

## Effect of a Grain Extract on Certain Digestive Physiological Indicators in Early Weaned Rabbits

Melinda Kovács<sup>1</sup>, Emma Kósa<sup>2</sup>, Péter Horn<sup>1</sup>, Zsolt Szendrői<sup>1</sup>, Gábor Milisits<sup>1</sup>

<sup>1</sup>Kaposvár University, Faculty of Animal Science, Kaposvár, Hungary

<sup>2</sup>Szent István University, Faculty of Veterinary Science, Budapest, Hungary

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### Abstract

The effect of a non-medicated diet with or without a grain extract feed additive (benzoquinones as main active ingredients) on the growth of rabbits and certain physiological indicators of the digestive tract was examined. One-day-old rabbits of average birth weight were distributed into litters of eight, and these litters were randomly divided into three groups (21-22 litters/group). The control group (Group C) received a basal diet. The diet fed to rabbits of Group IM, was supplemented with a feed additive containing natural basic ingredients (Immunovet-HBM, 1 kg/t); the diet fed to Group M was medicated (tiamulin, oxytetracycline and diclazuril). Three days prior to kindling and up to weaning at 21 days of age of the pups, the does were fed one of the three diets *ad libitum*. Young rabbits were allowed to consume the same diets beside their mother's milk before weaning.

Significant ( $p < 0.05$ ) difference in body weight between groups was detected only at the ages of 4 and 8 weeks. The pH of the gastric content remained significantly ( $p < 0.05$ ) higher in Group IM after weaning. Rabbits in Group IM showed the highest pancreatic enzyme (trypsin, lipase,  $\alpha$ -amylase) activities throughout the period studied. The composition of microflora of the caecum was only slightly altered by the treatment. The total volatile fatty acid content (tVFA) increased with age and from day 28 it was significantly ( $p < 0.05$ ) higher in the C and IM group than in M rabbits. The proportion of butyric acid was lower than that of propionic acid even on the 42<sup>nd</sup> day in Group M.

From the results of this study it is clear that the early weaning of rabbits can be accomplished by the use of a non-medicated diet without any decrease in weight gain. In our study the grain extract feed additive exerted a beneficial effect by increasing the pancreatic enzyme activity and maintaining a better VFA ratio.

*Digestive physiology, early weaning, grain extract, rabbit*

Mortality in meat rabbit production is primarily due to diseases of the digestive tract (Gidenne and Fortun-Lamothe 2002) and these diseases have a major impact on the welfare of animals. Digestive diseases of rabbits are rarely due to specific pathogenic agents. Acute diarrhoea develops as a result of non-specific enteropathy of multifactorial origin especially in the critical post-weaning period of life. Previous studies (Pascual 2001; Gidenne and Fortun-Lamothe 2002) indicate that changing feed in young rabbits from milk to solid feed markedly affects the maturation and development of the alimentary tract (ecosystem of the caecum, local immune system of the mucous membrane, enzyme activity, etc.) and determines the resistance of rabbits to enteropathogenic agents. These developmental changes during early weaning are not completely understood and necessitate more thorough studies on the developmental process of the digestive tract from birth to 42 days of age. Antibiotics are still widely used to reduce mortality, which has been implicated as a potential concern regarding food safety and human health. In recent years, with increasing concern over drug residues in meat products and increases in bacterial resistance due to the prophylactic use of antibiotics, the use of alternatives has received renewed emphasis. Therefore, the current experiment was conducted to study the effect of a non-medicated diet with or without a grain extract feed additive on the growth of rabbits and certain physiological indicators of the digestive tract.

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#### Address for correspondence:

Melinda Kovács  
University of Kaposvár  
H-7400 Kaposvár, Guba S.u. 40. Hungary

Phone/Fax: +36 82 505 970  
E-mail: kovacs.melinda@ke.hu  
<http://www.vfu.cz/acta-vet/actavet.htm>

## Materials and Methods

### Experimental animals and design

Three groups of Pannon White does and their progenies were used in the experiment. The does and the kits were housed in flat-deck cages in a closed building, with 16 light hours per day. After weaning, rabbits were reared in fattening cages made of wire mesh until slaughter (2–3 rabbits per cage), at 15–16 °C average temperature, a 16 L : 8 D lighting cycle and overpressure ventilation.

