



Sodium butyrate improves growth performance of weaned piglets during the first period after weaning

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Paper received October 26, 2001; accepted January 28, 2002.

ABSTRACT

The purpose of the present work was to evaluate whether the addition of sodium butyrate to feed could facilitate weaning and growth response in piglets. For 56 days two groups of 20 piglets (9.2 ± 1.4 kg LW) were fed an acidified basal diet (containing formic and lactic acid at 0.5 and 1.5 g/kg of feed, respectively) without (control group) or with sodium butyrate (SB) at 0.8 g/kg. Average daily gain (ADG), daily feed intake (DFI), feed efficiency (FE) and live weight (LW) were recorded. In the first two weeks, butyrate supplementation increased ADG (+20%; $P < 0.05$) and DFI (+16%; $P < 0.05$). During the subsequent period (15 to 35 days) animals fed SB had a higher DFI but lower feed efficiency (-10% and -14%, respectively; $P < 0.05$) than animals fed the control diet. No other benefits were observed thereafter. The data presented showed that the use of sodium butyrate facilitated only the initial phase of adaptation to a solid diet in piglets.

Key words: Butyric acid, Intestinal mucosa, Weaning, Nutrition, Swine

RIASSUNTO

IL SODIO BUTIRRATO FAVORISCE L'INCREMENTO PONDERALE DI SUINETTI NELLA PRIMA FASE DI POST-SVEZZAMENTO

Lo studio ha valutato eventuali effetti positivi del sodio butirrato durante le fasi di svezzamento e post-svezzamento in suinetti. Sono stati utilizzati due gruppi di 20 animali (9.2 ± 1.4 kg LW) alimentati per 56 giorni con una dieta standard acidificata (acido formico ed acido lattico, 0.5 e 1.5 g/kg di mangime rispettivamente) senza (controllo) o addizionata con sodio butirrato (SB) in ragione di 0.8 g/kg. I parametri valutati durante i 56 giorni di prova sono stati: incremento medio giornaliero (ADG), ingestione giornaliera (DFI), efficienza alimentare (FE) e peso vivo (LW). Nei primi 14 giorni, il gruppo trattato con SB ha riportato ADG e DFI maggiori del controllo (rispettivamente +20% e +16%; $P < 0.05$). Nel seguente periodo (dal 15^a al 35^a giorno) il gruppo SB ha fatto registrare una maggiore ingestione (+10%, $P < 0.05$) mentre l'efficienza alimentare è risultata minore (-14%, $P < 0.05$) rispetto al gruppo di controllo. Successivamente non sono emerse ulteriori differenze statisticamente significative. Il sodio butirrato alla dose testata sembra quindi essere di aiuto solamente nei primi giorni post-svezzamento quando tutto l'intestino subisce modificazioni sia nella struttura morfologica sia nella sua funzionalità, aiutando il suinetto nel momento del passaggio da una dieta liquida ad una solida.

Parole chiave: Acido butirrico, Mucosa intestinale, Svezzamento, Nutrizione, Suino

Introduction

The ever increasing economic and competitive demands on pork production during the last thirty years has forced the weaning age of piglets to 3 - 4 weeks and younger. Despite exciting progress in nutritional formulation and ingredient selection for nursery pig rations, the period of five to eight weeks after weaning continues to be a critical production stage in most pork production systems. Weaning time, regardless of the age of the piglet, presents the greatest dietary challenge a pig will encounter in its life (Odgaard, 2001). Along with social and environmental changes, these stressors lead to lower feed intake, resulting in a decrease in average daily gain, and contribute greatly to the onset of gastrointestinal diseases (Barnett *et al.*, 1989). Even the regrouping phase from pens to cages poses an additional threat to animal well-being. In the recent past, these problems were counteracted with widespread use of antibiotic substances and auxinic agents that may select antibiotic-resistant genes in intestinal pathogens with the possibility of a cross spread to human pathogen (Witte, 1998). These concerns culminated in the ban of the use of most antibiotics used as animal growth promoters and will probably lead to a complete ban of these molecules from animal feeding. As a consequence, there is an increasing need to find generally recognized as safe (GRAS) alternatives to modulate cecal microflora and control intestinal fermentation. Among these substances are some organic acids which are known to be very effective inhibitors of microbial growth and are therefore intentionally added to many foods as preservatives (Knochel and Gould, 1995; Podolak *et al.*, 1996). Adding organic acids such as citric, formic, fumaric, lactic or propionic acid to piglet diets has been reported to be helpful in overcoming problems of the post-weaning period (Falkowski and Aherne, 1984; Partanen and Mroz, 1999). Moreover, among the various metabolic functions, short chain fatty acids (SCFA) play a key role as an energy source, butyric acid being the most readily oxidized to CO₂ among all the other SCFA in the intestine (Fleming and Gill, 1997). Butyric acid was also shown to induce cell differentiation and to regulate the growth and pro-

liferation of normal colonic and ileal mucosa (Treem *et al.*, 1994), whereas it can actively reduce the growth rate of cells in colorectal cancer (Berry and Paraskeva, 1988). A deeper understanding of the role of butyric acid in the intestinal metabolism of food animals is needed in order to guarantee safe and efficient meat production. In this *in vivo* study we evaluated the effects of sodium butyrate on the growth performance of piglets receiving a diet in which other organic acids have been added.

