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Effect of different butyrate supplementations on growth and health of weaning pigs challenged or not with E. coli K88

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ABSTRACT - In a full factorial design (4 diets X challenge, Yes/No), 72 weaning pigs were assigned to one of the diets: Control; experimental diets, obtained with the addition of 2 g/kg free sodium butyrate (fNaB), or 0.6 g/kg fat-protected sodium butyrate (pNaB), or 2 g/kg INVE-NutriAd commercial mixture (Mix, based on 75 g/kg protected butyrate). Oral challenge with *Escherichia coli* K88 was done on 2/3 of pigs on d 7. Pigs were slaughtered on d 13. The mortality in challenged pigs, tended to be higher in control group (50.0%) than in the three supplemented groups (23.5%). Growth tended to be increased averagely by the supplements (P=0.100) after the challenge, that also significantly reduced growth. In general the diet did not affect the fecal shedding of *Escherichia coli* and Lactobacilli, the K88-specific IgA activity in blood, the morphology of oxyntic mucosa and the expression of H^+/K^+-ATPase gene. The supplementations tended to increase villous length of jejunum (P=0.101). On the whole, growth, villous height and surviving rate can be positively affected either when the supplementation is done by free butyrate, by protected butyrate or by the special Inve Nutri-Ad product and these effects are distributed both on pigs infected or not with *Escherichia coli* K88.

Key words: Weaning pig, Escherichia coli K88, Feeding, Butyrate.

Introduction – After early studies of Gälfi and Bakori (1990), research attention to dietary addition of sodium butyrate (SB) in piglet feed increased with the EU ban of growth-promoting antibiotics. However data from trials in piglets are conflicting, may be in relationship with the age and environmental conditions of pigs, the dose of supplementation etc. (Lallès *et al.*, 2009). The site of action could also be relevant considering that SB was detected only in the stomach (Gálfi and Bakori, 1990; Manzanilla *et al.*, 2006). This suggests that is quickly absorbed and/or metabolised in stomach or duodenum. A more local delayed action could be predicted with the use of protected SB. Our goal was to study the effect of different butyrate supplementations on growth and health of weaning pigs challenged or not with *Escherichia coli* K88ac (ETEC).

Material and methods – 72 pigs weaned at 21-28 d were obtained from litters where at least one pig was sacrificed and was positive for the susceptibility to ETEC intestinal adhesion, by in vitro test on the villi (Bosi *et al.*, 2004). This permitted to obtain most of the pigs prone to the ETEC intestinal adhesion. Four diets were formulated: basal diet (Control) without antimicrobials, zinc oxide, or any kind of growth promotant; experimental diets, obtained with the addition of 2 g/kg free sodium butyrate (fNaB) or 0.6 g/kg fat-protected sodium butyrate (pNaB) or 2 g/kg INVE-NutriAd commercial mixture (Mix, based on 75 g/kg butyrate in fat-protected form with yeasts -highly available beta-glucan, mannan-oligosaccharides and nucleotides-). The supplementation with pNaB was lower than with fNaB, because it was assumed that pNaB could be more effective in the intestinal tract. In a full factorial design (4 diets X challenge – Yes/No), pigs were divided into

4 control groups of 6 animals, and 4 challenged groups of 12 animals, balanced for litter and weight.

All the pigs were supplemented from d 0 to d 3 with colistin (250 mg/kg). On d 7, challenge groups received an ETEC oral dose of 5 mL of a 3^9 CFU/mL solution. The other groups received a placebo solution. Severity of diarrhea was characterized by using the fecal consistency score system (1 to 5: 1=hard, 5=watery faeces). Samples of feces were collected from all pigs for the determination of total Lactobacilli, *E. coli* and *E. coli* K88 plate counts on d 0, d 9, d10, d11. Blood samples from all pigs were collected before challenge, on d 11 and at sacrifice. Close samplings after challenge are justified by the usual rapid raise of K88-specific immune globulins, that were assessed as reported by Bosi *et al* (2004). All pigs were sacrificed on d 12 or 14, were deeply anaesthetized with sodium thiopenthal (10 mg/kg BW) and sacrificed by an intracardiac injection of Tanax® (0.5 mL/kg BW). The procedures were carried out according to the Italian law pertaining to experimental animals and approved by the Ethic-Scientific Committee for Experiments on Animals of the University of Bologna. The number bands after 16S ribosomal DGGE analysis of the DNA extracted from gastric and intestinal contents was assessed as reported by Konstantinov *et al.* (2008). Gastric parameters were assessed as reported by Bosi *et al.* (2006).

The individual data were analyzed by analysis of variance (GLM of SAS). A P value ≤0.10 was considered to represent a trend. Orthogonal pre-planned contrasts were tested for the effect of the diet.

