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Effect of Organic Acids on *Salmonella* Shedding and Colonization in Pigs on a Farm with High *Salmonella* Prevalence

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ABSTRACT

This study builds on the results of a previous study in which six commercial feed products based on organic acids were evaluated with respect to *Salmonella* contamination of piglets in an artificially challenged seeder model. In the present study, the efficacy of three of these commercial products was assessed for *Salmonella* reduction in fattening pigs on one closed farm with a natural high *Salmonella* prevalence. In each of four fattening compartments, one of the following feed treatments was evaluated during two consecutive fattening rounds: (i) butyric acid (active ingredients at 1.3 kg/ton of feed; supplement A1), (ii) a combination of short-chain organic acids (mixture of free acids and salts) and natural extracts (2.92 kg/ton; supplement A4), (iii) a 1:1 blend of two commercial products consisting of medium-chain fatty acids, lactic acid, and oregano oil (3.71 kg/ton; supplement A5+A6), and (iv) a control feed. On the farm, the *Salmonella* status of the fattening pigs was evaluated by taking fecal samples twice during the fattening period. At the slaughterhouse, samples were collected from the cecal contents and the ileocecal lymph nodes. *Salmonella* isolates were serotyped and characterized by pulsed-field gel electrophoresis. This farm had a particularly high number of pigs shedding *Salmonella* with a wide variety of sero- and pulsotypes. Only the feed blend based on the medium-chain fatty acids was able to significantly reduce *Salmonella* prevalence both on the farm and at the slaughterhouse. With this combined supplement, the *Salmonella* reduction in the feces at slaughter age, in cecal contents at slaughter, and the lymph nodes was 50, 36, and 67%, respectively, compared with the control animals. This promising finding calls for further investigation including cost-efficiency of this combined feed product and its effect on the animals.

Salmonella enterica subsp. *enterica* is one of the major bacterial foodborne pathogens in many industrialized countries. The European Food Safety Authority and the European Centre for Disease Prevention and Control *(13)* reported 20.4 confirmed cases per 100,000 European Union (EU) inhabitants in 2012. The most important serotypes of this *S. enterica* subspecies are Enteritidis and Typhimurium, which are mainly associated with the laying hen industry and pig production, respectively *(8)*. Many efforts have been made in the last 10 years to reduce *Salmonella* Enteritidis in the poultry industry, for example by vaccination programs in certain EU member states *(10)*. The relative importance of pork as a source of human salmonellosis has therefore been increasing in recent years *(8)*. One way to combat *Salmonella* in the pork industry is to decrease the number of pigs that are shedding or carrying *Salmonella* on the farm, which would reduce the risk of carcass *Salmonella* contamination during slaughter. Antimicrobial agents such as organic acids that are added to feed or water are often promising in vitro; however, in vivo studies often yield conflicting results *(24)*. The present study builds on that of Michiels et al. *(22),* in which the effect of six commercial products (A1 to A6) on *Salmonella* contamination and shedding was evaluated in an artificially challenged seeder model with weaned piglets. The active ingredients were mainly organic acids such as short-chain fatty acids (SCFAs), benzoic acid, and medium-chain fatty acids (MCFAs) and in a few cases also essential oils and other natural extracts. Two products, supplemented at more than 2.7 g of active ingredients per kg of feed, seemed to reduce *Salmonella* shedding and colonization in piglets. Both products contain SCFAs; one also contains benzoic acid, and the other also contains an essential oil. The aim of the present study was to assess the efficacy of three additives selected from this previous study for reducing *Salmonella* contamination in a field study on a closed farm with a high natural *Salmonella* prevalence. Results were compared with those of a control treatment without the additives. The supplemented and control feed was administered during two consecutive fattening rounds, and samples were collected for *Salmonella* evaluation both on the farm and at the slaughterhouse where the treated and control pigs were slaughtered. *Salmonella* isolates were further characterized

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to determine whether a shift in serotypes or genotypes had occurred in response to the feed treatments.

