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THE EFFECT OF ORGANIC ACIDS, MEDIUM CHAIN FATTY ACIDS AND YEAST CELL WALLS ON PRODUCTIVITY, PHYSIOLOGICAL STATE AND INTESTINAL HISTOMORPHOLOGY OF RABBITS

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Abstract. The study was conducted to investigate the effect of organic acids, medium chain fatty acids and yeast cell walls on the productivity, physiological state and intestinal histomorphology of the rabbits. The study was conducted with 14 Californian breed rabbits. The rabbits (77–161 days old) were assigned to two treatment groups (7 rabbits per each treatment group). The dietary treatments were 1) control diet, 2) diet supplemented with the additive *Luscent* (dosage 1 kg/t of feed) - from INNOV AD nv/sa, Belgium – a commercially available product that medium chain fatty acids, Yeast cell wall (*Saccharomyces cerevisiae*), butyric acids esters, plant extracts. The rabbits were kept in individual wire-net cages with grid floors and an individual vessel for watering and feeding. Storage conditions were the same for both groups. Rabbits were fed twice a day (*ad libitum*). During the feeding trial the following parameters were analysed: rabbits' performance, blood parameters, development of intestinal tract, pH and dry matter content in different parts of the intestine, the villus height and the crypt depth. The inclusion of organic acids, medium chain fatty acids and yeast cell walls in the compound feeds increased the rabbit's body weight, weight gain, growth rate and decreased feed intake. The feed additive *Luscent* had a positive effect on the length of the intestinal tract, the villus height in the duodenum.
Keywords: rabbits, organic acids, yeast cell wall, digestive processes

Introduction. The rabbit farming is an important emerging enterprise in many countries of the World. In 2006 European banned the use of antibiotics as growth enhancer in animal diet, and it poses a serious challenge for rabbit meat producers. Because of the very complex and unique digestion of rabbit, this species is susceptible to enteric diseases, particularly after weaning (Kalma et al., 2016). Due to the growing concern about using antibiotics in livestock feeds, it is essential to identify alternatives to antibiotic growth promoters (AGP) in the feed industry. The current candidate replacements include organic acids, probiotics, prebiotics (mainly oligosaccharides and polysaccharides products), plant extracts (essential oils), nucleotide and enzymes, etc. (Cheng et al., 2014).

Several organic acids have been reported to improve growth performance (for example, increased palatability, feed efficiency, mineral absorption, phytate-P utilization) when they are supplemented in non-ruminant diets (Papatsiros, 2011; Partanen and Mroz, 1999). In addition, organic acids are believed to have antimicrobial activity and they have been suggested for the control intestinal microbial growth (Davidson, 2001; Cheng et al., 2014). The antimicrobial activity of organic acids is basically the same, irrespective of acting in food, feed, or gut lumen (Diebold and Eidelsburger, 2006).

Prebiotic feed additives are also possible strategy of enhancing animal health and improving productive performance in the absence of antibiotic growth promoters. Prebiotics selectively stimulate the beneficial bacteria of the caecum microflora. Prebiotic mannanoligosaccharides (MOS) can be derived from the outer layer of yeast cell walls (YCW), which contain a mix of the oligosaccharides along with β-glucans and mannoproteins (The European Association for Specialty Yeast Products. 2012). The inclusion of YCW components from Saccharomyces cerevisiae (S. cerevisiae) can also lead to favorable direct effects on the intestinal mucosa itself (e.g., increasing villi height and goblet cell density) and an improvement in animal productivity (Zhang et al., 2005; Sohail et al., 2012).

Supplementation of rabbit feeds with prebiotics increases volatile fatty acids in the caeca of weanling rabbits, decreasing the caecal ammonia concentration. In addition, to stimulate the beneficial microflora of the gut, prebiotics may prevent the adhesion of pathogens to the mucosa and stimulate the immune response (Forchielli and Walker, 2005; Falçao-e-Cunha et al., 2007). Several authors (Aguilar et al., 1996; Lebas et al., 1996) have observed a decrease in mortality and an improvement in performance when oligosaccharides are added to rabbit feed.