One-day-old pups of average birth weight were distributed into litters of eight, and these litters were randomly divided into three groups (21–22 litters/group). The litter size was maintained constant until day 21 by replacing dead rabbits with rabbits of similar age and body weight from replacement litters. A total of 512 rabbits were used in the experiment. Feed consumption of the litters and individual body weights (BW) were measured at 3, 4, 8 and 11 weeks.

Eight rabbits per group (altogether 144) were randomly chosen at days 7, 14, 21, 28, 35 and 42 of age. These rabbits were euthanised at 11:00 h by an overdose of carbon dioxide, bled, weighed, and the weight of the kidneys, liver, heart and lungs was measured. After removing the gastrointestinal tract, the weight and pH of the gastric, small intestinal and caecal content, as well as the weight and length of the empty stomach, small intestine, caecum and colon were measured. The research protocol, including procedures for the care and treatment of the animals, was reviewed and approved by the Animal Use and Care Administrative Advisory Committee of the Municipal Veterinary Service for Animal Protection (Protocol No. 00618/007/SOM/2003).

### Diets

Three days prior to kindling and up to weaning at 21 days of age of the young, the does were fed one of the three diets *ad libitum*. The control group (Group C) received a basal diet containing 9.7 MJ DE/kg, 16% crude protein, 4.2% crude fat and 31.6% NDF. The diet fed to rabbits of Group IM was supplemented with a feed additive containing natural basic ingredients (Immunovet-HBM, 1 kg/t), while the diet fed to Group M was medicated (50 mg/kg tiamulin, 500 mg/kg oxytetracycline, 1 mg/kg diclazuril). Young rabbits consumed the same diets beside their mother's milk before weaning, and thereafter during the fattening period. The main active ingredients of the grain extract were benzoquinones (methoxy-p-benzoquinone and 2,6-dimethoxy-p-benzoquinone) produced during the fermentation of wheat germ with yeast, as well as other, hitherto precisely not identified biologically active substances that have antioxidant, immune stimulatory, roborant properties and affect/stimulate the formation of certain growth factors (e.g. IGF-1 and IGF-2) and cytokines (Szende et al. 1998).

### Enzyme activity assays

The frozen pancreatic tissue was cleared from fat and homogenized in ice cold saline, in a Potter-Elvehjem homogenizer. The homogenized pancreatic content was diluted with distilled water and centrifuged at  $10,000 \times g$  for 10 min; then the supernatants were used for enzyme assays. 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 Schön et al. (1961). To test for the activity of  $\alpha$ -amylase the Phadebas Amylase Test (Pharmacia Diagnostic AB, Uppsala) was used. 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) and the hydrolytic products were detected with the Folin-Ciocalteu reagent. Enzyme activity data were expressed in katal (kat, 1 kat = 1 mol/s).

### Microbiological examination

For microbiological examinations, a dilution series (with 0.9% NaCl) was made from 1 g of caecal chyme, the dilutions were smeared onto the surface of the selective culture media. Total aerobic germ count was determined on blood agar after incubation at 37 °C for 48 h in an LP-104 type thermostat (LMIM, Esztergom, Hungary). The obligate anaerobe bacteria (incl. *Bacteroides*) were cultured on Schaedler's agar (Sharlan Chemie, Barcelona, Spain), the selectivity of which was increased by the addition of esculin (Merck, Darmstadt, Germany), neomycin (Merck, Darmstadt, Germany) and Fe-ammonium citrate (Sharlan Chemie, Barcelona, Spain). The gamma sterile Petri dishes (Biolab, Budapest) were placed into Anaerocult culture dishes (Merck, Darmstadt, Germany), in which the anaerobic conditions were ensured with the help of an "Anaerocult A" (Merck, Darmstadt, Germany) gasifying bag. Subsequently the samples were incubated in an LP 104 type thermostat (LMIM, Esztergom, Hungary) at 37 °C for 96 h. Coliforms were cultured on a Chromocult differentiation medium (Merck, Darmstadt, Germany). The samples were incubated at 37 °C for 24 h in a thermostat under aerobic conditions. After the incubation time had elapsed, the colonies were counted with a Titriplaque colony counter (LMIM, Esztergom, Hungary). The colony counts were expressed in log<sub>10</sub> colony forming units (CFU) related to 1 g of sample.

