SODIUM BUTYRATE AS A NATURAL GROWTH PROMOTER FOR RABBITS

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Abstract - The authors investigated the impact of sodium butyrate supplementation on animal production, digestibility of nutrients and gut flora under farm conditions involving 30 NZW male and female rabbits per trial group weaned at six wk of age. Feeding trials were conducted in one control (Group 1) and in two experimental groups (Group 2 and Group 3; 0.15% and 0.30% sodium butyrate supplementation, respectively). The trial lasted until ten wk of age of animals. Feeding trials were followed by metabolism trials involving 5 males per group, over the period of 10-12 wk of age in order to determine digestible coefficients of feed nutrients. At the end of the trial the 15 rabbits were killed for examination of chymus at distal ileum and caecum as well as gastric fundus sample. Results of feeding trials showed that feed supplementation with 0.15% and 0.30% sodium butyrate significantly improved production parameters ($P< 0.05$ and $P< 0.01$, respectively), although there was practically no difference between the two sodium butyrate supplemented groups. Metabolism trial showed that sodium butyrate supplementation resulted in significantly ($P< 0.01$ and $P< 0.001$, respectively) improved digestibility of all major nutrients. On the basis of bacteriologic, microbiologic and chemical examinations the most advantageous form of eubiosis was found in Group 2 animals fed .15% sodium butyrate supplemented diet. In conclusion, 0.15% sodium butyrate supplementation in feed (non-foreign substance), first of all by sustaining the gut flora balance, appears to be most suitable for the purpose of substituting some foreign feed additives in feeding rabbits.

INTRODUCTION

The breakdown site of the indigestible part of feed crude fibre, starch and sugars is the colon in monogastric animals, resulting in the formation of volatile fatty acids, especially butyric acid, propionic acid and acetic acid that are the primary energy sources for colon microbes. Butyric acid is likely to play an important role in the growth and differentiation of colon (PASCALE *et al.*, 1992; GAMET *et al.*, 1993). Earlier trials (BOKORI and GALFI, 1990) showed definite advantages of pigs fed diets containing 0.17% sodium butyrate. Average weight gain increased and specific feed conversion improved. The results of microbiologic investigations of gut bacteria showed a decrease in the ratio of harmful coliforms but an increase of lactobacilli.

Cyniclomyces guttulatus is a yeast inhabiting the rabbit's gut found with a high incidence (almost 100%) in the faeces of healthy rabbits, especially when carbohydrate rich diet components were fed. /Cyniclomyces guttulatus was also isolated in chinchillas.

This yeast can be cultured neither on common fungus medium nor under aerobic conditions. 1% yeast-extract can be isolated in liquid medium adjusted to pH 3-4 on adding hydrochloric acid, and containing 1% peptone and 2% malt-extract. The above mentioned Agar solidified medium in CO_2-enriched air gives rise to colonies of whitish-butter colour at 37°C but not at room temperature. To maintain the colonies, since cultures have very limited life span, it is essential to transfer them every two weeks.

Earlier studies have showed that the site of multiplication of Cyniclomyces guttulatus was the gastric fundus where budding cells were found in the gastric mucosa. Multiplying cells in the small and large intestine chymus were rarely found.

At low temperature (15-25°C) either on Agar plate or in the faeces some of the cells were found to contain one to six ascospores which are more resistant than the vegetative cells.

In conclusion, these findings have revealed that the Cyniclomyces guttulatus is a natural member of gut flora in rabbits and plays an important role in maintaining gut flora balance. However, future research emphasis in the field will be needed to gain more in-depth understanding regarding its role.

This trial was conducted under farm condition to investigate the impact of sodium butyrate supplemented feed on the performance, nutrients' digestibility and gut flora in growing rabbits from the time of weaning.

MATERIAL AND METHODS

One control (Group 1) and two trial groups (Group 2 and Group 3) were used in the feeding trial which involved 30 NZW male and female rabbits (858 g average body weight) per group weaned at 6 wk of age and kept in individual two-level hutches. Control animals were fed ad lib granulated breeding rabbit compound alone (Table 1). Group 2 and Group 3 diets were supplemented with 0.15% and 0.30% of sodium butyrate of 75% concentration, respectively. The trial lasted four weeks, until 10 wk of age of the animals. Body weight and feed intake were measured, specific feed conversion was calculated, mortality and its diagnosis were registered individually during each week of the trial.

