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SHORT COMMUNICATION

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Use of a new form of protected sodium butyrate to control *Salmonella* infection in fattening pigs

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Abstract

A field trial on a commercial pig farm was carried out to assess the efficacy of the addition in the diet of fattening pigs of a new form of sodium butyrate protected with a sodium salt of coconut fatty acid distillate (3 kg/ton of feed) to control *Salmonella* spp. infection. Around 50 pigs were assigned to treatment group and 50 kept as controls. During the fattening period pigs were monthly sampled (serum and feces), and after slaughter fecal and mesenteric lymph nodes samples were collected. No differences in the proportion of shedders were observed between the sodium butyrate and the control groups, but a significant reduction in the number of infected pigs (61% *vs.* 4%; $p<0.01$) and in the median ELISA Optical Density percentage values (55.9% *vs.* 19.4%; $p<0.01$) at slaughter was observed in pigs under treatment compared to the controls. In addition, an overall significant association between seropositivity and *Salmonella* shedding and infection was detected. Results from this study add more evidences on the positive effect of butyrate on the control of pig salmonellosis.

Additional keywords: swine; organic acids; salmonellosis; feed; zoonoses.

Abbreviations used: CG (control group); MLN (mesenteric lymph nodes); OA (organic acids); OD% (optical density %); SFCA (short-chain fatty acids); TG (treatment group).

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Introduction

Subclinical *Salmonella* infection is common in fattening pigs, and infected pigs are responsible for *Salmonella* carcass contamination at slaughter, making the consumption of pig meat and products thereof a main vehicle of human infections (EFSA & ECDC, 2017). However, considering the alarming increase of antimicrobial resistance worldwide, the preventative use of antibiotics for controlling this pig infection is not advisable (OJEU, 2015).

A vast market of feed additives (*i.e.* essential oils, organic acids, plant extracts, etc.) with potential antimicrobial effects against enteric infections has been developed in recent times. Among organic acids (OA), the butyric acid, a component of the short-chain fatty acids (SFCA) obtained by degradation of fiber by colonic microbiota, has been one of the most studied due to its antimicrobial properties and down-regulating effects in some *Salmonella* virulence genes (Gantois *et al.*, 2006). However, results are often variable and difficult to compare due to different study designs (*i.e.* dosage or treatment period, production phase, level of exposure, etc.) (Creus *et al.*, 2007; De Busser *et al.*, 2009; Michiels *et al.*, 2012; Walia *et al.*, 2016, 2017; Lynch *et al.*, 2017).

In a recent article we reported a form of sodium butyrate protected with vegetable fat that showed some promising results for the control of *Salmonella* infection in pigs (Casanova-Higes *et al.*, 2017). In the current study we present the results of an additional field trial assessing the efficacy of the same sodium butyrate

formula protected with a different encapsulation, *i.e.* a sodium salt of coconut fatty acid distillate. This distillate is composed by lauric acid along with capric and caprylic acids, which have been also described as fatty acids with antimicrobial properties (Rossi *et al.*, 2010; Dayrit, 2015).

Material and methods

The trial was carried out between October 2016 and February 2017 in a small (100 pigs, 8 pens) commercial *Salmonella*-contaminated (identified through serology and microbiology of previous batches) fattening unit located in the NE of Spain. The additive used (DICOSAN+, Norel S.A., Madrid, Spain) is a form of sodium butyrate protected with a sodium salt of coconut fatty acid distillate. Lauric acid is the most abundant fatty acid in coconut oil (45-53%) along with capric and caprylic acids (≈10%).

DICOSAN+ was administrated to the feed in a single dose of 3 kg/ton of feed. The compound feed was provided in 40-kg bags and manually administered to four randomly selected pens (50 animals, treatment group: TG) by the farmer, who was unaware of the treatment allocation. The remaining four pens were fed with the same regular diet without the fatty acid (control group: CG). The treatment was initiated 15 days after pigs entered into the fattening unit (≈10 weeks-old pigs) and lasted until slaughter (3.5 months later).

Serum samples from all pigs were collected after 30, 60 and 90 days on the fattening unit, and within the last week before slaughter (≈ 110 days). On-farm fecal samples (a minimum of 25 g of feces) were collected in sterile containers along with the blood from approximately 25 pigs per group. Since fecal material was collected either after spontaneous defecation or by rectal stimulation, to reduce the risk of environmental contamination of the sample, the final number of sampled pigs varied between 24 and 26 per group, but at least 5 pigs from each pen were included in each sampling to make sure the sampling of all pens (Table 1). At slaughter, fecal and mesenteric lymph nodes (MLN) samples were collected from all pigs after evisceration.