### VFA and pH measurement

The pH of the gastric, intestinal and caecal content was measured with a manual automatic pH meter (OP-110, Radelkis, Hungary). About 3 g of caecal chyme were homogenized with 4.5 ml metaphosphoric acid (4.16%), then centrifuged at  $10,000 \times g$  for 10 min and filtrated. The concentration of volatile fatty acids was measured by gas chromatography (Shimadzu GC 2010, Japan). 2-ethyl-butyrates (FLUKA Chemie GmbH, Buchs, Switzerland) was used as internal standard. Parameters: Nukol 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m capillary column (Supelco, Bellefonte, PA, USA), FID detector, 1 : 50 Split ratio, 1  $\mu$ l injected volume, helium 0.84 ml/min. Parameters of the detector: air 400 ml/min, hydrogen 47 ml/min, temperature: injector 250 °C, detector 250 °C, column 150 °C.

### Statistical analyses

Statistical analysis of the data obtained was carried out by the SPSS statistical software package using the version 10.0. Effect of treatment, age and their interaction was analyzed by the following general linear model, using liveweight as covariate:

$$y_{ijkl} = \mu + T_i + A_j + TA_{ij} + W_k + e_{ijkl}$$

where  $\mu$  = mean,  $T_i$  = effect of treatment,  $A_j$  = effect of age,  $TA_{ij}$  = interaction of treatment and age,  $W_k$  = effect of liveweight,  $e_{ijkl}$  = random error.

The significance of between group differences was tested by the Tukey *post hoc* test.

## Results

Production variables (i.e. body weight, feed consumption and conversion) of the whole stock involved in the experiment were estimated (data not shown). Significant difference between groups was detected only in body weight at ages of 4 and 8 weeks; namely, the body weight of rabbits in Group IM (553 g at week 4 and 1,624 g at week 8) was significantly ( $P < 0.05$ ) higher than that of rabbits in Group C (529 and 1,589 g, respectively) and M (503 and 1,548 g, respectively). No significant difference attributable to treatments in feed consumption and feed conversion could be demonstrated. Feed conversion between days 21-42 was 3.16-3.18 g feed/g BW. Mortality between weeks 4 to 11 was 8.9, 8.0 and 5.3 % in Group C, IM and M, respectively.

After birth the relative weight (i.e. % of BW) of the heart + kidneys + lung was higher (2.6-3.5%), and decreased with age till day 35 (1.9-2.1%). In contrast, the relative weight of liver increased with age from 2.8-3.6% to 4.4-4.8%. The increase in liver weight began on day 14 and lasted till day 35. There was a considerable and significant increase in GI weight between days 21 and 28, i.e. after weaning (Table 1). Significant difference between treatments was found only in the gastrointestinal tract weight/body weight on day 42, which was the highest in M rabbits.

Table 1. Relative weight (% BW) of the GI tract (n = 8, mean  $\pm$  SEM)

Group	Age (days)						Interaction
	7	14	21	28	35	42	
C	4.8 $\pm$ 0.1 <sup>a</sup>	5.4 $\pm$ 0.1 <sup>a</sup>	7.3 $\pm$ 0.2 <sup>b</sup>	10.1 $\pm$ 0.3 <sup>c</sup>	9.9 $\pm$ 0.3 <sup>c</sup>	9.9 $\pm$ 0.3 <sup>cA</sup>	N.S.
IM	4.5 $\pm$ 0.1 <sup>a</sup>	5.3 $\pm$ 0.1 <sup>ab</sup>	6.7 $\pm$ 0.3 <sup>b</sup>	10.1 $\pm$ 0.4 <sup>c</sup>	9.3 $\pm$ 0.5 <sup>c</sup>	10.1 $\pm$ 0.3 <sup>cA</sup>	
M	4.5 $\pm$ 0.1 <sup>a</sup>	5.6 $\pm$ 0.1 <sup>ab</sup>	6.9 $\pm$ 0.2 <sup>b</sup>	10.7 $\pm$ 0.5 <sup>c</sup>	10.7 $\pm$ 0.8 <sup>c</sup>	12.0 $\pm$ 0.4 <sup>cB</sup>	