Table 1 : The nutritive value of the rabbit diet (%)

Dry matter	93.55	Ether extract	2.35
Ash	8.05	Crude fiber	14.01
Organic matter	85.50	N-free extract	52.64
Crude protein		16.50	

The feeding trial was followed by metabolism trial to determine the nutrients' digestibility coefficients in the diet, involving five male rabbits of 10-12 wk of age per group.

At the end of the metabolism trial 15 rabbits were overslept for the examination of the distal ileum chymus sample (10 cm from caecum) and caecum, as well as of the wall of the gastric fundus. The following parameters of the ileum chymus were determined at 37°C: total germ number (CFU*/g), coliforms (CFU/g), lactic acid producers (CFU/g), anaerobe decomposers (CFU/g), acetic acid, propionic acid, i-butyric acid, n-butyric acid, i-valeric acid, n-valeric acid and lactic acid. The presence of Cyniclomyces guttulatus in the mucus membrane near the gastric pylorus was investigated by native microscopic and culture methods.

RESULTS AND DISCUSSION

Table 2 : The results of the feeding trial (means ± s; n = 30)

Groups		Weeks 6.	7.	8.	9.	10.	6-10.
				Body weight (g)			
1		830	1027	1227[a]	1411[a]	1640[a]	-
	s	129	136	138	157	154	-
2.		882	1095	1328[b]	1536[c]	1787[c]	-
	s	120	146	160	179	190	-
3.		863	1092	1326[c]	1536[c]	1780[c]	-
	s	126	123	149	154	178	-
				Feed intake (g)			
1.			556	623	655	829[a]	2670[a]
	s		85	79	118	99	172
2.			534	592	649	758[b]	2529[b]
	s		131	109	154	123	169
3.			530	583	643	746[c]	2504[c]
	s		119	115	116	109	283
				Feed conversion (kg/kg))			
1.			2,82	3,11[a]	3,56[a]	3,62[a]	3,29[a]
	s		0,91	0,66	0,78	0,89	0,85
2.			2,51	2,54[c]	3,12[b]	3,02[c]	2,80[b]
	s		1,00	0,86	0,90	0,54	0,82
3.			2,31	2,49[c]	3,06[b]	3,06[c]	2,73[b]
	s		0,56	0,57	0,91	0,90	0,73

a-b: P<0.05; a-c: P<0.01

Results of feeding trial are summarised in Table 2. Group 2 and Group 3 animals' weight gain was significantly higher than that of controls. No differences were observed between Group 2 and Group 3. For the above weight gain, Group 2 and Group 3 consumed significantly less ($P< 0.05$) feed resulting in better specific feed conversion than the control animals ($P< 0.05$). No differences were observed in this respect between Group 2 and Group 3, either.

In the control group two animals, while in Group 2 and Group 3 none died.

Results from the feeding trial have demonstrated that 0.15% and 0.30 % sodium butyrate supplementation significantly improved performance parameters, however, butyric acid supplementation above 0.15% is not advantageous. Low death rate number cannot be related to treatments.

Results of metabolism trial are summarised in Table 3. These data show that sodium butyrate supplementation significantly ($P< 0.01$ and $P< 0.001$, respectively) improved digestibility of all nutrients. There is no considerable difference between Group 2 and Group 3. The results suggest that the improvement in production parameters can be explained by better nutrients' digestibility.

Results of microbiologic investigations are summarised in Table 4, while Table 6 contains the results of chemical investigations. It has been found that germ number in rabbits' chymus showed a CFU/g value which is about 1-2 exponent less than that of pigs and poultry. Results of ileum chymus (Table 4) have showed that total germ number in the control group and in Group 2 was nearly the same, while there was a considerable (by an

*: CFU = Colony Forming Unit

order of magnitude) increase in Group 3. Number of coliforms markedly decreased in Group 2 (to the limit of traceability: 10^2/g),but in Group 3 the detected level exceeded that of the controls. A slight decrease in the number of lactic acid producers was found in the trial groups. The number of anaerobe sporophytes (Clostridium) was found to be at its lowest in the control group followed by Group 2 and Group 3 with a characteristically increasing trend.