Scientific literature provides non-sufficient data on different organic acid additives consisting of organic acids, medium chain fatty acids and yeast cell walls on the productivity, physiological state and intestinal histomorphology of rabbits.

The aim of this study was to investigate the influence organic acids, medium chain fatty acids and yeast cell walls in rabbit's diets on rabbit's productivity, physiological state and intestinal histomorphology.

Materials and methods

The study was conducted in 2016–2017 in the individual rabbit breeding farm, which had about 400 of rabbits, in the Animal productivity laboratory under the Institute of Animal Rearing Technologies and Pathology centre by the Department of Veterinary Pathology Academy of Lithuanian University of Health Sciences.

Feeding trial was conducted with Californian breed rabbits of 77–161 days old. The rabbits were sampled by weight. The rabbit's weight was 1.54 kg at start of the study. During the study, 14 rabbits were divided into two groups, 7 rabbits in each.

The dietary treatments were 1) control diet, 2) diet supplemented with the additive *Luscent* (*Luscent*- from INNOV AD nv/sa, Belgium – a commercially available product that contains medium chain fatty acids, yeast cell wall *(Saccharomyces cerevisiae),* butyric acids esters, plant extracts) dosed at 1 kg/t of feed.

Table 1. **Nutrition indicators of compound feed**

Indicators	Value
Digestive energy, kcal	2370.70
Metabolized energy, kcal	2257.20
Crude protein*, %	16.40
Crude fibre*, %	16.39
Moisture*, %	10.65
Starch*, %	9.56
Sugar, %	4.38
Total lysine, %	0.65
Methonine + cistine, %	0.65
Triptophane, %	0.20
Linolenic acid, %	1.04
Threonine, %	0.61
Total methionine, %	0.39
Avalable phosphorus, %	0.37
Calcium*, %	1.29
Phosphorus*, %	0.59
Sodium, %	0.25
Chlorine, %	0.54

*analyzed values

Contents of the premix: vit. A – 10.08 TV, vit. D3 – 1.14 TV, vit. E – 50.30 mg/kg, vit. K3 – 0.99 mg/kg, vit. B1 – 3.71 mg/kg, vit. B2 – 2.80 mg/kg, vit. B5 – 9.80 mg/kg, vit. B12 – 0.01 mg/kg, nicotinic acid – 20.40 mg/kg, folic acid – 0.22 mg/kg, choline chloride – 170.00 mg/kg, magnesium – 76.28 mg/kg, iron – 317.00 mg/kg, zinc – 110.89 mg/kg, copper – 19.16 mg/kg, cobalt – 0.29 mg/kg, iodine – 0.67 mg/kg, selenium – 0.31 mg/kg.

The rabbits were stored in individual wire cages with grid floors and an individual vessel for watering and feeding. Storage conditions were the same for all groups. Rabbits were fed twice a day (*ad libitum*).

The main components of the compound feed were the following: hay, corn, oats, wheat, sunflower, vegetable oils and minerals.

Characteristics of additive used in the study

Luscent – medium chain fatty acids (4.44%), essential oils (0.37%), plant extracts (4.76%), Yeast cell wall (12.50%), HSCAS hydrated sodium calcium aluminosilicates (38.30%), Butyric acid esters (15.00%), Antioxydants and Chelators (3.20%), Calcium propionate (E282) (2.00%), Anticaking (15.99%), Natural plant gum (3.00%), Carrier (0.44%).

Zootechnical methods

During the feeding study it was found: individual rabbit weight; rabbits daily weight gain; daily feed intake; feed conversion ratio; growth rate (GR) by formula:

GR = (FW-IW) / IW; where FW - final weight; IW - initial weight (Handa et al., 1995).