MATERIALS AND METHODS

Description of the farm. A Belgian farm with persistent *Salmonella* contamination was selected based on samples taken on this farm before the start of the study. Samples from all overshoes worn in the various farm compartments (e.g., farrowing, nursery, and fattening units) tested positive for *Salmonella*. The serological results of the official *Salmonella* program in Belgium indicated that the farm had elevated sample-to-positive result (S/P) ratios for *Salmonella* in preceding years. The official program guidelines state that a farm is considered a *Salmonella* risk when three consecutive samplings have an S/P value above 0.6. Although this scenario had not occurred at this farm, the average S/P ratio was quite high: 0.73 in the 3 years before the beginning of the study and 0.83 during the study *(11)*. This farrow-to-finish farm, a so-called closed farm, had 280 sows in a 3-week system. Piglets were weaned at 28 days of age, and each group of piglets remained in the nursery for approximately 40 days. Almost all piglets were fattened on the farm, and the surplus animals were sold. Each of the four identical fattening compartments had a separate entrance and separate feed silo. Each compartment consisted of 25 pens with 14 pigs, for approximately 350 animals per compartment. The compartments were filled at different times with pigs weighing around 25 kg. The fattening phase had a duration of approximately 4.5 months. These pigs were kept on a four-stage feeding schedule, which included four successive feeds given each appropriate ages. All feeds were based on cereals, toasted soybeans, soybean meal, rapeseed meal, wheat by-products, minerals, and vitamins. Around 3 weeks before the end of the cycle, the heaviest pigs were removed from the pens and sold for slaughter (normally one-third of the total pigs in the unit). This removal ensured that there was enough room for the remaining pigs so that all pigs reached the appropriate slaughter weight. Pigs were fasted overnight before transport to the slaughterhouse.

Experimental set up and sampling. The farm had four identical fattening compartments. One compartment was used for the control pigs, and the other three were allocated to one of the three treatments. Each treatment, including the control, was evaluated during two consecutive rounds of fattening (round 1 and round 2). The repetition of each treatment was carried out in the same compartment. The study started in August 2011; round 1 finished in January 2012, and round 2 finished in May 2012. The animals received the control diet or the supplemented feed during the entire fattening period (from 25 kg of body weight until slaughter). The control diet consisted of the basal diet, and the supplemented feeds consisted of the same basal diet supplemented with one of the three commercial additives. The exact composition of the commercial products was described by Michiels et al. *(22)*. Based on the results of this seeder model study and the type of product, the following supplements were chosen to evaluate in the field: products A1, A4, and A5+A6. Product A1 contained butyric acid, and the active ingredients were added at 1.30 kg/ton of feed. This product was chosen for testing under field conditions based on the highest reduction in *Salmonella* counts in the ileocecal lymph nodes in the seeder model. Product A4 contained salts of formic, sorbic, acetic, and proprionic acid in free acid form and natural extracts as active compounds; excluding the natural extracts, the active compounds were added at 2.92 kg/ton of feed. This product performed best in the seeder model; it significantly reduced the amount and duration of *Salmonella* shedding after challenge. Products A5 and A6 were combined because both contained mainly MCFAs but did not reduce *Salmonella* shedding and colonization in young piglets in the seeder model when added separately to the feed, probably because of the low concentrations in the feed *(22)*. Therefore, we combined the two products at the doses tested in the seeder experiment, for a final active ingredients concentration of 3.71 kg/ton *(22)*. This combined supplement A5 + A6 consisted of triglycerides with caproic and caprylic acids, the free acids caproic, capric, caprylic, lauric, and lactic acid, and coated oregano oil. With the exception of the oregano oil, none of the other ingredients in the three supplements were coated.

Before the piglets were transferred to the fattening phase and the supplementation of feed with the organic acids started, the growing unit was checked for *Salmonella* by testing samples from overshoes. During each fattening round, the *Salmonella* status was evaluated in feces from animals that were 40 to 50 kg (first fecal samples, approximately 5 weeks after supplementing the feed), in feces from animals that were 90 to 100 kg before removal to the slaughterhouse (second fecal samples), in cecal contents at slaughter, and in ileocecal lymph nodes at slaughter. At each sampling event at the farm, approximately 75 fecal samples were collected (95% confidence interval [CI], error 10%, expected prevalence 50%). No samples were collected in isolated pens used to house sick animals. Three fecal samples were collected from three pigs per pen; the same pigs were not necessarily sampled on both sampling occasions. From each animal, a fecal sample was aseptically collected directly from the rectum and stored individually in a plastic bag. All animals in this study were slaughtered in the same slaughterhouse. The samples were collected when the largest number of pigs was slaughtered and not on the large pigs previously removed. In the slaughterhouse, animals from the experimental farm were slaughtered upon arrival, limiting the time between loading at the farm and actual slaughter. At slaughter, 68 animals were sampled (95% CI, error 10%, expected prevalence 50%). For each sampled animal, the tissue including the ileocecal lymph nodes was excised and stored individually. The cecum of the corresponding animal was tied off prior to removal and also stored individually. All samples were processed in the laboratory on the day of collection. In total, 43 pairs of overshoes were collected in the growing unit, 583 fecal samples were collected 5 weeks after the start of the treatment (first fecal sample), 580 fecal samples were collected at the end of the fattening period (second fecal sample), 533 cecal samples were collected at slaughter, and 545 samples of the lymph nodes were collected at slaughter.

