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Effect of dietary addition of free or fat-protected calcium formate on growth, intestinal morphology and health of *Escherichia coli* k88 challenged weaning pigs

P. Bosi[1], G. Sarli[2], L. Casini[1], S. De Filippi[1], P. Trevisi[1], M. Mazzoni[1], G. Merialdi[3]

[1] Dipartimento Protezione e Valorizzazione Agroalimentare, Università di Bologna, Reggio Emilia, Italy

[2] Dipartimento Sanità Pubblica Veterinaria e Patologia Animale, Università di Bologna, Italy

[3] Istituto Zooprofilattico Sperimentale "Bruno Umbertini", Sezione di Reggio Emilia, Italy

Corresponding author: Paolo Bosi. Dipartimento Protezione e Valorizzazione Agroalimentare. Via F.lli Rosselli 107, 42100 Reggio Emilia, Italy – Tel: + 39 0522 290522 – Fax: +39 0522 290523 – Email: paolo.bosi@unibo.it

RIASSUNTO – Effetto dell'integrazione della dieta con formiato libero o protetto sulla crescita, la morfologia intestinale e la salute di suini in svezzamento stimolati per os con *Escherichia* coli K88. *Sessanta suini, svezzati a 21 giorni (d 0), sono stati divisi in 3 gruppi, bilanciati per numero, peso e nidiata ed alimentati con: dieta standard (C), C + 1,2% calcio formiato, C + 1,2% calcio formiato protetto con grasso. Al giorno 2, i suini sono stati stimolati oralmente con 1,5 ml di una sospensione con 10^{10} CFU Escherichia coli K88 O148 e sacrificati ai giorni 7 e 8. L'impiego di formiato libero ha migliorato la crescita e l'ingestione, il formiato protetto ho prodotto solo una tendenza al miglioramento. Le due fonti di formiato hanno ridotto l'incidenza di diarrea, l'escrezione fecale di E. coli, le IgA anti E. coli K88 nella saliva e aumentato l'altezza dei villi del piccolo intestino. L'acido formico ha una funzione di promotore di crescita anche in suini stressati con E. coli enterotossigenico. La sua azione è stata però legata principalmente all'effetto sulla persistenza di E. coli totali. Non è emersa una particolare convenienza dall'uso di formiato protetto.*

Key words: weaning pig, Escherichia coli K88, feeding, formate.

INTRODUCTION – The practice of adding organic acid to diets for weaning pigs is quite established. An improvement of growth after weaning has been averagely demonstrated with a meta-analysis of feeding trials (Partanen and Mroz, 1999). However data showing that organic acids can positively counteract the presence of post-weaning diarrhoea and of enterotoxigenic *E. coli* k88 (ETEC) (Tsiloyiannis *et al.*, 2001) contrast with the absence of positive results in ETEC challenged pigs (Risley *et al.*, 1993). It cannot be excluded that differences in the response to acidifiers can depend on individual susceptibility to ETEC. We aimed to test the efficacy of calcium formate using a challenge model already established and considering the individual sensitivity of intestinal villous to ETEC adhesion (Bosi *et al.*, 2004). We also wanted to test if fat-protection of calcium formate could improve resistance to ETEC, by concentrating the action of the acidifier in the intestine.

