Galactomannans for the control of *Salmonella* infection in fattening pigs

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Abstract

The use of plant-derived products with antimicrobial activity appears as an alternative for the control of pig salmonellosis. The results of 3 field trials aimed at assessing the efficacy of different concentrations of a galactomannan (Salmosan®) in the control of *Samonella* infection in fattening pigs are presented. It was found that the addition of ≥2 kg/T of Salmosan® to the regular diet of pigs during the entire fattening period was able to decrease significantly the prevalence of *Salmonella* infection and shedding at slaughter. This galactomannan could be considered a good complementary tool along with good hygiene and biosecurity for the control of this infection in the pig farm.

Introduction

Among the main zoonotic diseases transmitted by pigs and pork products in developed countries one of the most important is salmonellosis. In the USA this infection was considered the main bacterial foodborne illness in 2011 (1), while in the European Union (EU) it was ranked as the second after campylobacteriosis (2). After years of control programmes against poultry salmonellosis, pigs are now becoming a major source of infection for humans in the EU (2).

Salmonella spp. are characterized by a great environmental and antimicrobial resistance. In addition, detection of asymptomatically *Salmonella*-infected pigs is not straightforward and vaccines are not usually used against zoonotic *Salmonella*. Thus, high standards on biosecurity and hygiene along the entire food chain are of utmost importance for the control of this infection (3).

The use of some non-digestible oligosaccharides has been proposed as a new alternative for the control of salmonellosis in weaning and postweaning pigs. These are usually plant or yeast-derived products that have shown some antimicrobial effects either through modulation of beneficial microbiota, thus favoring competitive exclusion, bacteriocin production, etc., or through the enhancement of the intestinal defense system by some immunomodulatory action. Of particular interest are the mannan-oligosaccharides due to their ability to bind to mannose-specific lectin of gram-negative pathogens that express Type-1 fimbriae, thus blocking the adhesion of these bacteria and excreting them from the intestine. However, when these products have been tested results have been inconsistent (4-6).

In this study we show the results of 3 field trials aimed at assessing the effect that the addition to the diet of fattening pigs of different concentrations of a galactomannan obtained from carob bean gum (*Ceratonia silicua*) had on the prevalence and seroprevalence of *Salmonella* spp.

Material and methods

Three different doses (0.5, 2 and 3 kg per Ton of feed) of a galactomannan (Salmosan®, ITPSA, Barcelona, Spain) were used during the entire period of fattening in 3 field trials carried out in a small commercial fattening unit previously identified as positive for *Salmonella*. This product had shown promising results against salmonellosis in previous trials on weaning pigs (7). The fattening unit was divided in 8 pens (12-14 tagged pigs/pen). Four of these pens were randomly chosen as treatment pens and pigs from these pens composed the treatment group (T). Pigs from the other 4 pens formed the control group (C). The farmer was unaware of the pen allocation.

All pigs were given in-feed colistin (120 ppm) for 2 weeks after arriving. Fifteen days after colistin treatment, serum (about 50 pigs/group) and fecal samples (20-25 pigs/group) were collected to confirm the absence of significant differences in

group seroprevalence and fecal prevalence. At 60 and 90 days of the fattening period serum and fecal samples were collected again from a similar number of pigs. One week before slaughter serum was collected from a minimum of 40 pigs in each group (except in the first trial), and mesenteric lymph nodes (MLN) and feces (except in the first trial) were collected at slaughter. The ISO 6579:2002 and the Herdcheck® Swine *Salmonella* ELISA (IDEXX Lab., cutoff %OD≥40%) were used for microbiological and serological analyses, respectively.

Univariable chi-squared analysis was used to assess the presence of differences in shedding, seroprevalence and prevalence between C and T at 60 and 90 days and at slaughter. A random-effects logistic regression (re-LG) with pen as the random factor and trial as the confounding factor was performed to measure the overall effect of the different doses of Salmosan* on the prevalence of infection and shedding at slaughter.

Results

Bacteriological results

Microbiological results for the C and T in the 3 trials are presented in Table 1. No significant differences were observed at any time when the lowest dose (0.5 kg/T) was used, but at higher doses (2 and 3 kg/T) the prevalences of infection and of shedding at slaughter were significantly lower for T.

Doses of 2 or 3 kg/T of Salmosan* supplemented during the entire fattening period decreased significantly the prevalence of infection after taking into account pig allocation to pens and adjusting by trial (Table 2). A dose-response trend was observed, with a higher reduction when a higher dose was used. According to results in Table 2, the efficacy of Salmosan* could reach from 90% (2k/T) to 99% (3 kg/T) when compared to the control group. Similar results were observed for *Salmonella* shedding.

Seroprevalence

No differences were observed between C and T after colistin treatment in any of the trials. When the highest dose (3 kg/T) was used, a significant lower seroprevalence was observed in T compared to C after 60 days of fattening. No differences at any time were observed for the other two trials (Table 3).

Discussion

The use of antibiotics as growth promoters or with a preventative use is officially forbidden in the EU. Thus, the control of enteric bacterial infections must be based on strict hygiene and biosecurity measures and the use of vaccines when available. No vaccines are commonly used for the control of salmonellosis in fattening pigs in the EU so far. Thus, different products, such as mannan-oligosaccharides, with antimicrobial properties are being considered as potential alternative for the control of this infection. Mannan-oligosacharides can be obtained from a wide variety of natural sources and their diverse biological composition and processing make their overall antimicrobial effect variable (4, 6).

