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Effect of organic acids and mannanoligosaccharide on excretion of *Salmonella typhimurium* in experimentally infected growing pigs

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ABSTRACT

The effect of organic acids and mannanoligosaccharide addition to the diet was assessed in pigs orally inoculated with *Salmonella typhimurium*. Forty-six growers were distributed among four treatments: Basal Diet (BD); BD + encapsulated organic acids; BD + free organic acids; BD + mannanoligosaccharide. Seroconversion was monitored, and feces and tissue samples were tested for *Salmonella* isolation. No treatment prevented the carrier state, but a tendency of lower fecal excretion was observed in the group treated with mannanoligosaccharide.

Dietary additives have been proposed as alternative tools to be adopted for reduction of *Salmonella* carrier pigs (Visscher et al., 2005; Creus et al., 2007; Papenbrock et al., 2009.). Therefore, the aim of this study was to evaluate the effect of either organic acid or mannanoligosaccharide dietary addition on *Salmonella typhimurium* carriage and fecal shedding in challenged growers.

The experiment was conducted in a randomized complete block design divided in two consecutive periods. Each block included all treatments, which were composed of six animals each, housed in separated pens in the same room at the biosafety facilities of Embrapa Swine and Poultry Research Center, Brazil. Forty-eight *Salmonella*-negative pigs (43-day-old, 11.39 ± 1.6 kg body weight) were allotted to one of the following treatments: a basal diet without additives (CON, control), and basal diets added with a blend of encapsulated organic acid (EOA, fumaric acid 20%, citric acid 10%, malic acid 10%, phosphoric acid 10% ; Tetracid® TM-500, Jefo Nutrition Inc.), or a blend of short chain free organic acids (SOA, formic acid 26%, propionic acid 10%, plant fatty acids 18%; Selacid Green Growth® Selko Latin America Ltda.) or prebiotic (MOS, mannanoligosaccharide 12%, Bio-Mos® Alltech Biotechnology Ltda.). The additives were mixed with the basal diet in a proportion of 2 kg/ton of feed, as recommended by the manufacturers. Pigs had ad *libitum* access to water and feed throughout the experiment. The protocol was approved by the Research Ethics Committee of Universidade Federal do Rio Grande do Sul (number 2007–962).

Two weeks after housing, pigs were orally challenged (day 0 post-inoculation, 0 PI) with a 10 mL-dose of a *S. typhimurium* suspension (106 colony forming units/mL). Two pigs from the control group died due to *Streptococcus suis* meningitis before the inoculation and were not replaced. Blood and fecal samples were collected on days 14 and 7 before inoculation and on days 0, 3, 7, 14, 21, 28 PI. On day 35PI, animals were weighed, euthanized, and samples of blood and organs were collected. Samples were submitted to the ISO6579 *Salmonella* isolation protocol. Fecal samples were tested following the ISO6579 Annex D (ISO, 2007). Quantification of *Salmonella* was performed in feces samples by the Most Probable Number method, according to BAM (2003). Serum samples were tested by an indirect IgG-ELISA assay based on somatic antigen 1, 4, 5 and 12 of *Salmonella* (Kich et al., 2007). Results were analyzed by the MIXED or the FREQ procedures of SAS (2002).

All treatment groups were infected, as evidenced by the isolation of *Salmonella* and by the seroconversion. The mean pig live weight increased in the four groups from 11.39 (±1.6) on the day of housing to 52.11(±5.5) on day 35PI. Seroconversion started on day 7PI, and IgG titles increased significantly ($P < 0.05$) until day 35PI in all groups.

The frequency of *Salmonella* excretion varied from 100% (10/10) on day 7PI in the control group to 25% (3/12) on day 28PI in the MOS group. No statistical difference was observed between groups throughout the experiment (Table 1). Results of *Salmonella* quantification showed interaction between treatment and

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Table 1
Frequencies of *Salmonella* fecal excretion in pigs fed with a basal diet (CON) or with a diet added of encapsulated organic acids (EOA), non-encapsulated organic acids (SOA) or mannanoligosaccharide (MOS), between days 3 and 28 after oral inoculation of *Salmonella Typhimurium* (DPI).