Bacteriological analysis. In the laboratory, each fecal sample was thoroughly homogenized using a sterile spatula, and 11 g of feces was added to 99 ml of buffered peptone water (BPW; CM509, Oxoid, Basingstoke, UK). After homogenization with a stomacher, 10 ml of sample was transferred to an empty sterile tube. This 10-ml subsample and the remaining 100 ml, corresponding to 1 and 10 g of feces, respectively, were separately enriched and further processed. This process also was used for the cecal contents collected in the slaughterhouse after the ceca were opened aseptically to collect the cecal digesta. Overshoes were homogenized in 225 ml of BPW. Lymph nodes were aseptically excised from each carcass and stored at −20°C until further processing. At analysis, the lymph nodes were weighed and diluted 10 times in BPW.

After incubation of the preenrichment medium (BPW) at 37°C for 20 h, 100 μl was plated on the center of modified semisolid Rappaport-Vassiliadis agar plates supplemented with novobiocin (CM0910 + SR0 SR0161, Oxoid). After incubation for 24 to 48 h at

TABLE 1. Salmonella *prevalence in feces during the two fattening rounds (*n = *75 per additive and round) and in cecal contents and lymph nodes (*n = *68 per additive and round) at slaughter compared with controls*

	Prevalence (%)[b]											
	Feces at the farm						Slaughterhouse					
	Sample 1			Sample 2			Cecal contents			Ileocecal lymph nodes		
Additive[a]	Mean ± SE (95% CI)		% of control	Mean ± SE (95% CI)[c]		% of control	Mean ± SE (95% CI)		% of control	Mean ± SE (95% CI)		% of control
A1												
Round 1	32 ± 5.5	(22–44)	128	17 ± 4.4†	(10–27)	113	33 ± 5.8	(23–45)	89	26 ± 5.4**	(17–38)	289
Round 2	31 ± 5.4	(21–42)	89	29 ± 5.4	(20–41)	74	79 ± 4.9**	(68–87)	214	69 ± 5.6**	(57–79)	288
Mean ± SE	31 ± 3.9	(24–39)	107	23 ± 3.5	(17–30)	88	58 ± 4.8**	(48–67)	161	47 ± 4.7**	(38–57)	313
A4												
Round 1	36 ± 5.7	(26–48)	144	19 ± 4.7†	(12–30)	127	60 ± 5.9**	(48–71)	162	10 ± 3.7	(5–20)	111
Round 2	51 ± 5.9*	(40–63)	146	35 ± 5.6†	(25–46)	90	51 ± 6.2	(39–63)	138	22 ± 5.0	(14–33)	92
Mean ± SE	44 ± 4.2*	(36–52)	152	27 ± 3.7†	(20–34)	104	56 ± 4.3**	(47–64)	156	15 ± 3.2	(10–23)	100
A5+A6												
Round 1	39 ± 5.7	(28–51)	156	19 ± 4.7†	(12–30)	127	21 ± 4.9*	(12–30)	54	4 ± 2.5	(1–13)	58
Round 2	33 ± 5.6	(23–45)	94	7 ± 3.1**‡	(3–16)	18	26 ± 5.5	(17–38)	70	4 ± 2.5**	(1–13)	17
Mean ± SE	36 ± 4.0	(28–44)	124	13 ± 2.8**‡	(8–20)	50	23 ± 3.7*	(16–31)	64	4 ± 1.8**	(2–10)	33
Total[d]												
Round 1	33 ± 2.7	(28–38)		18 ±2.2‡	(14–22)		38 ± 3.0	(32–43)		12 ± 2.0	(9–17)	
Round 2	37 ± 2.8	(32–43)		28 ± 2.6†	(23–33)		48 ± 3.1	(43–55)		30 ± 2.8	(25–36)	
Mean ± SE	35 ± 2.0	(31–39)		22 ± 1.7†	(19–26)		43 ± 2.1	(39–47)		21 ± 1.8	(18–25)	

