INFLUENCE OF DIETARY INCLUSION OF BUTYRIC ACID AND ORGANIC ACID SALT MIXTURE ON RABBITS' GROWTH PERFORMANCE AND DEVELOPMENT OF DIGESTIVE TRACT

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Abstract. The study was conducted to investigate the impact of butyric acid and organic acid salt mixture additives on growth performance and development of the digestive tract in rabbits. The study was conducted with 14 Californian breed rabbits. The rabbits (28–77 days old) were assigned to 2 treatment groups (7 rabbits per each treatment group). The dietary treatments were: 1) control diet, and 2) diet supplemented with a mixture (*Novibac*®, dosage 1 kg/t of feed, and *Novyrate*®, dosage 1 kg/t of feed) – from INNOV AD nv/sa, Belgium – a commercially available product that includes butyric acid, citric acid, calcium formate and propionate, silicic acid and zeolite. Rabbits were stored in individual wire 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, development of the intestinal tract, pH and dry matter content in different parts of the intestine. The inclusion of butyric acid and organic acid salt mixture in the compound feeds increased the body weight, feed intake as well as growth rate of rabbits and improved the feed conversion ratio. The additives did not have a significant effect on the pH value of different parts of the gastrointestinal tract of rabbits. The additives had a positive effect on the development of the intestine: the intestine length increased by 1% ($P>0.05$) and the intestine weight increased by 5% ($P<0.05$) in the experimental group compared with the control group.

Keywords: rabbits, butyric acid, organic acid salts, productivity, digestive tract

Introduction. Organic acids and salts have a long history in food and feed industries, which commonly use them as preservatives. Organic acids are routinely included in diets for monogastric animals in Europe in order to replace antibiotics as growth promoters (Falcão-e-Cunha et al., 2007). As a group of chemicals, organic acids are considered to be any organic carboxylic acid of the general structure R-COOH (including fatty acids and amino acids). Not all of these acids have effects on gut microflora. Organic acids (C1-C7) with specific antimicrobial activity are short-chain acids (C1–C7) and are widely distributed in nature as normal constituents of plants or animal tissues (Dibner and Buttin, 2002). Acidifiers have also been assayed for intensive rabbit production diets, as either organic acids or their salts with research being focused mainly on both health and productive performances (Leticia et al., 2005). However, because the importance of hindgut fermentation in feed utilisation is greater in rabbits than in young growing pigs, the possible effect of organic acids on the caecal environment cannot be overlooked.

Organic acids work in animals not only as a growth promoter but also as a meaningful tool of controlling all entrintic bacteria, both pathogenic and non-pathogenic (Naidu, 2000; Wolfenden et al., 2007). Moreover, organic acid feeding is believed to have several beneficial effects such as improving feed conversion ratio and growth performance, enhancing mineral absorption and speeding recovery from fatigue (Abdel-Fattah et al., 2008).

Organic acids – formic, fumaric, lactic, propionic and phosphoric – are investigated as potential growth promoters, having a positive effect on intestinal health and development of rabbits (Zdzislaw, 2005; Nezhad et al., 2007). Among other organic acids, citric acid is cheap, available, non-corrosive and has a greater growth promotion property. Its addition significantly increases live weight gain, feed conversion efficiency and availability of nutrients (Shen et al., 2005; Moghadam et al., 2006). It also increases the immunity of animals (Abdel-Fattah et al., 2008).

Scientific literature provides insufficient data on different organic acid additives consisting of butyric acid, citric acid, calcium formate and propionate, silicic acid and zeolite in rabbit productivity and physiological indicators.

The aim of this study was to investigate the impact of butyric acid and organic acid salt mixture on growth performance and development of the digestive tract in rabbits.

Materials and methods. The study was conducted in 2014–2015 in an individual X rabbit breeding farm, which had about 400 rabbits of different breeds, and in

the Animal Productivity Laboratory under the Institute of Animal Rearing Technologies of Veterinary Academy of the Lithuanian University of Health Sciences.