Physiological methods

At the end of the study (day 161), blood samples were collected from the *vena auricularis marginal*, using a vacutainer method (Venoject, Terumo Europe N.V., Leuven, Belgium) with lithium-heparin tubes to produce plasma. Blood samples were centrifuged for 20 min at 3000 rpm and the Following the plasma parameters were determined: total protein, ALT, AST, alkaline phosphatase, creatine, urea were determined Cobas Integra 400/700/800 (Biochemical Analysis System, Germany) blood analyzer.

At the end of the feeding test, 5 rabbits were selected from each group and slaughtered. The carcasses were prepared as reported by Blasco and Ouhayoun (1996). After removing the rabbit gastrointestinal tract, it was found the development of intestines (intestine length and intestinal weight). The intestine length of each rabbit was measured with a flexible tape on a glass surface (Lentle et al., 1998). The intestinal walls were washed with saline solution, drained with filter paper, and weighed; pH of duodenum, caecum, ileum and colon was measured by pH-meter "Inolab 730"; dry matter content of duodenum, caecum, ileum and colon was determined by the difference obtained by weighing the wet sample and the sample dried for 3 hours at 105° C (Naumann and Bassler, 1993).

Histomorphometry methods

Duodenum middle segments were taken from each group of rabbits (161 days of age, 5 rabbits from a group) for the study. They were fixed with 10 percent neutral formalin solution. Using standard histological procedures, the tissue was injected into paraffin, tissue sections of 4 µm thickness were cut by rotary microtome and then were painted with hematoxylin and eosin.

The histological preparations were examined using the Olympus BX63 microscope, Olympus DP72 video camera and the computer system "Image Pro Plus software (Olympus), duodenum villi height and crypt depth were morphometrically measured in the control and experimental groups of rabbits. Villi height measurements were carried out from the top of the papillae to villi-crypt connector), and the total mucosal thickness was measured from peak to villi mucosal muscle layer. In each sample, 10 measurements of papillae (best expressed) were made to measure the height and 10 measurements more in the same places were performed to measure the thickness of the mucosal lining. The thickness of the mucosal lining net the villi height is equal to the crypt depth ratio. Measurement unit - micrometer (μm).

Statistical analysis

The results of the experiment were analyzed using the 1-way ANOVA test, and significant differences between groups were determined by Duncan's multiple range test. Statistica 8.0. for Windows TM software was used. Differences were considered significant at P<0.05.

Results and discussion

When analyzing rabbits weights on the 161th day of age (Table 2), it was established that inserting of *Luscent* 1 kg/t of feed (Experimental group) into the compound feed their weights increased by 4%, as compared to the control group, but the data was not significant different (P>0.05).

The rabbits' daily weight gain during whole test period, i.e. from 77 to 161 days, increased by 8% in the experimental group as compared to the control group (P<0.05).

The analysis of the growth rate of rabbits (Table 2) showed that it increased by 9% in the experimental group as compared to the control group (P<0.05).

After insertion of organic acids, medium chain fatty acids and yeast cell walls (*Luscent* 1 kg/t) into the compound feed of rabbits, daily feed intake decreased by 2% per day as compared to the control group (P>0.05). The feed conversion ratio in the Experimental groups was by 5% lower than in the control group. The data are not statistically different.

Table 2. **Effect of organic acids, medium chain fatty acids and yeast cell walls on growth performance of rabbits**

Indices	Groups	
	Control	Experimental
Live weight, g:		
77 days of age	1549.29 ± 0.25	1543.43 ± 0.21
161 days of age	3353.71 ± 0.43	3500.57 ± 0.22
Daily weight gain, g:		
77–161 days of age	45.09 ± 0.51[a]	48.90 ± 0.75[b]
Growth rate, g	1.17 ± 0.32[a]	1.27 ± 0.38[b]
Daily feed intake, g:		
Interval of 77–161 days	96.95 ± 3.89	95.37 ± 4.58
Feed conversion ratio, kg/kg:		
Interval of 77–161 days	2.42 ± 0.12	2.29 ± 0.10
[a, b] – means within each rows with different superscripts are significantly different at P < 0.05.		