C = control, IM = supplemented with the grain extract, M = medicated

Significant differences ( $P < 0.05$ ) between <sup>a,b,c</sup> ages or <sup>A,B,C</sup> groups, N. S. = non significant

Table 2. Weight and pH of the gastric content (n = 8, mean  $\pm$  SEM)

Group	Age (days)						Interaction
	7	14	21	28	35	42	
Weight (g)							
C	16.8 $\pm$ 2.0 <sup>a</sup>	20.5 $\pm$ 3.5 <sup>a</sup>	19.4 $\pm$ 1.6 <sup>a</sup>	38.2 $\pm$ 3.8 <sup>b</sup>	59.2 $\pm$ 3.5 <sup>cA</sup>	62.3 $\pm$ 3.9 <sup>cA</sup>	***
IM	17.4 $\pm$ 1.5 <sup>a</sup>	23.1 $\pm$ 2.0 <sup>ab</sup>	21.6 $\pm$ 1.6 <sup>a</sup>	37.0 $\pm$ 4.1 <sup>bc</sup>	48.5 $\pm$ 2.4 <sup>cdAB</sup>	54.8 $\pm$ 5.3 <sup>dA</sup>	
M	10.8 $\pm$ 1.4 <sup>a</sup>	18.6 $\pm$ 2.6 <sup>bc</sup>	21.8 $\pm$ 1.9 <sup>bc</sup>	25.6 $\pm$ 2.4 <sup>ac</sup>	41.7 $\pm$ 2.2 <sup>bb</sup>	28.2 $\pm$ 4.0 <sup>bcB</sup>	
pH							
C	4.8 $\pm$ 0.1 <sup>a</sup>	5.7 $\pm$ 0.1 <sup>b</sup>	5.3 $\pm$ 0.1 <sup>ab</sup>	2.5 $\pm$ 0.3 <sup>cA</sup>	2.1 $\pm$ 0.1 <sup>cd</sup>	1.7 $\pm$ 0.1 <sup>d</sup>	*
IM	4.6 $\pm$ 0.2 <sup>a</sup>	5.5 $\pm$ 0.1 <sup>b</sup>	4.8 $\pm$ 0.1 <sup>a</sup>	2.4 $\pm$ 0.2 <sup>cAB</sup>	2.0 $\pm$ 0.2 <sup>c</sup>	2.1 $\pm$ 0.1 <sup>c</sup>	
M	4.2 $\pm$ 0.2 <sup>a</sup>	5.6 $\pm$ 0.1 <sup>b</sup>	5.0 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.1 <sup>cB</sup>	1.9 $\pm$ 0.1 <sup>c</sup>	1.6 $\pm$ 0.1 <sup>c</sup>	

Significant differences ( $P < 0.05$ ) between <sup>a,b,c</sup> ages or <sup>A,B,C</sup> groups, \*  $P < 0.05$ , \*\*\*  $P < 0.001$

The weight of the gastric content (Table 2) was the lowest in Group M both prior to and after weaning. On day 21 a transient decrease could be observed in the other two groups,

Table 3. Changes of pancreas tissue hydrolase activity (n = 8, mean ± SEM)