We tried different doses of a galactomannan obtained from carob bean gum (Salmosan®) added to the regular diet of pigs for the entire period of fattening to assess its effect on *Salmonella* shedding, prevalence and seroprevalence. The results indicated that the addition of ≥2 kg/T of Salmosan® was able to significantly decrease the prevalence of *Salmonella* infection and shedding at slaughter. In trial 2 this was supported by a significant lower number of seroconverting pigs (cutoff %OD>40) before slaughter. In trial 3, however, despite a very large number of control pigs were infected and shed *Salmonella* at slaughter, the proportion of seroconverting pigs was very low in both groups. This finding could be explained if *Salmonella* infection occurred mostly at the end of the fattening period, during the transport to the slaughter or at lairage (9), as seroconversion is seen around 2 weeks after infection (8). It could also be explained by a delayed onset of seroconversion that may happen with certain *Salmonella* strains (10).

The apparent and unexpected difference in seroprevalence between C and T in trial 3 (0% *vs.* 7.8%; p=0.12, respectively) was most likely due to the intrinsic variability of the ELISA used, as three animals from C had ODs higher than 36% but lower than 40%, and thus were deemed test negative.

Conclusion

In view of the bacteriological results, Salmosan® seems to be effective against *Salmonella* infection in fattening pigs. Its efficacy would be very high when doses ≥2 kg/T are used for the entire period of fattening, and may be useful even if infection occurs during the transport to the slaughterhouse or at lairage. Overall, this galactomannan could be considered

a complementary tool along with good hygiene and biosecurity for the control of this infection in the pig farm. Further investigation is warranted to confirm these findings.

Table 1. Microbiological results (ISO 6579:2002) for fecal samples after 60 (60d) and 90 (90d) days in the fattening unit and for mesenteric lymph nodes (MLN) and fecal samples (Fecal) at slaughter.

		Fattening unit						Slaughter					
		60d			90 d			MLN			Fecal		
		N	No. + (%)	*p*	N	No. + (%)	*p*	N	No. + (%)	*p*	N	No. + (%)	*p*
T1	C	19	4 (21)	0.41	45	2 (4.2)	0.99	42	23 (54.8)	0.25			
	T	28	3 (10)		44	1 (2.3)		49	21 (42.9)				
T2	C	25	3 (12)	0.60	25	2 (8)	0.48	47	37 (78.7)	<0.01	45	26 (57.8)	<0.01
	T	25	1 (4)		25	0 (0)		39	0 (0)		39	1 (2.6)	
T3	C	26	7 (26.9)	0.15	28	0 (0)	1	49	20 (40.8)	<0.01	49	34 (69.4)	<0.01
	T	23	2 (8.7)		27	1 (3.7)		51	3 (5.9)		51	6 (11.8)	

T1: Trial 1 (0.5 kg/T); T2: Trial 2 (3 kg/T); T3: Trial 3 (2 kg/T). C: control group; T: treatment group.

Table 2. Results of the random-effect logistic regression on the effect of Salmosan® on *Salmonella* infection and shedding at slaughter.*

	Logistic regression parameters					
	Infection (MLN)			Shedding (Fecal)		
Factor	*P*	Odds Ratio (OR)	95%CI (OR)	*P*	Odds Ratio (OR)	95%CI (OR)
Doses						
0 kg /T feed		1			1	
0.5 kg /T feed	0.55	0.73	0.26, 2.07	-	-	-
2 kg /T feed	<0.01	0.09	0.04, 017	<0.01	0.06	0.02, 0.13
3 kg /T feed	<0.01	0.008	0.001, 0.07	<0.01	0.018	0.001, 0.23
Trial[a]						
1 (April 2009)		1		-	-	-
2 (May 2010)	0.15	3.1	0.64, 15.6		1	
3 (Feb. 2013)	0.47	0.68	0.25, 1.91	0.64	1.51	0.25, 8.86

*Pen as random factor and trial as confounding factor. No fecal samples collected in Trial 1 (0.5 kg/t.)

Table 3. Serological results (Herdcheck® *Salmonella* ELISA, cutoff %OD ≥40) for the three trials after 60 (60d) and 90 (90d) days in the fattening unit and one week previous slaughter (Slaughter).

		60 d			90d			Slaughter		
		N	No. + (%)	*p*	N	No. + (%)	*p*	N	No. + (%)	*p*
T1	C	55	3 (5.5)	0.98	55	4 (7.3)	0.46	-	-	-
	T	56	3 (5.4)		53	6 (11.3)		-	-	-
T2	C	55	18 (32.7)	<0.01	53	13 (24.5)	0.06	40	15 (37.5)	<0.01
	T	56	6 (10.7)		55	6 (10.9)		40	4 (10)	
T3	C	51	1 (1.9)	1	51	0 (0)	0.12	49	0 (0)	0.12
	T	51	2 (3.9)		50	3 (6)		51	4 (7.8)	

T1: Trial 1 (0.5 kg/T); T2: Trial 2 (3 kg/T); T3: Trial 3 (2 kg/T). C: control group; T: treatment group.

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