EFFECT OF THE ADDITION OF PROTECTED SODIUM BUTYRATE TO THE FEED ON *SALMONELLA* SPP. INFECTION DYNAMICS IN FATTENING PIGS

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Abstract

The effectiveness of a new form of sodium butyrate protected with sodium salt of coconut fatty acid distillate for the control of *Salmonella* infection in fattening pigs was assessed. A dose of 3 kg/T of this product was added to the basal diet of a group of pigs for the whole fattening period while another group within the same fattening unit remained without treatment. A significant reduction in the number of infected pigs (4% *vs*. 61%; $P<0.01$) and in the median OD% values (19.4 *vs*. 55.9) at slaughter were observed in the pigs under treatment compared to the controls. Also, a significant association between high OD% values and *Salmonella* shedding and infection was detected. The use of this form of protected sodium butyrate may be useful to reduce *Salmonella* shedding and infection in slaughter pigs.

Introduction

In the pig industry, the use of antimicrobials may have favored the selection for antimicrobial resistance (AR) (Davies *et al*., 2010). The emergence of AR has prompted European Health Authorities to reconsider their use for meat production, reinforcing the search for alternative products for the control of enteric infections in pigs and triggering new EU antibiotics regulations.

Organic acids (OA) might be beneficial for the productive performance of fattening pigs (Partanen *et al.,* 1999), but they are also known for their *in vitro* capacity to inhibit the growth and proliferation of Gram-negative pathogens such as *Salmonella* spp. (Gantois *et al*., 2006). Therefore, OA are seen as an alternative to antibiotics to reduce the burden of pig salmonellosis.

Results on the effectiveness of OA for the control of pig salmonellosis are variable (Creus *et al.,* 2007; Walia *et al*., 2016), which is likely associated to different study designs or the use of different acids, blends, or doses. There is a clear need for more research on the use of OA for *Salmonella* reduction in fattening pigs to get a better idea of the effectiveness of the different types of OA available in the market. In particular, on new forms of OA (i.e. encapsulated or protected OA) that may act on the more distal part of the gastrointestinal tract (Piva *et al*., 2007). The effectiveness of protected sodium butyrate (PSB) in reducing *Salmonella* has been shown previously in chicken (Fernández-Rubio *et al*., 2009). Thus, a field trial was carried out to assess the effect of the addition of PBS to the feed on *Salmonella* infection dynamics in fattening pigs from an area of high *Salmonella* infection prevalence.

Material and methods

This study was carried out in a small (8 pens, ≈110 pigs) commercial *Salmonella*-infected fattening unit located in Spain. A new form of sodium butyrate protected with sodium salt of coconut fatty acid distillate (DICOSAN+, Norel SA, Spain) was added to the feed (3 kg/T) in 4 randomly selected pens (approx. 50 animals, treatment group - TG). The remaining 4 pens were fed with the same regular diet without the treatment (control group -CG). The treatment with the protected sodium butyrate (PSB) was initiated after finishing the in-feed antibiotic treatment.

Serum samples from all pigs were collected after 30 (beginning of the treatment with PSB), 60 and 90 days on the fattening unit, and within three days before slaughter, to check for the presence of antibodies against *Salmonella* spp. On-farm fecal samples (OFFS) were collected along with the blood from 25 pigs per group, after spontaneous defecation. At slaughter, fecal (FSS) and mesenteric lymph nodes (MLN) samples from all pigs were collected after evisceration. Bacteriology from individual fecal samples and mesenteric lymph nodes (MLN) was performed following the EN ISO 6579:2002/A1:2007. For serology an indirect ELISA (HerdCheck Swine *Salmonella*, IDEXX Laboratories, ME, USA) was used and three cut-off values considered (%OD ≥10, ≥20 y ≥40).

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, and infection prevalence (proportion of MLN-positive pigs) at slaughter in each trial individually. A one-tailed P-value ≤0.05 was considered for significance. A repeated measures analysis was used to estimate differences in median OD% values in each group after taking into account sampling times and the interaction treatment*time. Statistical analyses were performed with STATA software (STATA, StataCorp, L.P., USA).

Results

Both groups showed a large proportion of shedders on the first sampling, but somewhat higher for the CG. The proportion of shedders decreased significantly in the following samplings in both groups and virtually no significant differences between them were observed along the fattening period (Figure 1). However, at slaughter, the proportion of infected pigs (MLN+) was significantly higher for the CG compared to the TG (61% vs. 4%; P<0.01).

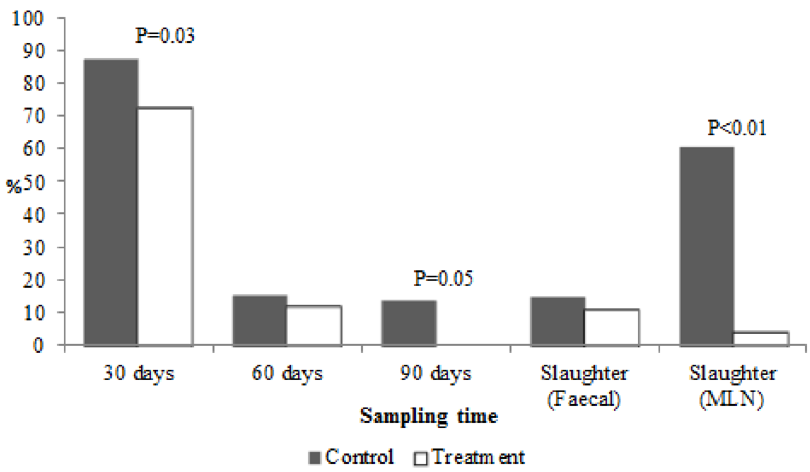

Figure 1. Summary results of bacteriology on fecal samples for the control (CG) and treatment (TG) groups at different sampling times.

