Effect of dietary butyrate supplementation on the production performance and parasitology of growing rabbits

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Abstract. The effect of a dietary butyrate supplementation on the production of fattening rabbits was examined. The control group (n=70 rabbits) was fed with granulated diet whereas the diet of butyrate group (n=70 rabbit) was supplemented with 0.2% of butyrate. The mortality rate was examined on a larger population (n=1050 rabbits/group). Butyrate group had lower weight gain at the ages of 46-52 and 60-66 days (-20 and -17 %, respectively; P>0.001) but higher weight gain at 53-59 days of age (+13%; P<0.05). Butyrate group consumed less feed than the control group between 38-45, 46-52 and 60-66 days (-2.4%, P<0.001; -5.7%, P<0.01; -4.9%, P<0.05, respectively). The feed conversion ratio of the butyrate group was worse between 46-52 days of age (+19%; P<0.01) but favourable at the ages of 53-59 and 67-73 days (-15% and -9%, respectively; P<0.05). Concerning the whole fattening period the weight gain, the feed intake and the feed conversion ratio of the groups did not differ. In the larger examined population, 4.0% and 6.3 % mortality was observed in the control and butyrate groups, respectively. The parasitological tests resulted only negative samples independently of groups. It can be concluded that dietary butyrate supplementation has not improved the performance of the growing rabbits.

1 Introduction

The Hungarian rabbit population consists of around 100 thousand rabbit does and their offspring. In the two rabbit slaughterhouses almost 4 million rabbits were slaughtered in 2022 [1]. One of the biggest problems facing the sector is the treatment and prevention of gastrointestinal diseases in rabbits, which is the basic condition of economical production.

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Diarrhoea is a common symptom among fattening rabbits and can result in high rate of mortality. Digestive illnesses can be caused by a number of reasons, such as improper feed (too high carbo-hydrate level, too low fiber content, change of diets without transition period) and infections caused by bacteria, viruses or parasites [2].

The epizootic rabbit enteropathy (ERE) has been caused high mortality on rabbit farms in Hungary and also all around the world. The ERE is a complex, serious syndrome of the digestive system, that mostly affects weaned rabbits and has caused great financial losses on rabbit farms since 1997 [3, 4]. Diarrhoea in post weaning period can affect up to 100% of the rabbit population and the mortality rate, in the absence of proper treatment can increase to 30-80% [5]. Based on practical and experimental observations the typical symptoms of ERE are the total loss of hunger, the reduction in water intake, liquid filled stomach, expanded small intestine, abdomen tension, obstipathy, alternating constipation and/or diarrhoea, mucin in the stomach (formerly named as mucoid enteropathy) and also during pathological examination a full bladder can be observed [6].

There have been several scientists trying to identify the cause of ERE and also to find the parasites, responsible for the emergence of ERE but no unequivocal answer has been given [7, 8]. It was established already in 2000 by Coudert *et al.* [9] that a coccidial infection even at a very low dose can strengthen the symptoms of ERE that leads to worse weight gain and higher mortality rate.

The volatile fatty acids (VFA) produced in the caecum provide 12-40% of the maintenance energy requirements of rabbits. The different VFA-s play roles in physiological functions, such as inflammatory reactions, the mucous membrane of the colon and the parasite protection as well [10]. A comparative analysis of the microbiota in the caecum highlighted significant differences between healthy rabbits and rabbits suffering from ERE [11]. Based on these, the relationship between VFA concentration and the microbiota in caecum deserves deep investigation. Jin *et al.* [12] reported a decreased level of VFA and butyrate concentration in the caecum of rabbits suffered from ERE, which was attributed to the decreased number of *Lactobacillus Alistipes* and other fibrolitical bacteria and that of the butyrate producing bacteria, such as Eubacteria and Facelibactaria.

Butyrate is produced in the colon through microbial fermentation of dietary fiber and it is an important energy source for cells lining the colon of mammals (colonocytes) [13]. As a short-chain fatty acid, butyrate is metabolized by mitochondria as an energy source through fatty acid metabolism [14]. Butyrate has numerous effects on energy homeostasis, inflammation, and immune function in humans. It has a key role in immune homeostasis both locally (in the gut) and systemically and it is a critical mediator of the colonic inflammatory response [15].

Since to the best of our knowledge, dietary butyrate supplementation of rabbits has not yet been studied, our aim was to examine its effect on production performance and parasitological infection of growing rabbits.