The study was carried out in accordance with the Law of the Republic of Lithuania on animal care, storage and use No. XI-2271 of 3 October 2012 (Valstybės žinios, 3 October 2012, No. 122 (151)) as well as secondary legislation – the Order of the Lithuanian State Food and Veterinary Service approving storage, maintenance and operation of animals used for experimental and other scientific research (Valstybės žinios, 24 September 2015, No. B1-872). The research was conducted in line with the EU Directive 2010/63 of the EU and EC recommendations 2007/526 EC on handling and storage of animals used for experimental and other purposes.

The feeding trial was conducted with Californian breed rabbits of 28–77 days old. The rabbits were sampled by weight. The rabbits' weight was 452±34g at the beginning of the study. During the study, 14 rabbits were divided into 2 groups, 7 rabbits in each.

The dietary treatments were: 1) control diet, and 2) diet supplemented with a mixture (*Novibac*® and *Novyrate*® − from INNOV AD nv/sa, Belgium – a commercially available product that contains butyric acid, citric acid, calcium formate and propionate, silicic acid, zeolite) dosed at *Novibac*® 1 kg/t + *Novyrate*® 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
Methionine + cystine, %	0.65
Tryptophan, %	0.20
Linolenic acid, %	1.04
Threonine, %	0.61
Total methionine, %	0.39
Available phosphorus, %	0.37
Calcium*, %	1.29
Phosphorus*, %	0.59
Sodium, %	0.25
Chlorine, %	0.54
*analysed 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 both groups. The 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 preparations used in the study

Novyrate® consists of salts of fatty acids (butyric acid of 98%) (100%).

Novibac® consists of calcium formate (55.0%), calcium propionate (10.0%), citric acid (10.0%), medium-chain fatty acids (0.50%), plant extracts (0.30%) silicic acid precipitated and joined together (2.0%), zeolite (22.20%).

Zootechnical methods

During the feeding study, the following parameters were determined: individual rabbit weight; rabbit's 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 feeding test, 5 rabbits were selected from each group and slaughtered in accordance with the recommendations for euthanasia of experimental animals (Close et al., 1997). After removing the rabbit's gastrointestinal tract, the development of intestines (intestine length and intestinal weight) was determined. 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 the duodenum, the caecum, the ileum and the colon was measured by pH-meter Inolab 730. Dry matter content of the duodenum, the caecum, the ileum and the 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).

Statistical analysis. The results of the experiment were analysed using the 1-way ANOVA test, and significant differences between the 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 analysing the rabbits' weights on day 77 of age (Table 2), it was established that inclusion of *Novibac*® 1 kg/t and *Novyrate*® 1 kg/t into the compound feed (experimental group) increased their weights by 5% ($P<0.05$), as compared with the control group.

The rabbits' daily weight gain during the whole test period, i.e. from day 28 to 77, increased by 8% in the experimental group as compared with the control group ($P<0.05$).

The analysis of the growth rate of rabbits (Table 2) showed that it increased by 8% in the experimental group as compared with the control group ($P<0.05$).

Table 2. **Effect of butyric acid and organic acid salt mixture (*Nobivac*® and *Novyrate*®) on growth performance of rabbits**

Indices	Group	
	Control	Experimental
Live weight, g:		
77 days of age	1296.60±47.39[a]	1356.20±46.24[b]
Daily weight gain, g:		
28–77 days of age	16.60±0.75[a]	17.85±0.94[b]
Growth rate, g	1.68±0.42[a]	1.82±0.34[b]
Daily feed intake, g:		
Interval of 28–77 days	78.25±10.54[a]	80.58±9.47[b]
Feed conversion ratio, kg/kg:		
Interval of 28–77 days	4.71±0.60	4.51±0.49
[a, b] – means within each row with different superscripts are significantly different at P<0.05		

After inclusion of *Novibac*® 1 kg/t and *Novyrate*® 1 kg/t into the compound feed of rabbits (experimental group), daily feed intake increased by 3% per day as compared with the control group (P<0.05). The feed conversion ratio in the experimental groups was by 4% lower than in the control group. The data are not statistically different.

The analysis of the effect of the above mentioned additives added to the rabbits' compound feed showed that *Novibac*® 1 kg/t and *Novyrate*® 1 kg/t had a positive impact on liveability of rabbits.