Material and methods

Animals and Housing

The trial was carried out at the Centro Ricerche per la Zootecnia e l'Ambiente, (CERZOO, Piacenza - Italy). The study was conducted using 40 Gorzagri hybrid piglets (castrated males and females weighing 9.2±1.4 kg LW) divided into two homogenous groups and fed a conventional non-medicated diet without (CTR) or with sodium butyrate (SB) at 0.8 g/kg. This amount was supplied as VF APPETITE 50 (OrSell s.r.l., Carpi, Modena - Italy). Formic and lactic acid at 0.5 and 1.5 g/kg of feed, respectively, were added to both diets. The animals were weaned at 28 days of age and began to receive the experimental diets two weeks thereafter. They were reared in flat-deck cages in rooms with a controlled climate (T=26°C; RH=65%) and natural daylight during the first 35 days of the study. The animals were then transferred to a grower room with a concrete slatted floor for the remaining 21 days. The barn was carefully cleaned and disinfected with solutions considered atoxic for animals before bringing the piglets in. The "all in - all out" procedure carried out at least 7 days before the transfer of the animals from the piggery to the production barn, ensured the perfect sanitary conditions of the barn. No adaptation period was observed. Immediately after their arrival in CERZOO, the piglets' health was examined by the Veterinarian responsible for animal welfare; they were weighed, ear tagged and fed the experimental diets.

The CTR and the SB diets were prepared by CERZOO personnel using a horizontal mixer. The

Table 1. Composition (g/kg feed) of the diets.

	Control diet	Sodium Butyrate diet
Corn meal	323.2	319.2
Barley meal	70.0	70.0
Fish meal	30.0	30.0
Non dehulled barley flakes	250.0	250.0
Whey powder	50.0	50.0
Soy-bean meal 44%	210.0	210.0
Soya oil	20.0	20.0
L - Lysine HCl	5.0	5.0
DL - Methionine	1.0	1.0
Calcium carbonate	3.0	3.0
Dicalcium phosphate	27.0	27.0
Sodium chloride	1.8	1.8
Vit. trace element premix ⁽¹⁾	5.0	5.0
Preservative ⁽²⁾	4.0	4.0
VF APPETITE 50 ⁽³⁾	0.0	4.0

⁽¹⁾ Composition/kg feed: vit. A: 15000 IU; vit D3: 1700 IU; vit E: 35 mg; Fe: 200 mg; Cu: 170 mg; Zn: 200 mg.

⁽²⁾ Composition/kg feed: formic acid (E270) 0.5 g; lactic acid (E236) 1.5 g; colloidal silica (E551b) and vegetable carrier 2 g.

⁽³⁾ Composition/kg feed: sodium butyrate 0.8g, vegetable carrier 3.2 g.

SB premix was weighed with a technical balance, and mixed with commercial meal feedstuffs produced by Mangimi Ferrari (Sarmato, Piacenza - Italy). The final composition and the analytical characteristics of the diets are shown in tables 1 and 2, respectively. The animals had free access to drinking water and were fed *ad libitum* for the first 35 days of trial. The animals were then fed for the remaining 21 days at 9% of their metabolic weight (live weight^{0.75}) with a weekly adjustment of the intake based on the hypothetical gain.

Recorded parameters

Feed and water were not withheld before the animals were individually weighed at the beginning of the study and after 14, 35 and 56 days. The health status of the animals and clinical signs

Table 2. Analytical characteristics (g/kg feed) of the diets.

Analytical parameter	Control diet	Sodium Butyrate diet
Dry matter	884.8	884.0
Crude protein	181.6	181.5
Ether extract	51.6	55.0
Crude fiber	49.9	46.6
Ash	63.1	64.8
Starch	423.0	422.5
Lysine	15.5	15.5
Methionine	7.8	7.8
Threonine	7.8	7.8
Tryptophan	2.2	2.2
Digestible Energy ⁽¹⁾ MJ/kg	13.5	13.5
Net Energy ⁽²⁾ MJ/kg	10.7	10.7
Inhibitory substances in feed	Negative	

⁽¹⁾ According to Whittemore (1980).

⁽²⁾ According to Noblet et al. (1994).

were recorded by the Veterinarian in charge at CERZOO. Feed consumption was recorded per pen to allow the calculation of feed efficiency.