Group	Age (days)						Interaction
	7	14	21	28	35	42	
<i>α</i> -Amylase (kkat/mg protein)							
C	53.3 ± 3.2 <sup>a</sup>	61.7 ± 3.2 <sup>a</sup>	70.0 ± 3.1 <sup>abA</sup>	100.0 ± 5.3 <sup>bcA</sup>	128.3 ± 6.4 <sup>cA</sup>	171.7 ± 8.5 <sup>dA</sup>	***
IM	51.7 ± 1.6 <sup>a</sup>	65.0 ± 3.2 <sup>a</sup>	131.7 ± 3.3 <sup>bb</sup>	173.3 ± 10.2 <sup>cb</sup>	213.3 ± 11.7 <sup>db</sup>	246.7 ± 10.6 <sup>db</sup>	
M	66.7 ± 1.6 <sup>ab</sup>	60.0 ± 5.1 <sup>a</sup>	98.3 ± 5.3 <sup>bcA</sup>	126.7 ± 6.7 <sup>cdA</sup>	158.3 ± 8.5 <sup>deA</sup>	186.7 ± 10.7 <sup>eA</sup>	
Lipase (kat/mg protein)							
C	710.0 ± 6.4 <sup>a</sup>	755.0 ± 6.1 <sup>abA</sup>	791.7 ± 10.7 <sup>baA</sup>	1023.3 ± 14.9 <sup>cA</sup>	1063.3 ± 15.0 <sup>cA</sup>	1155.0 ± 15.3 <sup>dA</sup>	***
IM	661.7 ± 5.3 <sup>a</sup>	853.3 ± 10.7 <sup>bb</sup>	956.7 ± 12.8 <sup>cb</sup>	1155.0 ± 13.9 <sup>db</sup>	1286.7 ± 12.8 <sup>db</sup>	1346.7 ± 14.9 <sup>db</sup>	
M	686.7 ± 5.1 <sup>a</sup>	826.7 ± 10.8 <sup>bb</sup>	870.0 ± 10.2 <sup>bc</sup>	1006.7 ± 16.0 <sup>cA</sup>	1100.0 ± 13.3 <sup>dA</sup>	1206.7 ± 13.2 <sup>eA</sup>	
Trypsin (kat/mg protein)							
C	590.0 ± 10.1 <sup>aA</sup>	603.3 ± 11.2 <sup>a</sup>	616.7 ± 10.6 <sup>abA</sup>	670.0 ± 11.2 <sup>bcA</sup>	700.0 ± 11.7 <sup>cdA</sup>	756.7 ± 10.7 <sup>dA</sup>	***
IM	553.3 ± 10.7 <sup>aAB</sup>	606.7 ± 10.6 <sup>a</sup>	726.7 ± 10.0 <sup>bb</sup>	788.3 ± 11.6 <sup>cb</sup>	840.0 ± 10.7 <sup>cb</sup>	953.3 ± 11.2 <sup>db</sup>	
M	525.0 ± 10.7 <sup>ab</sup>	585.0 ± 10.9 <sup>b</sup>	653.3 ± 10.8 <sup>cA</sup>	745.0 ± 11.7 <sup>db</sup>	798.3 ± 12.8 <sup>deB</sup>	838.3 ± 11.5 <sup>ec</sup>	

Significant differences ( $P < 0.05$ ) between <sup>a,b,c</sup> ages or <sup>A,B,C</sup> groups, \*\*\*  $P < 0.001$

which was presumably attributable to the lack of appetite associated with weaning.

The pH value of  $< 2$ , typical of adult rabbits, developed by the first week after weaning (28 days of age) in Group M, whereas in Group C this decrease was slower. In IM rabbits the pH remained slightly higher than in the other two groups.

During the experiment there was no significant difference between the groups in the weight of the small intestinal content, with the exception of day 42, when it was significantly ( $p < 0.05$ ) lower in Group M (data not shown). The pH of the chyme increased from an initial value of 6.0 up to day 21, after which it stabilized between pH 7.8–8.0 in all three groups.

The activity of pancreatic enzymes (trypsin, lipase,  $\alpha$ -amylase) increased with age in all groups (Table 3). The greatest change was observed in the activity of  $\alpha$ -amylase. In the control group the increase in  $\alpha$ -amylase activity could be observed following weaning. In Group IM and M the increase between days 14 and 21 already was significant, IM showing the highest activity throughout the following period. Increases in lipase activity could be observed after 21 days of age. Rabbits in Group IM showed the highest activity throughout the period studied. Differences in trypsin activity amongst the groups only became significant after 21 days.

Table 4. Composition of the caecal microflora expressed in log<sub>10</sub> count/g chyme (n = 8, mean ± SEM)

