued also of medicated feeding the beneficial effect of physiological process in digestive system.

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