The data recorded during the feeding phase were as follows: live weight at 0, 14, 35 and 56 days from the beginning of the study for each animal; average daily gains during the following periods: 0-14; 15-35; 0-35; 36-56 and 0-56 days for each animal; average feed intake and gain/feed ratio during three periods (0-14, 15-35, 0-35, 36-56 and 0-56 days) for each pen.

Chemical analysis of feedstuffs

The analyses were carried out in compliance with the analytical methods of the Italian Ministero dell'Agricoltura e Foreste (supplement n. 2 of 1975) for moisture, ash, starch and carbohydrates in animal feed. The crude protein content was determined according to the Gazzetta Ufficiale Serie Generale n. 92 of April 21, 1996; the crude fat according to the Directive EEC n. 84/4/EEC of December 12, 1983 (Gazzetta Ufficiale EC n. L 15 of January 1, 1984); and crude fiber according to the EEC Directive n. 92/89 of November 3, 1992.

Table 3. Piglets' growth performance (means \pm S.D.)⁽¹⁾.

		n.	Control diet	Sodium Butyrate diet
0-14 days				
ADG	g/d	20	216 \pm 69 a	259 \pm 75 b
Intake	"	4	295 \pm 14 a	351 \pm 10 b
Feed efficiency	g gain/kg feed	4	739 \pm 95	732 \pm 42
Live weight 14 d	kg	20	12.2 \pm 1.82 a	12.8 \pm 2.27 b
15-35 days				
ADG	g/d	20	421 \pm 68	409 \pm 101
Intake	"	4	637 \pm 30 a	704 \pm 32 b
Feed efficiency	g gain/kg feed	4	667 \pm 24 b	577 \pm 36 a
Live weight 35 d	Kg	20	21.1 \pm 2.97	21.4 \pm 4.08
36-56 days				
ADG	g/d	18	433 \pm 71	483 \pm 184
Intake	"	4	886 \pm 111	782 \pm 92
Feed efficiency	g gain/kg feed	4	545 \pm 33	590 \pm 119
0-56 days				
ADG	g/d	18	373 \pm 55	409 \pm 108
Intake	"	4	645 \pm 51	645 \pm 36
Feed efficiency	g gain/kg feed	4	549 \pm 13 b	524 \pm 16 a
Live weight 56 d	kg	18	30.5 \pm 3.8	31.9 \pm 7.0

¹ Means within a row with different letters differ significantly ($P < 0.05$).

Microbiological analysis of feedstuffs

Before beginning the study, a sample of basal feed was analyzed to evaluate the content of drugs or medicated premix for inhibitory substances. The sample was prepared as follows: 1) sample suspension in water (rate 1:10) and evaluation of pH and adjustment to pH 7; 2) a blotting paper disc saturated with sample water suspension was placed on Petri dishes containing plate count agar medium at pH=8 (Oxoid, Basingstoke, Hampshire, UK) inoculated with *Bacillus stearothermophilus* (DSN 1550). After incubation at 55°C for 3 h, the microbial growth was evaluated. If there was an inhibitory halo of microbial growth near the disc containing the sample, some inhibitory substance was present in the sample. Following the same procedure, the same medium at pH=7 was incubated with *Bacillus subtilis* (DSN 618) at 30°C for 24 h.

Statistical analysis

The data collected during the study were statistically processed. Live-weight, average daily gain, feed intake and feed efficiency were subjected to analysis of variance according to General Linear Model Procedure of the S.A.S. (SAS Institute, USA) statistical method (1996; ver. 6.12). The means were compared using Student's "t" test. The live weight recorded during the study and the average daily gain were covaried with the live weight of the piglets at the beginning of the study. The statistical model employed was the following:

$$Y_{ij} = \mu + \tau_i + \beta_j (x_{ij} - \bar{x}) + \varepsilon_{ij}$$

where μ was the general average, τ was the effect of the animal, β was the correction factor for the covariate, \bar{x} was the average of the observations and ε was the effect of the error.

Animal exclusion criteria

All piglets were considered suitable for the study by the Veterinary responsible for the animals' health status according to the exclusion criteria decided in the study protocol. More precisely, there was no occurrence of any clinical conditions which could have compromised the health conditions of the animals or required the need of pharmaceutical treatment for pathologies after the beginning of the study.

Declaration of compliance with legislation

Animals employed in this study were reared and treated in compliance with the Directive 86/609/EEC relative to the protection of animals used for experimental or other scientific purposes, according to the Italian Legislation with Legislative Decree (D.Lgs) January 27, 1992 n. 116. The research center where the study was conducted was authorized by the Ministry of Health (with Ministerial Decree n° 253/95-A of 18th August 1995) to employ animals for experimental purposes or other scientific purposes according to item 12 of the above mentioned Legislative Decree.