Group	Age (days)						Interaction
	7	14	21	28	35	42	
Total aerobic germ count							
C	3.6 ± 0.3	5.6 ± 0.4	4.1 ± 0.5	4.4 ± 0.3	4.3 ± 0.5	5.2 ± 0.3	*
IM	5.0 ± 0.5	4.8 ± 0.4	5.4 ± 0.5	4.6 ± 0.4	3.7 ± 0.3	5.7 ± 0.3	
M	4.6 ± 0.5 <sup>ab</sup>	5.6 ± 0.4 <sup>bc</sup>	5.2 ± 0.5 <sup>ab</sup>	3.1 ± 0.1 <sup>b</sup>	3.1 ± 0.1 <sup>b</sup>	4.4 ± 0.6 <sup>bc</sup>	
Strictly anaerobic bacteria							
C	8.2 ± 0.2	8.1 ± 0.1	8.3 ± 1.4	9.2 ± 0.2	8.2 ± 0.5	8.2 ± 0.2	N. S.
IM	7.8 ± 0.1	9.4 ± 0.4	10.0 ± 0.1	8.9 ± 0.4	8.6 ± 0.1	8.7 ± 0.2	
M	8.3 ± 0.2	8.4 ± 0.8	10.1 ± 0.1	9.9 ± 0.3	8.5 ± 0.3	8.2 ± 0.2	
Coliforms							
C	2.0 ± 0.3 <sup>aA</sup>	3.5 ± 0.5 <sup>b</sup>	3.5 ± 0.4 <sup>b</sup>	3.1 ± 0.6 <sup>b</sup>	< 2	< 2	N. S.
IM	3.6 ± 0.5 <sup>B</sup>	3.3 ± 0.4	5.0 ± 1.3	2.0 ± 1.7	< 2	< 2	
M	2.5 ± 0.4 <sup>aAB</sup>	4.0 ± 0.8 <sup>b</sup>	3.1 ± 0.2 <sup>ab</sup>	< 2	< 2	< 2	

Significant differences ( $P < 0.05$ ) between <sup>a,b,c</sup> ages or <sup>A,B,C</sup> groups, N. S. = non significant

\*  $P < 0.05$

Composition of the microflora of the caecum was slightly altered by the treatment (Table 4). The number of the anaerobic bacteria growing on the Schaedler agar remained relatively constant throughout the study. The coliform count remained low in all cases. Significant difference between groups could be established only in one case (in the coliform count on day 7).

There were no inter-group differences in the weight and pH of the caecal content (data not shown).

The total volatile fatty acid content (tVFA) increased with age and from day 28 it was significantly higher in the C and IM groups than in M rabbits (Table 5).

Table 5. Volatile fatty acid content of the caecal chyme (n = 8, mean ± SEM)

Group	Age (days)						Interaction
	7	14	21	28	35	42	
tVFA (mmol/kg)							
C	17.0 ± 4.7 <sup>a</sup>	18.6 ± 5.7 <sup>a</sup>	52.1 ± 7.2 <sup>a</sup>	93.3 ± 7.0 <sup>bA</sup>	85.1 ± 7.4 <sup>bA</sup>	104.2 ± 5.9 <sup>bA</sup>	***
IM	8.5 ± 1.9 <sup>a</sup>	20.9 ± 2.5 <sup>a</sup>	59.9 ± 11.0 <sup>b</sup>	79.9 ± 6.2 <sup>bAB</sup>	75.1 ± 8.4 <sup>bAB</sup>	68.0 ± 7.4 <sup>bB</sup>	
M	9.3 ± 2.1 <sup>bc</sup>	23.0 ± 3.6 <sup>ab</sup>	58.9 ± 7.0 <sup>b</sup>	52.2 ± 3.2 <sup>bB</sup>	47.1 ± 5.6 <sup>bcB</sup>	40.2 ± 5.0 <sup>bcB</sup>	
Acetic acid (mol%)							
C	72.7 ± 4.3	73.0 ± 4.9 <sup>A</sup>	73.2 ± 1.9	74.9 ± 1.3	78.1 ± 1.2	76.6 ± 1.4	*
IM	75.0 ± 6.0	76.9 ± 3.9 <sup>AB</sup>	72.1 ± 1.7	78.1 ± 0.7	77.8 ± 1.4	76.7 ± 1.6	
M	86.8 ± 4.0 <sup>ab</sup>	87.6 ± 1.6 <sup>ab</sup>	75.6 ± 1.7 <sup>b</sup>	78.8 ± 1.6 <sup>ab</sup>	77.0 ± 1.3 <sup>ab</sup>	75.1 ± 0.7 <sup>b</sup>	
Propionic acid (mol%)							
C	14.6 ± 1.2 <sup>ab</sup>	17.0 ± 1.5 <sup>a</sup>	15.0 ± 1.3 <sup>ab</sup>	13.7 ± 2.3 <sup>ab</sup>	8.9 ± 1.0 <sup>b</sup>	8.3 ± 0.5 <sup>b</sup>	**
IM	15.9 ± 3.9	14.4 ± 2.2	16.6 ± 0.8	11.7 ± 1.0	10.1 ± 1.0	9.9 ± 0.9	
M	7.9 ± 1.7	8.8 ± 1.2	15.0 ± 1.9	13.5 ± 1.7	12.8 ± 1.7	12.7 ± 1.2	
Butyric acid (mol%)							
C	4.3 ± 0.9 <sup>a</sup>	5.1 ± 1.2 <sup>a</sup>	7.3 ± 0.4 <sup>ab</sup>	10.1 ± 1.4 <sup>abc</sup>	12.2 ± 1.6 <sup>bc</sup>	13.2 ± 0.9 <sup>c</sup>	N. S.
IM	5.2 ± 1.8 <sup>a</sup>	4.7 ± 0.6 <sup>a</sup>	5.5 ± 0.8 <sup>a</sup>	9.0 ± 1.0 <sup>ab</sup>	9.5 ± 0.6 <sup>ab</sup>	11.9 ± 1.2 <sup>b</sup>	
M	2.6 ± 1.0 <sup>b</sup>	1.4 ± 0.4 <sup>a</sup>	5.2 ± 0.8 <sup>ab</sup>	6.6 ± 0.8 <sup>ab</sup>	8.8 ± 0.8 <sup>b</sup>	9.8 ± 1.3 <sup>b</sup>	