The weight gain and feed conversion ratio were improved in the study of Azza and other scientists (2008), when using fumaric, citric and malic acids of 0.5% or mixtures thereof. Cardinali with colleagues (2008) performed a study with weaned rabbits of 28 days old and found that formic acid and lactic acid (5 g/kg) had a positive effect on weight gain in the second phase of fattening and on the feed conversion degree. Similar results were found by Radwan and Abdel-Khalek (2007) in the experiment with rabbits fed compound feed supplemented with acetic and lactic acids of 0.5%, and a positive impact of butyric acid on those indicators was demonstrated (Hassanin et al., 2015).

Table 3. **Effect of butyric acid and organic acid salt mixture (*Nobivac*® and *Novyrate*®) on pH value in the gastrointestinal tract of rabbits**

Parts of digestive tracts	Groups	
	Control	Experimental
Duodenum	6.92±0.07	6.75±0.10
Caecum	6.28±0.15	6.33±0.07
Ileum	7.07±0.06	6.98±0.05
Colon	6.47±0.03	6.45±0.07

The effect of butyric acid and organic acid salt mixture on pH value in the gastrointestinal tract of rabbits is shown in Table 3. A positive tendency to a lower pH in the content of the duodenum and the ileum was observed in the experimental group (P>0.05). A decrease in pH in the intestinal tract creates an acid milieu killing many pathological organisms, which may cause various diseases (Gibson, 2004). The decreasing pH is an indicator showing the higher production of SCFA caused by an increased microbial fermentation (Steenfeldt et al., 1998).

Table 4. **Effect of butyric acid and organic acid salt mixture (*Nobivac*® and *Novyrate*®) on dry matter concentration in the gastrointestinal tract of rabbits, %**

Parts of digestive tracts	Groups	
	Control	Experimental
Duodenum	16.23±1.89	16.16±2.03
Caecum	20.14±0.98[a]	22.58±1.89[b]
Ileum	8.97±1.00	9.89±1.16
Colon	18.45±1.25[a]	20.81±1.72[b]
[a, b] – means within each row with different superscripts are significantly different at P<0.05		

Our results differ from those obtained by Zhu and other researchers (2014), when drinking water was supplemented with the mixture of organic acids and caused a decrease in the pH of the stomach, but the changes in the caecum pH were not observed. Amaefule et al. (2011) found decreased pH in the small intestine without significant changes in the pH of the caecum.

The analysis of the dry matter content of the rabbits' gastrointestinal segments showed (Table 4) that butyric acid and organic acid salt mixture (experimental group) increased by 2.44% in the caecum (P<0.05), by 0.92% in the ileum (P>0.05), by 2.36% in the colon content (P<0.05), as compared with the control group.

These results coincide with Romero's (2011) results, where higher quantity of dry matter in the caecum was established when using formic acid of 0.4% and citric acid of 0.2% (P=0.045).

Table 5. **Effect of butyric acid and organic acid salt mixture (*Nobivac*® and *Novyrate*®) on intestine development of rabbits**

Indices	Control	Experimental
Weight of intestine, g	270.3±7.05[a]	283.1±5.36[b]
Length of intestine, cm	499.7±10.10	503.00±23.99
[a, b] – means within each row with different superscripts are significantly different at P<0.05		

The analysis of the rabbits' intestinal weight and length (Table 5) showed that butyric acid and organic acid salt mixture (*Novibac*® and *Novyrate*®) had a positive impact on the said indicators: the rabbits' intestinal weight of the experimental group was higher by 5% (P<0.05), and the length of the intestine was longer by 1% (P>0.05), as compared with the control group.

There is a positive effect of additives on intestinal development of rabbits in the experimental group. This is in line with the studies conducted by Radwan and Abdel-Khalek (2007) and Uddin with colleagues (2014). Meanwhile, Dorra with other researchers (2013) found no effect of the mixture of formic and propionic acids on the development of the intestine.

Conclusions

The results of this study clearly demonstrate that supplementation of butyric acid and organic acid salt mixture in the rabbits' diets had a positive effect on growth performance, dry matter content of the gastrointestinal tract and intestinal development of rabbits.

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