The trial was conducted in compliance with the Good Laboratory Practices guidelines (Ministry of Health certification number 08/09/97 of December 24, 1997) reconfirmed by Ministerial inspection on June 8, 2000.

Results

All pigs were in good health before and throughout the experimental period. Firm feces were observed throughout the study with no occurrence of diarrhea. Statistical differences between the feeding treatments were already obtained after 14 days of the trial. At 14 days the piglets fed SB had a higher ADG than the CTR animals (259 vs 216 g/d; +20%; $P<0.05$) and a higher live weight (12.8 kg vs 12.2 kg; +4.9%, $P<0.05$). This difference was no longer significant after 35 days. There was only a tendency toward a difference in the period 0-35 days ($P<0.11$) between piglets fed with or without SB (365 g/d vs 316 g/d; +15.5%). This dif-

ference was no longer appreciable at the end of the study. The feed intake (table 3) of the animals fed SB resulted higher than that of the control animals in the period 0-14 and 15-35 days, with a difference of 19% ($P<0.01$) in the first period and 10.5% ($P<0.05$) in the second period. This difference disappeared when the animals were reared in the grower room (from 36 to 56 days of trial). At the end of this period, piglets fed the diet containing SB showed only a tendency toward a higher final live weight (+1.4 kg) compared to animals fed the CTR diet ($P=0.17$).

Discussion and conclusions

For several decades, antibiotic growth promoters have been used in diets for young and growing pigs in order to reduce the incidence of post-weaning diarrhea and enhance growth performance (Partanen, 2001). Due to the spreading of antibiotic resistance in a number of pathogenic bacteria, precautionary actions have been taken in the European Union to exclude several antibiotics from pig diets (EC, 1998). This raised a concern for producers as any increase in the incidence of diarrhea would threaten the health status of the animal as well as production efficiency and might eventually result in an increase in production costs. The susceptibility of weaned piglets to diarrhea is related to several factors (Hampson, 1994); one of these is the immaturity of digestive tract. At weaning the small intestine of the piglet generally undergoes a reduction in villous height and an increase in crypt depth, that are associated with a decreased capacity of absorption. Such scenario may lead to mal-absorption, increased intestinal fermentation, post-weaning diarrhea and reduction of feed intake that would in turn further decrease the nutrient supply (Pluske *et al.*, 1995). Changes in gut morphology are important as they can affect growth rate. Short chain fatty acids (SCFA) produced by microbial fermentation from dietary fiber stimulate epithelial cell proliferation resulting in a larger absorptive surface (Sakata, 1988). Moreover, the fact that normal colonic epithelia derive 60 to 70 % of their energy supply from SCFA, particularly from butyric acid (Schepbach *et al.*, 1992) must be considered. The

latter induces cell differentiation and regulates the growth and the proliferation of normal colonic mucosa (Treem *et al.*, 1994) while suppressing the growth of cancer cells (Clausen *et al.*, 1991).

The beneficial effects of butyric acid were appreciable in the first period of the study (0 to 14 days) with higher ADG (+20%; $P < 0.05$) and higher daily feed intake (+16%; $P < 0.05$). A higher feed intake was also recorded during the second phase (15 - 35 days), although it was not associated with a higher ADG. This loss of feed efficiency is likely to be connected to an effective response of the intestinal architecture to SB only during the first phase (0 - 14 days). Conversely, in the subsequent period SB might have stimulated feed intake without an equally effective utilization of nutrients. The improved growth performance could be associated with the beneficial effect of butyric acid on the proliferation of the intestinal epithelium. This is of greater biological value to the weaning period when the weight of the small and large intestine increases 3 times faster than that of the whole body mass growth (Sakata and Setoyama, 1997). In the present study, the supplied amount of 5 ($\mu\text{mol/g}$ DM feed of butyric acid could have been of biological significance for the small intestine where the baseline value for butyric acid is about 4 $\mu\text{mol/g}$ DM. Conversely, cecal concentrations of butyric acid are about 240 $\mu\text{mol/g}$ DM (Piva *et al.*, 2002). As such, even in the unlikely event of the entire amount of SB reaching the hindgut, the addition of SB at the tested dose would have had no incidence in colonocyte metabolism. This would in turn substantiate the efficacy of SB limited to the post-weaning period, when the villi structure is more negatively affected by the transition to solid feed and when it may benefit from the growth modulation effect of SB (Hodin *et al.*, 1997). Other studies have shown positive effects of butyric acid on the ileal villi and cecal crypts structure (Galfi and Bokori, 1990; Piva *et al.*, 2002). To confirm this hypothesis morphometric measurements of the small intestine are needed. Furthermore, additional research is needed to significantly increase butyric acid concentration in the lower gut to enhance the energy supply to colonocytes.

We gratefully acknowledge the support from OrSell s.r.l., Modena - Italy

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