Improved zootechnical indicators suggest that the optimal bacterial flora development has led to a good nutrient utilization. The positive effect of organic acids on rabbit growth indicators was established by Debi and other scientists (2010) using citric acid on 30–86 days, by Romero and colleagues (2011) using the mixture of microencapsulated formiate and citric acids on 28–77 days and others (Radwan et al., 2007; Hassanin et al., 2015). However, the impact of organic acids in the feed on rabbits' productivity remains unclear.

Kalma et al. (2016) and Hassanin et al. (2015) reported that dietary yeast *(Saccharomyces cerevisiae)* and medium chain fatty acids in rabbits diet increases zootechnical parameters of rabbits.

Table 3. **Effect of organic acids, medium chain fatty acids and yeast cell walls on blood parameters of rabbits**

Parameter	Groups	
	Control	Experimental
AST, U/l	161.83 ± 146.19[a]	56.53 ± 17.11[b]
ALT, U/l	111.00 ± 27.13	77.47 ± 14.58
Alkaline phosphatase, U/l	274.78 ± 154.20[a]	152.68 ± 47.81[b]
Creatine, μmol/l	85.90 ± 12.16[a]	105.80 ± 6.40[b]
Urea, mmol/l	5.73 ± 0.92	6.33 ± 0.57
[a, b] – means within each rows with different superscripts are significantly different at P < 0.05.		

The analysis on blood parameters of the rabbits showed (Table 3) that dietary supplementation of analysed additive in diets at the rate of 1 g/kg increased the amount of creatine − by 19.90 μmol/l (P<0.05) and decreased AST − by 105.30 U/l (P<0.05), ALT − by 33.53 U/l (P>0.05), alkaline phosphatase − by 122.10 U/l (P<0.05) compared to the control group.

These results are in agreement with those of El Kerdawy (1996). While, Abdel Azeem et al. (2000) showed that level of AST was reduced in growing rabbits fed citric acid, although ALT was not significantly affected. On the other hand, Grassmann and Klasna (1986) reported that dietary addition of 3% citric acid significantly increased the activities of both AST and ALT enzymes.

The effect of organic acids, medium chain fatty acids and yeast cell walls on pH value in the gastrointestinal tract of rabbits is shown in Table 4. A tendency to a lower pH in the content of duodenum, caecum, ileum and colon was observed in the experimental group (P>0.05).

A group of scientists (Romero et al., 2011) found in their study that organic acid did not affect pH of the caecum as compared to the control group.

Table 4. **Effect of organic acids, medium chain fatty acids and yeast cell walls on pH value in the gastrointestinal tract of rabbits**

Parts of digestive tracts	Groups	
	Control	Experimental
Duodenum	7.05 ± 0.11	6.85 ± 0.15
Caecum	6.48 ± 0.06	5.99 ± 0.13
Ileum	7.15 ± 0.14	6.77 ± 0.09
Colon	6.72 ± 0.07	6.37 ± 0.09

With use of benzoic acid, no effect on rabbits' stomach or small intestine pH was seen. Ribeiro and others (2012) noticed no effect of sodium butyrate on gastric pH. This trend suggests in part that organic acids of the digestive tract of rabbits are not working as expected. A degree of specificity of the rabbit gastrointestinal tract (small gastric motility and prolonged retention in the stomach feed time), as well as cecotrophy distinguish them from other animals. Perhaps this was due to the fact that in a number of studies the expected results in relation to the pH of the digestive tract were not obtained.

During the study of Cardinali et al. (2008), the effect of formic acid and lactic acid (5 g/kg) on the caecal pH was found. It was shown that the use of organic acid increased fermentative activity in caecum - pH of caecum content was 5.82, while in the control group the pH was 6.03.

Nevertheless, several studies have clearly confirmed organic acids effect on gastrointestinal pH. A recent study with butyric acid (sodium butyrate) has proved that if inserted into the feed it reduced caecal pH.