[a] Product A1, butyric acid (active ingredient at 1.3 kg/ton of feed); product A4, a combination of short-chain organic acids (mixture of free acids and salts) and natural extracts (2.92 kg/ton); product A5+A6, a 1:1 blend of two commercial products consisting of medium-chain fatty acids, lactic acid, and oregano oil (3.71 kg/ton).

[b] Values are estimated marginal means ± standard error. Asterisks indicate means that are significantly different from those for the control for experimental treatments: * $P < 0.05$, ** $P < 0.001$.

[c] Daggers indicate means that are significantly different from those for the first fecal sample collected: † $P < 0.05$, ‡ $P < 0.001$.

[d] All treatments: control, product A1, product A4, and product A5+A6.

41.5°C, a loopful of culture from the edge of the white migration zone was plated onto xylose lysine deoxycholate agar plates (CM469, Oxoid). Plates were evaluated for typical *Salmonella* colonies after 24 h of incubation at 37°C. From each plate, one suspect *Salmonella* colony was further identified and characterized as described by Rasschaert et al. *(28)*. A multiplex PCR assay was used to confirm the *Salmonella* genus identification and to further identify the *Salmonella* Typhimurium isolates *(1, 19)*. All *Salmonella* isolates belonging to other serotypes were grouped according to their serotype by enterobacterial repetitive intergenic consensus PCR *(27)*. At least two isolates per cluster of identical fingerprints were serotyped at the Belgian *Salmonella* reference laboratory according to the White-Kauffman-LeMinor scheme. An animal was considered to be colonized with *Salmonella* when *Salmonella* was isolated from either 10 g of feces or 1 g of cecal contents.

Molecular typing. A subset of 291 isolates (from feces, cecal digesta, and ileocecal lymph nodes) were further characterized by pulsed-field gel electrophoresis with *Xba*I as the restriction enzyme. The isolates were chosen to represent the six most abundant serotypes, the four compartments, all sample types, and both fattening rounds. The PulseNet protocol *(4)* was followed. Fingerprints with one band of difference were considered different pulsotypes. Each pulsotype was indicated by a number (e.g., 1.1), in which the first number referred to the serotype and the second number referred to the pulsotype within that serotype.

Statistical analysis. Data were analyzed using the SPSS Statistics version 20.0 program for Windows (2010, SPSS Inc., IBM, Armonk, NY). Dichotomous variables (presence or absence of *Salmonella* in feces, cecal digesta, and ileocecal lymph nodes) were analyzed using the generalized linear model procedure with the binary logistic model. The response variable was the dichotomous variable representing the likelihood of *Salmonella* presence. Predictors used were treatment (four), fattening round (two), sampling time (two), and their interactions for fecal samples; and treatment (four), fattening round (two), and their interactions for the samples of cecal digesta and ileocecal lymph nodes. Interaction terms were omitted when not significant ($P > 0.05$). Pig was used as the experimental unit. Data are represented as estimated marginal means ± the standard errors.

RESULTS

Of the 43 pairs of overshoes worn in the growing unit before the piglets were moved to the fattening compartments, all were positive for *Salmonella*. These *Salmonella* isolates were not further typed and were not included in the statistical analysis. In total, 2,241 samples were taken from the fattening pigs (feces, cecal contents, and ileocecal lymph nodes), and 680 (30.3%) of these samples were positive for *Salmonella*. An overview of the percentage of positive samples per treatment, per round, and per sample type is given in Table 1.

More pigs were shedding *Salmonella* in the beginning of the fattening period (mean, 35%) than at the end of the fattening period (mean, 22%). In each round of slaughtered pigs, the number of *Salmonella*-colonized ceca at the slaughterhouse was consistently higher than the corresponding number of *Salmonella*-shedding pigs at slaughter age. On average, 43% of the tested ceca were *Salmonella* positive. However, a large variation was seen, from 21 to 79%. In total, 21% of the lymph nodes were infected with *Salmonella,* but a high level of variation was found between and within the different treatment groups (4 to 69%).