Table 3 : The nutrients' digestibility of the diets, % (means ± s; n = 5)

Diets		Dry matter	Organic matter	Crude protein	Ether extract	Crude fiber	N-free extract
1.		66,08[a]	66,78[a]	79,94[a]	67,34[a]	26,68[a]	76,05[a]
	s	2,28	2,40	1,34	1,68	4,91	3,98
2.		76,54[d]	77,46[d]	79,52[d]	75,84[d]	52,02[d]	83,84[d]
	s	0,48	0,85	0,31	1,39	1,18	2,00
3.		75,21[d]	76,68[d]	78,71[d]	71,96[d]	50,06[d]	81,60[d]
	s	1,49	1,47	2,37	1,42	2,05	1,19

a-d: P<0.01; e-d: P<0.001

Table 4 : Results of microbiological investigations (means ± s; n = 5)

Intestine wall	Ileum			
Cyniclomyces guttulatus	Total germ number	Coliformes	Lactic acid producers	Clostridium
1. ++++	$5,3x10^5$	$9,5x10^4$	$4,9x10^5$	$1,0x10^1$
2. ++++	$5,2x10^5$	$1,1x10^2$	$1,8x10^4$	$2,2x10^2$
3. ++++	$8,6x10^6$	$2,5x10^5$	$4,1x10^4$	$1,0x10^3$

Caecum			
Total germ number	Coliformes	Lactic acid producers	Clostridium species
1. $7,8x10^5$	$4,5x10^5$	$1,9x10^6$	$4,5x10^2$
2. $1,2x10^6$	$1,0x10^2$	$3,9x10^4$	$6,0x10^2$
3. $5,3x10^5$	$5,8x10^4$	$2,4x10^5$	$3,4x10^3$

Table 5 : Relations (rations) of germ groups in the ileum chymus

	Group 1	Group 2	Group 3
Total germ n. / Coliformes	$\frac{5,3x10^5}{9,5x10^4}$ = 5,6	$\frac{5,2x10^5}{1,1x10^2}$ = 4727	$\frac{8,6x10^6}{2,5x10^5}$ = 34
Lactic acid p. / Coliformes	$\frac{4,9x10^5}{9,5x10^4}$ = 5,2	$\frac{2,8x10^4}{1,1x10^2}$ = 225	$\frac{3,2x10^4}{2,5x10^5}$ = 6,3
Total germ n. / Clostridium	$\frac{5,3x10^5}{1,0x10^2}$ = 5300	$\frac{5,2x10^5}{2,2x10^2}$ = 2364	$\frac{8,6x10^6}{1,0x10^3}$ = 8600

Investigation data, along with theoretical assumptions, provided evidence that it is not the absolute number of germs alone but the ratio of the germ groups that indicates the balance of gut flora (eubiosis) or its momentary state. The most frequently used germ group ratios are as follows: total germ/coliform ratio, lactic acid producer/coliform ratio and total germ/Clostridium ratio. Germ group ratios in ileum chymus are listed in Table 5. Total germ/coliform and lactic acid producer/coliform ratios were found to be the most favourable in Group 2, but there was no difference in total germ/Clostridium ratio. Considering all the circumstances, the most favourable form of eubiosis was found in Group 2, but the likelihood of dysbiosis is rather low in the other two groups, too. Investigations of caecum chymus showed (Table 4) slight increase in total germ number in Group 2 compared to controls, followed by a decrease in Group 3. It should be noted that changes in caecum volatile fatty acid concentration (acetic acid, propionic acid and n-valeric acid) characteristically follows the changes in total germ number in each of the trial groups (Table 6). The number of coliforms, like in the ileum, dramatically decreased in Group 2 (to the limit of traceability: 10^2/g), but increased in group 3. The number of lactic acid producers was found to be the highest in the control group. It markedly decreased in Group 2 and less so in Group 3. The number of Clostridiums showed a slight but definite increase in the different groups in the following order: Group 1, Group 2 and Group 3.

Table 6 : The volatile fatty acid and lactic acid content of caecum, mg/cm³ (means ± s; n = 5)

Group		AA	PA	IBA	NBA	IVA	NVA	LA
1.		5,03	0,97	0,018	3,004	0,023	0,174	0,072
	s	0,44	0,24	0,050	1,052	0,004	0,048	0,039
2.		4,54	0,87	0,032	3,770	0,032	0,172	0,076
	s	1,47	0,22	0,016	0,490	0,020	0,360	0,020
3.		6,55	1,32	0,058	3,340	0,057	0,227	0,050
	s	2,08	0,66	0,040	1,380	0,038	0,073	0,000

AA:) acetic acid; PA: propionic acid; IBA: iso butiyric acid; NBA: n-butyric acid; IVA: iso valeric acid; NVA: n-valeric acid; LA: lactic acid.

