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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 Gälf 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 (Gälfi 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° 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 faeces were collected from all pigs for the determination of total Lactobacilli, E. coli and E. coli K88

plate counts on d 0, d 9, d 10, d 11. Blood samples from all pigs were collected before challenge, on d 11 and at sacrifice. Close samplings after challenge were 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; 0/6; 0/6 and 6/12; 3/12; 3/12; 2/10K88 respectively for un-challenged and challenged pigs of groups Control; fNaB; pNaB; Mix (aOne piglet died before challenge after a bone fracture; bincluding two pigs that were suppressed due to the extreme cachexia; ctwo 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%) (χ2 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 (+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           | Orthogonal contrasts, P | Challenge |
|----------------|----------------|-------------------------|-----------|
|                | Control       | fNaB                    | pNaB      | Mix | SEM | Control | Mix | fNaB | Others | pNaB | N | Y | SEM     |
| - 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| 177.8         | 173.3                   | 169.9     | 179.7| 15.2| 0.850   | 0.656| 0.872| 175.5   | 174.8| 10.8 |
| d 0 to d7      | 336.1         | 345.1                   | 381.3     | 377.4| 43.1| 0.548   | 0.783| 0.545| 397.4   | 322.6| 30.5 |
| d 7 to end     |               |                         |           |     |     |         |     |      |         |     |    |   |         |

*First level interactions among the three factors were always p>0.10. 1p<0.05; 2p=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 | Orthogonal contrasts, P | Challenge |
|----------------|------|-------------------------|-----------|
|                | Control | fNaB | pNaB | Mix | SEM | p | No | Yes | SEM | 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. 1p<0.05; 2p=0.09.

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