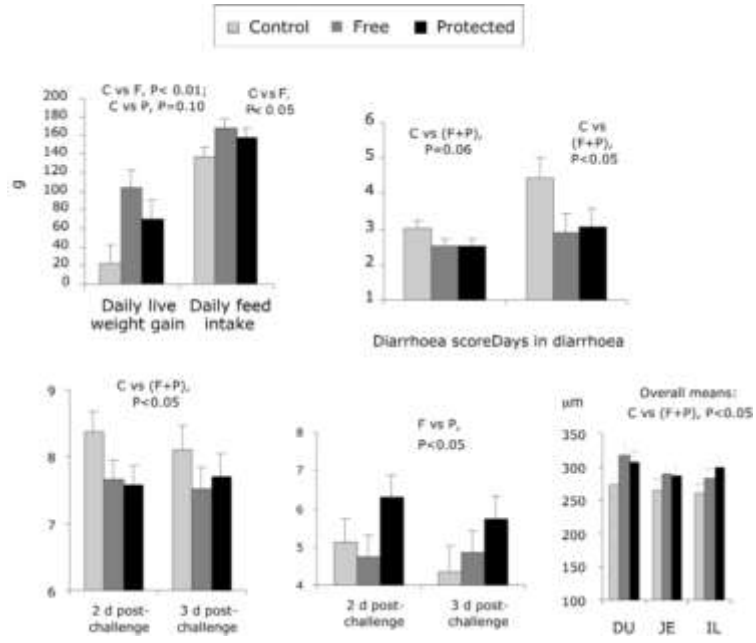
MATERIALS AND METHODS – Sixty pigs, weaned at 21 days (day 0), were divided into three groups, balanced for number, litter and live weight and fed: standard balanced diet (C), C + 1.2% of free calcium formate (F), C + 1.2% of fat-protected calcium formate (P). In F and P diets, monosodium phosphate and calcium

formate were partial substituted for dicalcium phosphate and calcium sulphate of diet C. Pigs were individually housed, orally challenged with 1.5 ml of a 10^{10} CFU *Escherichia coli* K88 O148 (F4) suspension on day 2, and sacrificed on day 7 or 8.

Diarrhoea score and days in diarrhoea; faecal excretion of total *E. coli* and K88 *E. coli*; villus height in duodenum, jejunum and ileum, sensitivity of intestinal villous to ETEC adhesion, total IgA and IgA anti *E. coli* K88 in blood, saliva and jejunum secretion were measured as reported by Bosi *et al.* (2004).

On samples of the gastric wall (at greater curvature) and of the jejune wall, a RT-PCR was done for quantification of expression of TNFα gene, by an absolute quantitative realtime PCR assay performed with a LightCycler instrument (Roche Mannheim, Germany). All the data were analysed by analysis of variance using the GLM procedure of SAS with a 3-factor design, including diet, cycle, sensitivity of intestinal villous to ETEC adhesion, and 1st level interaction.

Figure 1. Effect of free or fat-protected formate on daily live weight gain, feed intake; diarrhoea score and day in diarroea; faecal excretion of total *E. coli* and K88 *E. coli*; villus height in duodenum (du), jejunum (je) and ileum (il) (LSM+SEM).



RESULTS AND CONCLUSIONS – The factors "sensitivity of intestinal villous to ETEC adhesion" and cycle of the experiment did not interact with the diet and will not be discussed here. Four subjects (2 of C diet, 1 of each experimental diet) died after the challenge, for colibacillosis. The supplementation with free calcium formate improved growth (P<0.01) and feed intake (P<0.05) (Figure 1), while only a trend of increase of growth was observed for fat-protected formate (P<0.10). Both forms of formate addition reduced diarrhoea score, days in diarrhoea, total *E. coli* faecal excretion (P<0.05), and increased the average of villous heights in duodenum, jejunum and ileum (P<0.05). IgA anti *E. coli* K88 were reduced by formate treatments (P<0.05), in saliva but not in blood and in jejunum secretion (data not in figure). In saliva, the total IgA activity tended to be reduced by acidifiers (P=0.07). The expression of TNFα gene in stomach and jejunum wall was not affected by the diet (data not shown). The pH of stomach was not affected, while free formate reduced average pH in ileum, colon and cecum, compared to the other dietary treatments.

It is confirmed that formic acid (actually calcium formate) has a growth promoting action for the young pig, in agreement with Partanen and Mroz (1999). This action is explained by a reduction of total *E. coli* persistence. In contrast to our data, the absence of improvement in the experiments by Risley *et al.* (1993) can be explained by the different organic acids or by the dose of supplementation. Our data concerning the use of fat-protected formate are not conclusive. Some positive effect was seen on health, but not on growth. The degree of protection was not tested, but data could lead to suspect that the protection was not homogeneous; the reduction of IgA in saliva observed also with the addition of fat-protected formate could be explained by the presence of some free formate in the product too. The use of this fat-protection is justified only to overcome the negative effect of free formate on gastric morphology (Mazzoni *et al.*, 2004).

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