Table 5. **Effect of organic acids, medium chain fatty acids and yeast cell walls on dry matter concentration in the gastrointestinal tract of rabbits, %**

Parts of digestive tracts	Groups	
	Control	Experimental
Duodenum	15.42 ± 1.23	14.92 ± 1.48
Caecum	20.82 ± 1.62	19.34 ± 1.34
Ileum	9.38 ± 1.53	10.89 ± 1.41
Colon	21.59 ± 1.75	20.45 ± 1.28

The analysis of the dry matter content of the rabbits' gastrointestinal segments showed (Table 5) that organic acids, medium chain fatty acids and yeast cell walls in ileum increased by 1.51%, but decreased in caecum - by 1.48%, in colon – by 1.14% ($P>0.05$), as compared to the control group.

Increased quantity of dry contents in caecum can be caused by the used feed, since in fibre is a good barrier to water, and this water with the fibre is pushed farther into the large intestine, and then a large part of it is removed with solid faeces.

The analysis of the rabbits' intestinal weight and length (Table 6) showed that the length of intestine of experimental group was longer by 8% ($P<0.05$), but weight of the intestine was lower by 12% ($P<0.05$) as compared to the control group.

Table 6. **Effect of organic acids, medium chain fatty acids and yeast cell walls on intestine development of rabbits**

Indices	Control	Experimental
Weight of intestine, g	431.75 ± 103.34^{a}	381.75 ± 30.49^{b}
Length of intestine, cm	460.50 ± 18.38^{a}	496.75 ± 43.28^{b}
$^{a, b}$ – means within each rows with different superscripts are significantly different at $P < 0.05$.		

There is a positive effect of additives on the experimental group of the rabbits' length of intestine. This is in line with the early conducted our experiment (Kliseviciute et al., 2016).

Table 7. **Effect of organic acids, medium chain fatty acids and yeast cell walls on duodenum histomorphology of rabbits, µm**

Parts of digestive tracts	Groups	
	Control	Experimental
Crypt depth	312.95 ± 17.22^{a}	227.63 ± 14.23^{b}
Villus height	1364.85 ± 102.39^{a}	1385.00 ± 100.26^{b}
$^{a, b}$ – means within each rows with different superscripts are significantly different at $P < 0.05$		

The results of intestinal histomorphometry measurements of rabbits are presented in Table 7. Supplementation at 1 kg/t a mixture of analysed additive in diets for rabbits increased the height of duodenum villi by 20.15 µm ($P<0.05$), but decreased the depth of crypts – 85.32 µm ($P<0.05$) as compared to the control group.

Regarding the relations between performance and gut morphology, Vieira et al. (2008) reported that significant correlations were not observed between performance and villus height or crypt depth. But, Awad et al. (2009) concluded that villus condition is a common criteria measurement for investigation of the effects of nutrition on gut physiology. Longer villus could be considered as an indicator of an active functioning of intestinal villi. Increased villi height provides more surface area for nutrients absorption. On the contrary, reduction in villus height can reduce nutrient absorption due to the decrease in the intestinal surface area for absorption. Thus, reduction in nutrient absorption, decreased resistance to disease and lower growth performance and increase in secretion of gastrointestinal tract are the negative consequences of deeper crypt and shorter villi (Xu et al., 2003). Also, Loddi et al. (2004) and Pelicano et al. (2005) explained that increased villus heights with most of the organic acids was attributed to the fact that organic acids reduce the growth of many pathogenic or nonpathogenic intestinal bacteria, decreasing the intestinal colonization and infectious processes, ultimately decreasing the inflammatory reactions at the intestinal mucosa, which increases the villus height and functions of secretion, digestion and absorption of nutrients by the mucosa.

The use of organic acids and medium chain fatty acids was proven to have a positive impact on growing rabbits' duodenal papillae. In the control group, the papillae length was 349 µm, while in the experimental group the length was 395 µm ($P < 0.05$) (Hassan et al. 2010).

Conclusions

The results of this study clearly demonstrate that supplementation of organic acids, medium chain fatty acids and yeast cell walls in the rabbits' diets had positive effect on growth performance, blood parameters, on the length of the intestinal tract, the villus height in the duodenum of rabbits.

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