The median OD% value for both groups was similar at 30 days, but in subsequent samplings median OD% values remained significantly lower for the treatment group (Figure 2). A significant overall interaction between treatment and time was observed. For the TG, median OD% rose significantly from the first (OD%=14.9) to the second sampling (OD%=29.5), but they started to decrease after that. Median OD% values at 90 days on fattening and at slaughter were similar to the median OD% value found for the first sampling on day 30 (14.9 and 19.4, respectively). However, in the CG an overall increasing trend of median OD% values was observed, from 20.2 on day 30 to 55.9% at slaughter. Differences in seroprevalence were also observed after 60 days on fattening when a cut-off value ≥40% was used. These differences remained significant in subsequent samplings for the 20% and 40% cut-off values as well.

It was observed a positive relationship between serology and shedding and serology and infection (MLN+). A seropositive pig (i.e. OD%≥40) had 9 times (OR= 9.2; 95%CI: 1.9, 45.3; *P*= 0.003) higher risk of shedding *Salmonella* at slaughter and 4 times (OR= 4.1; 95%CI: 1.4, 12.4; *P*=0.003) higher risk of being infected.

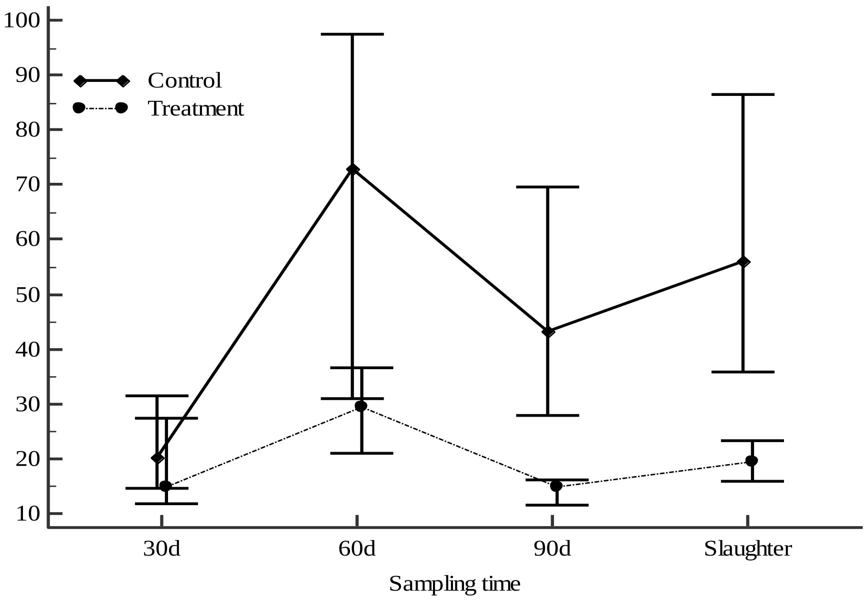

Figure 2. Median OD% values and their corresponding 95%CI for the control and treatment group at each sampling time.

Discussion

A very large percentage of pigs were shedding *Salmonella* on the first sampling after 30 days on fattening in both groups. This result seemed to be related to an early exposure of the farm unit to *Salmonella*. Further samplings showed a significant reduction in the level of shedding in both groups. This drop of *Salmonella* shedding along the fattening period seemed to be likely related to the adaptation of the pigs to the unit environment.

Given the large number of pigs shedding *Salmonella* on day 30 in both groups, a large number of infected (MLN+) pigs at slaughter was expected. Although this was true for the CG, as 60.7% of pigs resulted infected, it was not observed in the TG (4.3%). This finding suggested a protective effect of DICOSAN+ against *Salmonella* infection despite the high level of exposure to *Salmonella* of these pigs at the beginning of the fattening period. Serological results would further support this conclusion. After an initial increase of median OD% values in the TG, they significantly decreased over time despite the presence of *Salmonella* in the farm environment. The OD% values in the TG only increased from the first to the second sampling, suggesting that some time under treatment is required (probably a minimum of 3-4 weeks) in order for the product to be effective. On the contrary, in the CG, OD% values increased significantly from day 30 to slaughter, with a median OD% value at slaughter much higher than the maximum cut-off value usually considered for deeming a pig as seropositive (i.e. 40%).

The low number of pigs in both groups that were shedding at slaughter was an unexpected result and it may be the consequence of the short period of transport and lairage. Transport to slaughter and lairage are usually factors that prompt infected pigs to shed *Salmonella* due to the stress that they produce on the animals (Scherer *et al*., 2008). In this case, the transport lasted only for half an hour and the lairage was short as well (≈ 2 hours), which may have prevented high levels of stress in these pigs. In addition, the level of cleanliness of the lairage area was high as new facilities had been built for this slaughterhouse, which likely contributes to impair the transmission of the pathogen between pigs (Berends *et al*., 1997).

Conclusion

A significant reduction in the number of infected pigs and in OD% values were observed in the pigs under treatment, and high OD% values were positively associated to both *Salmonella* shedding and infection. Therefore, the use of DICOSAN+ at 3kg/T would appear as a potential strategy to reduce *Salmonella* shedding and infection in slaughter pigs when used during the whole fattening period.

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