Only supplement A5+A6, the blend of two commercial products that contained MCFAs, lactic acid. and oregano oil, significantly reduced the number of animals shedding *Salmonella* at slaughter age at the farm, the number of animals harboring *Salmonella* in the cecum at slaughter, and the number of animals with *Salmonella*-positive ileocecal lymph nodes at slaughter in comparison to the control group (Table 1). In the beginning of the fattening period, on average 36% of the pigs who received this product were shedding *Salmonella* compared with 30% of the pigs receiving the control diet. At slaughter age, a significantly lower number of the pigs receiving this blend were excreting *Salmonella* (13%, $P < 0.001$) compared with 27% of the control animals. The same observation was made for the results obtained at the slaughterhouse; a significantly lower number of ceca of the A5+A6 group were contaminated with *Salmonella* (23%, $P < 0.05$) compared with the ceca collected from the control animals (36%). The results were even more pronounced for the lymph nodes, 4% of lymph nodes were infected with *Salmonella* compared with 16% of lymph nodes in the control group ($P < 0.001$). Compared with the control animals, *Salmonella* reduction was 50% in the feces at slaughter age on the farm, 36% in cecal contents at slaughter, and 67% in the lymph nodes at slaughter. The other two feed treatments did not reduce the *Salmonella* prevalence and in some cases even increased the prevalence. For example, the number of *Salmonella*-positive ceca of the pigs receiving product A1 (butyric acid, 58%) or product A4 (SCFAs, 56%) was significantly higher than that of the control pigs (36%; $P < 0.001$).

The *Salmonella* serotypes most commonly isolated were Typhimurium (44.1% of the isolates), Derby (19.5%), Mbandaka (17%), Livingstone (8.5%), Agona (4.1%), and Anatum (3.3%). The prevalence of *Salmonella* Typhimurium decreased often during the fattening period, often in favor of *Salmonella* Derby and *Salmonella* Mbandaka (Table 2).

The *Salmonella* isolates of the six most abundant serotypes were highly diverse. In total, 36 different *Salmonella* pulsotypes were obtained (Table 3); 20 for Typhimurium, 4 for Derby, 5 for Mbandaka, 3 for Livingstone, 2 for Agona, and 2 for Anatum. For each serotype, one or two pulsotypes dominated throughout the field study and were found in all four compartments. Thus, no shift in pulsotypes was seen for pigs fed with supplements versus the control diet. For *Salmonella* Typhimurium only, some pulsotypes (6.3, 6.4, 6.6, and 6.7) were observed only in round 2 of treatment with A5+A6 (mainly containing MCFAs).

DISCUSSION

The potential of organic acids in feed or drinking water for reducing certain pathogens such as *Salmonella* in pigs and poultry has been assessed both in vitro and in vivo *(2, 24, 29–31)*. In most cases, the efficacy of these acids has been evaluated in vitro, often with promising results, or in short challenge studies with young animals, often with conflicting results. Long-term field studies are rather scarce *(2)*. Organic acids function both outside the animal and in the animal's gastrointestinal tract. These acids can decontaminate feed or drinking water by lowering the pH and may prevent the animal from becoming infected with *Salmonella* by lowering the pH of the upper gastrointestinal tract or by reducing *Salmonella* colonization in the lower gastrointestinal tract *(2, 31)*. Both SCFAs and MCFAs can have anti-*Salmonella* activity in vitro, but conflicting results have been obtained concerning the differential effect of chain length *(3, 21, 29, 32)*. Because synergistic effects of various acids and other components such as essential oils have been observed in vitro, many commercial products include blends of these chemicals.