Table 7 : Relations (rations) of germ groups in the caecum chymus

	Group 1	Group 2	Group 3
Total germ n. / Coliformes	$\frac{7,8x10^5}{4,5x10^5} = 1,7$	$\frac{1,2x10^6}{1,0x10^2} = 12000$	$\frac{5,3x10^5}{5,8x10^4} = 9,1$
Lactic acid p. / Coliformes	$\frac{1,9x10^6}{4,5x10^5} = 4,2$	$\frac{3,9x10^4}{1,0x10^2} = 390$	$\frac{2,4x10^5}{5,8x10^4} = 4,1$
Total germ n. / Clostridium	$\frac{7,8x10^5}{4,5x10^2} = 1733$	$\frac{1,2x10^6}{6,0x10^2} = 2000$	$\frac{5,3x10^5}{3,4x10^3} = 156$

The frequency of anaerobe sporophytes (Clostridium)shows a tendency to follow the changes in the concentration of i-butyric acid and i-valeric acid (Table 6). Ratios of germ groups are shown in Table 7. The results obtained in chemical investigations of digesta at the caecum show that ratios of total germ number/coliform, lactic acid producer/coliform and total germ number/clostridium are the most desirable in Group 2, and there was no possible dysbiosis in the other two groups, either.

Results from investigation of gastric mucus showed the presence of an obligate gut fungus (Cyniclomyces guttulatus) in all trial rabbits, regardless of the above mentioned two methods used. Therefore in Group 2 and Group 3 sodium butyrate concentration in the diet has no impact on the obligate gut fungus, a member of the natural gut flora in rabbits.
In conclusion, results of bacteriologic, microbiologic and chemical investigations showed more desirable gut flora balance in Group 2 (0.15% sodium butyrate supplementation) than in controls or in Group 3.

DISCUSSION

The results obtained in these feeding trials are in accordance with the previous ones (GALFI and BOKORI, 1990) in growing pigs, however, in the latter, butyrate supplementation resulted in increased ad lib feed intake in pigs as well as in broilers. In the present trial the impact of sodium supplementation resulted in decreased feed intake in rabbits (with improved feed conversion). PAPAS and HATFIELD (1978), by giving volatile fatty acids into the abomasum, brought about slight acidosis in sheep resulted in reduced appetite. Later research (PAPAS and HATFIELD, 1978) demonstrated that sodium salts of volatile fatty acids did not have the same impact therefore there was no decrease in feed intake. Since feed intake in rabbits is regulated according to energy level of the feed it is assumed that the primary reason behind reduced feed intake is not the lack of appetite but the improved efficiency of their energy utilization. KATOH and TSUDA (1987) showed that the impact of sodium butyrate injection resulted in increased amylase production. This finding confirms the above hypothesis, i.e. the improved efficiency of feed energy utilization verified by the significantly improved digestibility coefficient. Results from other trials (ROEDIGER *et al.*, 1988; BINDER and MEHTA, 1989; SCHAMER and LUTZ, 1990) demonstrated that butyrate enhanced the energy consuming process of sodium absorption. GULLANS *et al.* (1984) showed that the just as energy consuming gluconeogenesis and sodium transport are somehow competing for cell energy. Butyrate supplementation resulted in an increased rate of glucose synthesis without changing ATP-level. Besides the improved digestibility coefficients, this could be another possible reason for the more efficient utilization of feed energy and the decreased feed intake, thus for improved feed conversion.
It has been found (Settle, 1990) that the sodium butyrate slows down the progress of mucosal atrophy in the gut and also neutralizes the effects of certain enterotoxins, as well as significantly decreases the increased fluid and

sodium loss caused by them (RAMAKRISNA *et al.*, 1990). In contrast to acetates and propionates it takes a relatively longer time for butyrates to be absorbed meanwhile they help maintain and restore gut flora (Stephen, 1991). Sodium butyrate as a "differentiator" may contribute towards arresting abnormal cell proliferation (GAMET *et al.*, 1993). Our microbiologic investigation of small intestine revealed that the absolute number of bacteria was similar, though with a lower number of bacteria in caecum, to the values found in research literature (GIESECKE and HENDERICK, 1965). This could probably be accounted for by the fact that samples were taken early in the morning following the egestion of soft faeces. Irrespective of this, it can be concluded that the effect of 0.15% sodium butyrate supplementation resulted in dramatically decreased ratio of harmful gut coliforms. Considering all the circumstances, eubiosis was most desirable for the gut flora of group fed 0.15% sodium butyrate supplemented diet.

Microbe balance is a result of several processes which can only be influenced to some extent. Attempts to optimize farm animal production by altering gut flora are largely dependent upon empirical observations. Bearing all these in mind, actual results provided evidence that by maintaining the biologic gut flora balance, sodium butyrate, a non-foreign substance, can be considered to be effective in substituting some foreign feed additives in feeding rabbits.

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