Significant differences ( $P < 0.05$ ) between <sup>a,b,c</sup> ages or <sup>A,B,C</sup> groups, N. S. = non significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

The proportion of acetic acid within the tVFA was around 70–80%, and there were no major inter-group differences. The proportion of propionic and butyric acids within the tVFA, and the C3/C4 value indicating the ratio of the two volatile fatty acids as related to each other were different in the groups studied. In suckling rabbits the C3/C4 ratio is > 1. In groups C and IM after consumption of solid feed the percentage proportion (mol %) of propionic acid decreased while that of butyric acid increased, resulting in continuously decreasing C3/C4 values. In Group M the proportion of butyric acid was lower than that of propionic acid even on the 42<sup>nd</sup> day.

## Discussion

The digestive tract is able to adapt to the qualitative and quantitative nutritional changes around weaning. Growth of the body is rapid around weaning (i.e. between the ages of 7 and 35 days). The weight of the GI organs was found to concur with most of the relevant data in the literature (Lebas and Laplace 1972; Alus and Edwards 1977; Piattoni et al. 1997). The weight of the GI followed an almost linear curve between the ages of 7 and 28 days. It seems that early weaning and the subsequent higher consumption of solid feed between days 21 and 28 affected the weight of the digestive system.

According to Dojana et al. (1998) changes of enzyme activity are genetically determined; however, the time of weaning, the amount and composition of solid feed consumed can also influence enzyme activity. They found the highest lipase activity on d 15 with slight decline afterwards. Marounek et al. (1995) also observed the lipase activity to decrease with age. Similarly to the data presented in the current study, others also reported detection of the amylase activity in the pancreas before solid feed consumption. Corring et al. (1972) and Lebas et al. (1977) identified amylase in the pancreas at 1 and 7 days of age. Higher consumption of solid feed after weaning has been shown to stimulate amylase production in the pancreas. Scapilleno et al. (1999) reported a nearly 4% increase after weaning. Although the effect of changes in enzyme activities on the digestibility of different nutrients was not examined, the current study shows an earlier increase and a higher enzyme activity in the rabbits supplemented with grain extract.