Salmonella isolation from on-farm and slaughter fecal samples as well as from MLN samples was performed following the Standard ISO 6579:2002/ Amd 1:2007. Serum samples were kept at -20°C until serological test was performed. The Herdcheck Swine *Salmonella* ELISA test (IDEXX Laboratories, Westbrook, ME, US) was used for detection of specific antibodies (IgG) against *Salmonella* spp. and results recorded as optical density % (OD%) values following manufacturer´s instructions. Given the low specificity of the ELISA test (Vico *et al.*, 2010) and as suggested in previous studies (Nollet *et al.*, 2005), a cut-off value of OD% ≥40 was considered for seroprevalence estimates.

Repeated measures analysis was used to estimate differences in median OD% values in each group (STATA, StataCorp LP, USA). The effects of sampling time (within-subject factor), and treatment (between-subject factor), and the corresponding interactions among them, on OD% values during the fattening period were assessed by general linear models repeated measures analysis of variance (ANOVA). Fisher exact test was used to assess statistical differences between the CG and the TG regarding the proportion of *Salmonella* shedders at different sampling times, the infection prevalence (proportion of MLN-positive pigs)

Table 1. Microbiological results for *Salmonella* spp. isolation (ISO 6579:2002/ Amd 1:2007) for on-farm fecal samples (OF) after 30, 60 and 90 days in the fattening unit and for mesenteric lymph nodes (MLN) and fecal samples at slaughter (SF) for the Control (CG) and Treatment (TG) groups.

			CG	TG	*p**
Fattening unit	OF at 30d	No.	24	25	
		No. + (%)	22 (87.5)	17 (72)	0.07
	OF at 60d	No.	25	25	
		No. + (%)	4 (16)	3 (12)	1
	OF at 90d	No.	24	26	
		No. + (%)	3 (14.2)	0 (0)	0.1
Slaughter	SF	No.	45	46	
		No. + (%)	7 (15.5)	5 (10.8)	0.55
	MLN	No.	45	46	
		No. + (%)	17 (60.7)	2 (4.3)	0.0001

*p**: Fisher exact test, two-tailed.

and the seroprevalence at slaughter. Logistic regression analysis was used to assess, at pig level, the overall relationship between being a seropositive pig (OD% ≥40) and shedding and infection at slaughter. A two-tailed *p*-value ≤0.05 was considered for significance in all comparisons.

Results and discussion

The fatty acid considered was a new form of sodium butyrate protected with sodium salt of coconut fatty acid distillate, which contains several organic acids that had already shown some antimicrobial properties (Rossi *et al.*, 2010; Dayrit, 2015). Thus, we assessed whether this combination of acids may exert some control on pig *Salmonella* infection when used on a commercial fattening unit known to be consistently contaminated with *Salmonella* spp.

At the beginning of the trial (day 30) both the CG and the TG showed a large proportion of shedders (Table 1). Since recently infected pigs become *Salmonella* shedders quickly after infection and for a median of 14 days (Pires *et al.*, 2013), after which they usually turn into a stage of intermittent shedding, this finding would be compatible with an early exposure to *Salmonella* at the beginning of the fattening period. Further fecal samplings showed a reduction of the number of shedding pigs in both groups but no significant differences between them. This reduction in the proportion of shedders along the fattening period would be likely related to the expected intermittent shedding described above and usually occurring 2 or 3 weeks after the primary infection (Scherer *et al.*, 2008; Pires *et al.*, 2013). In the TG however, this reduction could be also associated, at least partially, with the destruction of the pathogen in the intestinal lumen due to the presence of the organic acids (*i.e.* through the lowering of extra- and intra- cellular pH). In fact, considering the large number of pigs shedding *Salmonella* on day 30 in the TG (72%), a large number of infected (MLN positive) pigs at slaughter would have been expected in this group. However, the proportion of infected pigs at slaughter in the TG was very low (4%) and significantly lower than that in the CG (61%), which supported the protective effect against infection of this form of organic acid (Rasschaert *et al.*, 2016). It appeared that the treatment could have prevented bacteria translocation to MLN despite the initial high levels of exposure to *Salmonella*.

The low number of pigs shedding *Salmonella* at slaughter was also an unexpected finding, particularly in the CG, as shedding in *Salmonella*-infected pigs is usually favored by stress-producing factors such as the transport to the slaughter and also the lairage (Rostagno *et al.*, 2003). This result may be partially explained by the short time periods for transport and lairage in this study. Transport lasted only for half an hour and lairage was less than 2 hours, which may have contributed to low levels of stress in these pigs. Regarding the TG, since the presence of *Salmonella* in MLN seems to be associated to shedding at slaughter (Casanova-Higes *et al.*, 2018), the low proportion of MLN-positive pigs in this group would explain the small proportion of shedders detected.