Results and Conclusions – Frequencies of subjects dead for colibacillosis, after the day of challenge, were: 1/6; 0/5[a]; 0/6; 0/6 and 6/12; 3/12; 3[b]/12; 2/10[c], respectively for un-challenged and challenged pigs of groups Control; fNaB; pNaB; Mix ([a]One piglet died before challenge after a bone fracture; [b]including two pigs that were suppressed due to the extreme cachexia; [c]two pigs died before the challenge). In challenged pigs, the frequency of dead animals of control group (50.0 %) tended to be higher than that in the pooled three supplemented groups (23.5%) (χ^2 test, P=0.087).

Dietary supplementations did not affect growth and feed intake before challenge, while after that, average daily gain tended to be increased by the supplements (P=0.100). The challenge reduced growth and tended to reduce feed intake, but did not interact with the diet (Table 1). The fecal score was impaired by the challenge, but not affected by the diet. The challenge caused a significant increase of total *E. coli* counts for all the post-challenge samplings, while this criteria was not affect by the dietary additions. However for d 9 the interaction of the diet with the challenge tended to be significant (P=0.060) and in pigs challenged with ETEC the supplemental additives averagely tended to increase the total *E. coli* counts (+20.7 %, P=0.053). Before the challenge K88-specific IgA activity in blood serum was in general very low. After the challenge, values were of course strongly affected by the ETEC challenge, but it is worthwhile to note that this effect was seen as so early as on d 4 post-challenge (P<0.05). The dietary supplementations did not change significantly the K88-specific IgA activity in blood serum.

At sacrifice, the three supplementations averagely tended to increase the villous length and crypt width of jejunum (P=0.101 and P=0.077), while the Mix product tended to increase the villous width (P=0.073). In general the challenge did not affect small intestinal morphology. Villus width increased in the distal tract (P<0.05) and crypt width in the proximal (P<0.01) (Table 2). In the stomach, the diet and the challenge did not affect the gland depth, the counts of parietal cells. The expression of the H^+/K^+-ATPase gene, involved in the acid secretion, was not changed by the diet.

The diet did not affect the number of bands in the DGGE profiles of DNA extracted from gastric and jejunum samples. The oral challenge with ETEC increased the variability in the stomach content (P<0.05), as indicated by the increased numbers of bands.

The results on growth, villous height and surviving rate after ETEC challenge indicate that there is a positive effect of supplementations. These effects are distributed both on challenged and not challenged pigs, as indicated by the absence of interaction, and do not depend on the specific formulation of butyrate (free, protected or in the special Inve Nutri-Ad product). Data do not support that positive effects on piglet performance after free or protected butyrate depend on a specific effect on numbers of total *E. coli* or ETEC in the gut.

Table 1. Effect of the diet and the challenge on average daily gain (ADG) and feed intake*.

Items	Diet				SEM	Orthogonal contrasts, P			Challenge		SEM
	Control	fNaB	pNaB	Mix		Control Vs. Others	Mix Vs. NaB	fNaB Vs. pNaB	N	Y	
- ADG, g											
d 0 to d7	7.0	37.0	8.7	14.3	19.5	0.585	0.713	0.297	17.5	16.0	13.8
d 7 to end	131.3	197.2	246.1	251.4	49.0	0.100	0.712	0.472	256.6	156.3	34.7
- Feed intake, g											
d 0 to d7	177.8	173.3	169.9	179.7	15.2	0.850	0.656	0.872	175.5	174.8	10.8[1]
d 7 to end	336.1	345.1	381.3	377.4	43.1	0.548	0.783	0.545	397.4	322.6	30.5[2]

**First level interactions among the three factors were always p>0.10. [1]p<0.05; [2]p=0.096.*

Table 2. Effect of the diet, the challenge and the tract of sampling (Prox and Dist: respectively at 25 % and 75% of the small intestinal length) on morphological measures*.

Items		Diet						Challenge				Tract			
		Control	fNaB	pNaB	Mix	SEM	p	No	Yes	SEM	p	Prox	Dist	SEM	p
Villous															
Length	µm	295	322	332	351	19.8[±]	0.270	331	319	14.5	0.559	328	322	12.6	0.654
Width	µm	93	94	97	104	4.4[#]	0.218	97	97	3.3	0.975	94	100	2.7	0.027
Crypt															
Depth	µm	43	45	44	45	1.7	0.928	43	45	1.3	0.350	44	45	1.1	0.472
Width	µm	208	238	225	224	9.6[¶]	0.229	216	232	7.0	0.112	235	212	6.1	<0.01

**First level interactions among the three factors were always P>0.10. [±]Control Vs. Others, P=0.101. [#]Mix Vs. NaB, p=0.073. [¶]Control Vs. Others, p=0.077.*

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