It is generally accepted that the antimicrobial effect of feed additives is mainly due to the altered microflora and microbial activity in the GI tract. In rabbits, the highest microbial activity is in the caecum. Microbial fermentation of plant carbohydrates results in volatile fatty acid (VFA) production which is absorbed and covers about 40% of the maintenance requirement (Marty and Vernay 1984). In this study, feeding antibiotic containing diet resulted in lower VFA production compared to the other two groups (i.e. C and IM). It could be presumably due to the slightly lower germ count or lower microbial activity as the main effect of the in-feed antibiotic. Enumeration of the bacteria may, however, not reflect their metabolic activity (Gidenne 1996). Higher VFA production could be beneficial in respect of better energy supply and better body weight gain as a consequence. Higher VFA content is favourable also because they provide the main metabolic fuel for the mucosa of the large intestine (Roediger 1986). The other difference between groups fed antibiotic containing (M) or antibiotic free (C and IM) diet was in the proportion of propionic and butyric acids within the tVFA, and the C3/C4 value. In Group M the proportion of butyric acid was lower than that of propionic acid even after weaning. So, in the other two groups fermentation resulted in higher butyric acid production. Butyrate has been shown to be the preferred substrate for normal colonocyte growth and promotes normal cell phenotype by stabilizing DNA and repair of damage (Leavitt et al. 1978). It has been shown that antibiotics reduce the rate of fermentation, reduce the amino acid catabolism and increase the efficiency of microbial protein production in relation to the amount of glucose fermented (Visek 1978). In our case the reduction in the fermentation was shown (by the lower VFA content of the caecal chyme); however, the positive effect, i.e. improvement in utilisation of protein and energy and a better production as a consequence could not be detected in rabbits fed the medicated diet.

Whether the higher propionate and lower butyrate activity in Group M may be explained by the different substrate supply in the large intestine due to different enzyme activities in the small intestine is not consistent. Different VFA production was not related to the altered pancreatic enzyme activity, rather with the altered activity of microbial enzymes due to different supplementation (grain extract vs. antibiotics).

From the results of this study it is clear that early weaning of rabbits may be accomplished using a non-medicated diet without any decrease in weight gain.

The benzoquinones have been examined mainly as immune-modulators, antimetastatic, apoptosis-enhancing and antioxidant chemical substances (Szende et al. 1998). In agreement with human studies the benzoquinones containing fermented wheat extract had a protective effect against *Mycoplasma gallisepticum* infection of chickens (Stipkovits et al. 2004). So, its application in animal production can be favourable especially in the case of suboptimal management conditions. In our case the grain extract feed additive exerted a beneficial effect presumably by increasing the pancreatic enzyme activity and maintaining a better VFA ratio, but further studies are needed to define the mode of action.

## Vliv obilného extraktu na vybrané fyziologické ukazatele trávení u časně odstavených králíků

V této studii byl zkoumán vliv nemedikované diety s nebo bez přídavku extraktu z obilných zrn (benzochinony jako hlavní aktivní ingredience) na růst králíků a vybrané fyziologické ukazatele trávicího traktu. Jednodenní králíci o průměrné porodní hmotnosti byli rozděleni do osmi vrhů, které byly poté náhodně rozděleny do tří skupin (21 - 22 mlád'at/skupina). Kontrolní skupině (C) byla podávána základní dieta. Krmivo podávané králíkům ze skupiny IM, byla doplněno o aditivní krmný přídatek obsahující přírodní ingredience (Immunovet HBM, 1 kg/t); skupina M byla krmena medikovaným krmivem s obsahem tiamulinu, oxytetracyklinu a diklazurilu. Tři dny před porodem až do odstavení mláďat ve 21 dnech stáří, byly ramlice krmeny jednou ze tří diet *ad libitum*. Před odstavením bylo mláďatům králíků kromě mateřského mléka umožněno konzumovat stejnou dietu s matkou.

U mláďat z jednotlivých skupin byl signifikantní rozdíl v tělesné hmotnosti pozorován pouze ve věku 4 a 8 týdnů. Hodnota pH žaludečního obsahu byla signifikantně zvýšená po odstavení mláďat ze skupiny IM. Králíci ze skupiny IM vykazovali také nejvyšší aktivity pankreatických enzymů (trypsin, lipáza,  $\alpha$ -amyláza) po celou dobu studie. Složení mikroflóry slepého střeva se u králíků z různých skupin odlišovalo pouze zanedbatelně vlivem podávání obohaceného krmiva. Celkový obsah celkových mastných kyselin (tVFA) stoupal s věkem a od 28. dne stáří byl významně vyšší u jedinců ze skupiny C a IM než u králíků ze skupiny M. Podíl kyseliny máselné byl oproti kyselině propionové nižší u králíků ze skupiny M dokonce už 42. den.

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