Seroprevalence was similar on day 30 for both groups (26.9% in the CG *vs.* 25% in the TG), which indicated that both groups started the trial from a similar epidemiological situation. In subsequent samplings seroprevalence remained consistently and significantly lower ($p<0.01$) for the TG (Fig. 1). The repeated measures analysis showed a significant overall interaction between treatment and time. For the TG, median OD% values rose significantly from the first (OD%=14.9) to the second sampling (OD%=28.4), but began to decrease after that. Median OD% values on day 90 and prior to slaughter (OD%=14.9 and 19.4, respectively) were similar to the median OD% value found on day 30 (Fig. 2). However, in the CG an overall increasing trend of OD% values was observed, from 19.4 on day 30 to 58.3 at slaughter (Fig. 2). At pig level, a positive relationship between seropositivity and shedding/infection at slaughter was observed. A seropositive pig (OD%≥40) had 9 times (OR= 9.2; 95%CI: 1.9, 45.3; $p=0.003$) higher odds of shedding *Salmonella* at slaughter and 4 times (OR= 4.1; 95%CI: 1.4, 12.4; $p=0.003$) higher odds of being infected than a seronegative pig (OD%<40). All together, these results suggested an overall preventive effect of this organic acid against further *Salmonella* exposure. The relationship observed between seropositivity on days prior to slaughter and shedding/infection supported previous hypothesis that on-farm seropositivity could

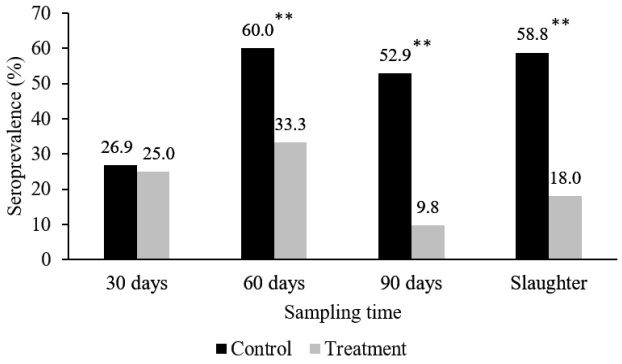

Figure 1. *Salmonella* seroprevalence (cut-off value OD% ≥40%; Herdcheck Swine *Salmonella* ELISA test, IDEXX Laboratories, US) at different sampling times in the fattening period in the control and treatment group. **: $p\leq0.01$

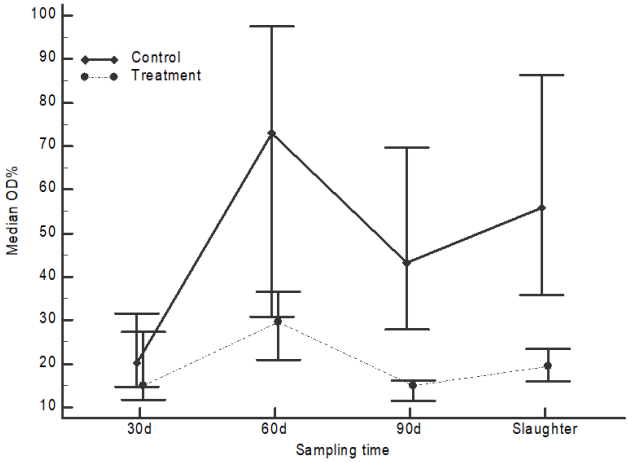

Figure 2. Median optical density percentage values (Median OD%) (Herdcheck Swine *Salmonella* ELISA test, IDEXX Laboratories, US) and their corresponding 95% confident intervals at the different sampling times (30, 60, 90 days on fattening and prior to slaughter) for the control and treatment groups.

be considered an indicator of the risk of *Salmonella* shedding at slaughter (Mainar-Jaime *et al.*, 2018).

Overall results from this study seem to be somewhat better than those previously obtained from a similar study with the same product protected with vegetable fat (Casanova-Higes *et al.*, 2017). The use of sodium salts of organic acids (such as lauric, capric and caprylic), besides protecting the sodium butyrate against its early dissociation in the stomach, may enhance the product antimicrobial effect, through interfering with cell membrane and cellular processes (Dayrit, 2015). As a result, a synergic effect could be expected from the combination of sodium butyrate with this protecting ingredient. These results add more evidences on the positive effect of butyrate on the control of pig salmonellosis, but more studies on dosage, treatment periods and duration will be required to optimize this approach.

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