2 Materials and methods

2.1 Animals, housing, feeding

The experiment was carried out at Dabas rabbit farm of Tetrabbit Ltd in Hungary. The rabbit stables were tempered at a constant 18-22 ºC and 16 L: 8D lighting schedule was applied. The fattening rabbits were kept in galvanized wire mesh cages (cage sizes: 86 x 38 x 30 cm, 5 rabbits / cage) from weaning to slaughter age (38–73 days of age). The weaned rabbits were randomly divided into two differently fed groups. The Control group (n = 70 rabbits) was fed with a commercially available (Cargill feed Inc.) granulated feed (Control diet; pellet size 3

mm). In case of Butyrate group (n = 70 rabbits) the same Control diet was supplemented with 0.2 % of butyric acid. Both groups were fed *ad libitum*. The ingredients and chemical components of the diets are shown in Table 1. The rabbits could drink water freely from nipple drinkers. The mortality rate was examined on a bigger population (n = 1050 rabbits/group) of fattening rabbits that were kept under the same housing conditions (in the same building and similar cages) and were fed with the same diets as the experimental groups.

Table 1. Ingredients and chemical components of the Control diet
(The diet of Butyrate group contained butyrate in 0.2 %)

Ingredients	
Alfalfa pellet, %	40.21
Wheat bran, %	30.00
Barley, %	15.14
Oat, %	5.00
Sunflower meal, %	5.00
Sugar beet pellet, %	2.45
Sunflower hay, %	0.50
Arbocell, %	0.50
Potassium carbonate, %	0.47
NaCl, %	0.41
Rabbit premix 0.3, %	0.30
L-Lisine HCl, %	0.09
DL-Methionine, %	0.01
Chemical composition	
DE, MJ/kg	9.2
Dry matter, %	89.4
Crude protein, %	15.6
Crude fat, %	2.7
Crude fibre, %	17.0
NDF, %	37.5
ADF, %	20.0
ADL, %	4.65

Notes: The diets contained 66 ppm Robenidine as coccidiostat

2.2 Production and parasitological data collection

During the test period the weight of the rabbits and the feed consumption per cage were measured weekly. Using these data, the daily weight gain of the rabbits, the average feed consumption and the feed conversion ratio were calculated. (The average daily weight gain of the rabbits was calculated as the weight of the rabbit at the end of the examined period minus the weight of the rabbit at the beginning of the period, divided by the number of days. The daily feed intake per animals in each cage was calculated as the amount of diet in the feeder at the beginning of the examined period minus the amount of the diet at the end of the period, divided by the number of animals per cage and the number of days. In case of mortality, it was assumed that the died animals did not consume any pellet for the 2 days preceding their death. The feed conversion ratio for each cage was calculated as the average daily feed intake divided by the average daily weight gain of the animals.) The mortality was daily checked.

Manure samples were collected according to a standardized method [16] and were analysed implementing a mixed sample floatation fluid method (McMaster method as recommended by the Royal Veterinary College and FAO; [17]) in the laboratory of S&K-Lap Ltd. A minimum of 2-5 g of manure per group was collected under the cages and were

mixed, i.e. the samples were not individual samples, but mixed samples of the individuals per group. The samples were examined within 48 hours after collection. The applied solution was a mixture of 400 g magnesium sulphate ($MgSO_4$) and one Liter of water. The samples were tested for the presence of *Eimeria oocist* and *Passalurus ambiguous* egg and larva. Subsequently, quantitative determination and morphological identification of Eimeria species were planned.

2.3 Statistical analysis

The normal distribution of data was checked by using Shapiro-Wilk Test, comparison of production data was carried out by the use of T-test, the mortality rates were compared by Chi-square test, by using R-project software. The difference between the means of the groups was considered significant when P-value < 0.05.

3 Results and discussion

In case of butyrate supplementation, a significantly decreased level of feed intake was detected in 3 periods of fattening compared to control group (Table 2). Rabbits in butyrate group consumed lower amount of diet by 2.4%, ($P < 0.001$), 5.7% ($P < 0.01$) and 4.9% ($P < 0.05$) between 38-45, 46-52 and 60-66 days of age, respectively. Although at a younger age rabbits in the Butyrate group ate less feed, there was no significant difference between the feed consumption of the groups in the last week of fattening. When taking the whole fattening period into account, significant difference cannot be verified in the feed consumption.

Table 2. Effect of the dietary butyrate supplementation on the feed intake of growing rabbits

Traits	Groups		SEM*	*P*-value
	Control	Butyrate		
Feed intake, g/day (n=14 cages/group)				
38-45 d	126	123	0.42	<0.001
46-52 d	159	150	1.74	0.008
53-59 d	169	161	2.59	0.128
60-66 d	183	174	2.37	0.046
67-73 d	200	196	2.48	0.384
38-73 d	**165**	**161**	**1.45**	**0.152**

Notes: *SEM: standard error of means

Despite the differences in feed consumption of the two dietary groups, the butyrate supplementation had no significant effect ($P>0.05$) on the body weight of rabbits in none of the tested ages (Table 3). The rabbits in the two groups reached approximately similar slaughter weight.