DPI	CON (n = 10)	EOA (n = 12)	SOA (n = 12)	MOS (n = 12)	P^*
3	7	11	10	10	0.6599
7	10	10	8	7	0.1136
14	8	7	6	5	0.3482
21	7	7	10	5	0.1804
28	7	5	6	3	0.1976

* P-value of Fisher's Exact Test by the FREQ procedure of SAS.

Table 2
Quantification of *Salmonella* ($\log_{10}$ ufc g^{-1}) in fecal samples from pigs fed a basal diet (CON) or a diet added of encapsulated organic acids (EOA), non-encapsulated organic acids (SOA) or mannanoligosaccharide (MOS), between days 3 and 28 after oral inoculation of *Salmonella Typhimurium* (DPI).

DPI	CON	EOA	SOA	MOS	P^*
3	0.61 ± 0.31**Ba**	5.75 ± 1.37**Ac**	4.39 ± 1.20**Ab**,**c**	1.99 ± 0.83**a**,**b**	0.0019
7	4.66 ± 1.07**A**	3.01 ± 0.81**B**	1.93 ± 0.78**AB**	2.05 ± 0.94	0.1671
14	1.61 ± 0.71**B**	2.06 ± 0.77**BC**	0.60 ± 0.28**B**	0.77 ± 0.53	0.2148
21	0.57 ± 0.20**B**	1.05 ± 0.34**CD**	1.46 ± 0.40**B**	0.64 ± 0.33	0.1143
28	0.99 ± 0.29**Ba**	0.64 ± 0.24 **Da**	0.91 ± 0.31**Ba**	0.23 ± 0.12**b**	0.0015
P	0.029	<0.01	0.0033	0.064	

Means followed by different lowercase letters (a, b, c) in the same line are different by t-test ($P < 0.05$).
Means followed by different capital letters (A, B, C, D) in the same column are different by t-test ($P < 0.05$).
* P-value of F test by the MIXED procedure of SAS for repeated measures.

Day-post-inoculation (DPI, P = 0.0056). Three groups (CON, EOA, SOA) presented an excretion peak of *Salmonella* after day 3PI, followed by a gradual decrease in fecal excretion until day 28PI (Table 2). The MOS group showed a trend of low excretion throughout the experiment, and on day 28PI had significantly less *Salmonella* in feces than the other groups.

Salmonella was isolated from various tissue samples in all groups, with a frequency ranging from 16.7% (liver from SOA and MOS) to 90% (lung from CON). Samples of cecum (67.3%) and lymph node (52.9%) were *Salmonella*-positive, and there was no statistical difference in the number of intestinal carriers between groups. Besides the fecal-oral route, invasion through the respiratory tract has been demonstrated (Oliveira et al., 2006), and may have also occurred in our study, since tonsil (84.58%) and lung (69.6%) samples presented the highest rates of isolation. These results may raise the hypothesis that additives acting in the gastrointestinal tract will not be able to completely prevent the infection of the *Salmonella*-exposed pigs.

Organic acids have been extensively used due to their beneficial effect on the performance of reared pigs and improvement of gut health (Walsh et al., 2007). Addition of organic acids (0.4–0.9% in-feed) has also been tested in weaned (Papenbrock et al., 2005; Taube et al., 2009) and market-age pig herds (Creus et al., 2007) to reduce the number of *Salmonella*-shedders. In spite of controlling *Salmonella* excretion, a substantial addition of acids to the diet may result in decrease of palatability, leading to depression of feed intake (Walsh et al., 2007). Thus, we tested organic acids in a concentration (0.1% in-feed) successfully used to increase growth performance in pigs. However, the low acid concentration will apparently not be sufficient to decrease *Salmonella* shedding rates.

A tendency towards a lower fecal number of *Salmonella* was observed in the group fed on diets with added MOS. The action mechanism of probiotic compounds is related to the stimulation of fermentative activity in the gut, leading to a beneficial change in the intestinal flora and to interference with *Salmonella* colonization (Martín-Peláez et al., 2010). These combined effects might have reduced the *Salmonella* shedding rate in our study. Therefore, the effect of mannanoligosaccharide treatment of carrier pigs upon the infection rates in sentinel pigs deserves further investigation.

Conflict of interest statement

None declared.

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