Table 3. Effect of the dietary butyrate supplementation on the body weight of growing rabbits

Traits	Groups		SEM*	*P*-value
	Control	Butyrate		
Body weight, g (n=70 rabbits/group)				
38 d	1272	1273	12.7	0.996
45 d	1620	1623	15.8	0.921
52 d	1896	1844	16.2	0.106
59 d	2231	2216	20.0	0.714
66 d	2569	2498	23.2	0.125
73 d	**2880**	**2835**	**25.9**	**0.389**

Notes:*SEM: standard error of means

Even though the body weight of the two groups did not differ, fluctuate tendencies can be detected in the weight gain of rabbits at different ages (Table 4). These trends only partially coincide with those observed in feed consumption. While rabbits fed with butyrate supplemented diet had lower weight gain between 46-52 and between 60-66 days of age (+20% and +17%, respectively; *P<0.001*) in the in-between period at 53-59 days of age the weight gain of the butyrate group was higher by 13% compared to the control group (P<0.05). Considering the whole fattening period though these differences levelled out and the weight gain of the two groups showed no significant difference.

Table 4. Effect of the dietary butyrate supplementation on the weight gain of growing rabbits

Traits	Groups		SEM*	*P*-value
	Control	Butyrate		
Weight gain, g/day (n=70 rabbits/group)				
38-45 d	49.7	50.1	1.02	0.834
46-52 d	39.4	31.5	0.56	<0.001
53-59 d	47.2	53.2	1.32	0.022
60-66 d	48.3	40.3	0.96	<0.001
67-73 d	44.4	47.5	0.90	0.081
38-73 d	**45.9**	**44.5**	**0.50**	**0.180**

Notes:*SEM: standard error of means

The detected differences in weight gain and feed consumption in certain periods resulted in differences in the feed conversion ratio as well. Whereas the feed conversion ratio of the butyrate group was significantly worse than that of the control group at the age of 46-52 days (+19%; P<0.01; Table 5) however the butyrate group had favourable feed utilization at the periods of 53-59 and 67-73 days of age (-15% and -9%, respectively; P < 0.05). Taking the whole growing period into account, the feed conversion ratios of the two groups were identical.

Table 5. Effect of the dietary butyrate supplementation on the feed conversion ratio of growing rabbits

Traits	Groups		SEM*	*P*-value
	Control	Butyrate		
Feed conversion ratio (n=14 cages/group)				
38-45 d	2.56	2.48	0.06	0.515
46-52 d	4.06	4.85	0.14	0.003
53-59 d	3.71	3.14	0.13	0.026
60-66 d	3.65	4.42	0.22	0.086
67-73 d	4.56	4.14	0.09	0.020
38-73 d	**3.63**	**3.63**	**0.06**	**0.961**

Notes:*SEM: standard error of means

Of all the rabbits examined in the experiment (n = 140) only 2 rabbits died in the control group (2.9%) and 1 rabbit in the butyrate group (1.4%). Diarrhoea did not occur in the animals involved in the experiment. The mortality rate was also examined on a larger population (n=1050 rabbits/group) of fattening rabbits that were kept under the same conditions and were fed with the same diets as the experimental groups. In the case of the larger population a significant difference was detected (P<0.05), as there was a 4.0% mortality rate in the control group and an unfavourable 6.3% mortality rate in the butyrate group considering the whole duration of the fattening period. It can be assumed that dietary butyrate supplementation had an adverse effect on mortality results, but there are no literature data to confirm or disprove this effect.

The parasitological tests showed an all in all very favourable picture as not any Eimeria oocytes or *Passalurus ambiguus* were detected from the faeces samples independently of the dietary group.

Taking the whole fattening period into consideration, the production performances of rabbits consuming butyrate supplemented diet did not differ from the Control group. Although the body weight of groups did not differ at any of the ages studied, in two of the five examined weeks the Butyrate group gained faster and in one in-between week the weight gain of Control group was higher. These differences were not identical to those seen in feed consumption, as the rabbits ate lower amount from butyrate supplemented diet at each examined week compared to Control diet, however the difference in feed intake was only significant in 3 weeks. Due to the variable trends described above, feed conversion ratio was favourable for Control group in two weeks of investigation and for Butyrate group in one week.

4 Conclusions

Based on the results, it cannot be stated that dietary butyrate supplementation clearly improved or worsened the production results of rabbits. Unambiguous trends could not be detected in the examined traits, except for feed consumption. In some periods of the fattening, the rabbits ate lower amount from the butyrate supplemented diet. In the case of the larger examined population dietary butyrate supplementation had an adverse effect on mortality. Parasitological infection could not be detected from manure samples independently of the dietary supplementation. As a conclusion the use of butyrate supplementation of the diet of growing rabbits cannot be recommended.

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