Based on the results of a challenge trial in which several organic acids were evaluated in a seeder model *(22),* the aim of the present study was to evaluate the effect of some of these organic acids on the *Salmonella* shedding and colonization degree of pigs in a field study on a *Salmonella*-positive farm. As demonstrated previously, challenge studies and field studies may have conflicting results even when the same organic acid mixture is used *(5, 20)*. The present study is one of the first long-term studies to describe the effect of feed supplemented with organic acids on the reduction of *Salmonella* under field conditions. These kinds of feed additives are already on the market for supporting general gut health or reducing pathogens such as *Salmonella*. Field trials may provide more definitive results; however, several methodological issues arise. When natural infection occurs in a pig, *Salmonella* is excreted at quite low and variable levels over time. This makes detection by direct culture and quantification of the shedding difficult. Therefore, isolation of *Salmonella* from fecal samples is recommended according ISO 6579 Annex D *(17),* which includes two enrichment steps, as performed in the present study. Direct plating is possible only when high numbers of *Salmonella* are expected, as often occurs in challenge studies in which pigs are challenged with high doses, e.g., 9 log CFU in the previous seeder model study *(22)*. The detection limit in that study was 100 CFU/g of feces, which is too high for a field trial with natural *Salmonella* colonization. Instead, we chose to determine the prevalence of *Salmonella*-colonized animals. On each of two occasions, samples were collected from approximately 75 animals. Determination of prevalence also has been used in other intervention studies *(5, 7, 9)*. In our study design, the pig was the experimental unit. Based on the total number of animals in each compartment (350 animals), we calculated that 75 fecal samples per sampling time should be collected (95% CI, error 10%, expected prevalence 50%). Three random fecal

TABLE 2. *Distribution of* Salmonella *serotypes per feed treatment, fattening round, and sample type*

Feed treatment[a]	Round	Sample type	% (no.) of isolates with each serotype								
			Typhimurium	Derby	Mbandaka	Livingstone	Agona	Anatum	Enteritidis	Senftenberg	Unknown
Control	1	Feces, sample 1	61.9 (13)	4.8 (1)	33.3 (7)						
		Feces, sample 2	33.3 (4)	25 (3)	33.3 (4)				8.3 (1)		
		Cecal contents	33.3 (9)	18.5 (5)	48.1 (13)						
		Ileocecal lymph nodes	42.9 (3)		14.3 (1)						42.9 (3)
	2	Feces, sample 1	70.4 (19)		18.5 (5)	7.4 (2)	3.7 (1)				
		Feces, sample 2	38.7 (12)	9.7 (3)	29 (9)	12.9 (4)	9.7 (3)				
		Cecal contents	4.2 (1)	4.2 (1)	41.7 (10)	29.2 (7)	20.8 (5)				
		Ileocecal lymph nodes	25.0 (4)		31.3 (5)	25.0 (4)	12.5 (2)	6.3 (1)			
A1	1	Feces, sample 1	62.5 (15)	4.2 (1)	4.2 (1)			29.2 (7)			
		Feces, sample 2	58.3 (7)		8.3 (1)		16.6 (2)	16.6 (2)			
		Cecal contents	26.1 (6)	21.7 (5)	4.3 (1)	17.4 (4)		30.4 (7)			
		Ileocecal lymph nodes	61.1 (11)			5.6 (1)		11.1 (2)			22.2 (4)
	2	Feces, sample 1	66.7 (16)	4.2 (1)	25 (6)	4.2 (1)					
		Feces, sample 2	28.6 (6)	23.8 (5)	38.1 (8)	9.5 (2)					
		Cecal contents	20.0 (12)	45.0 (27)	11.7 (7)	5.0 (3)	11.7 (7)				6.7 (4)
		Ileocecal lymph nodes	8.5 (4)	68.1 (32)	4.3 (2)	8.5 (4)	6.4 (3)				4.3 (2)
A4	1	Feces, sample 1	78.6 (22)	7.1 (2)	14.2 (4)						
		Feces, sample 2	71.4 (10)	21.4 (3)							7.1 (1)
		Cecal contents	64.3 (27)	4.8 (2)	11.9 (5)	19.0 (8)					
		Ileocecal lymph nodes	71.4 (5)		28.6 (2)						
	2	Feces, sample 1	42.0 (16)	13.2 (5)	15.8 (6)	23.7 (9)		5.3 (2)			
		Feces, sample 2	20.0 (5)	48.0 (12)	8.0(2)	12.0 (3)	12.0 (3)				
		Cecal contents	21.2 (7)	63.6 (21)	9.1 (3)	3.0 (1)				3.0 (1)	
		Ileocecal lymph nodes		46.7 (7)	46.7 (7)	6.7 (1)					
A5+A6	1	Feces, sample 1	75.9 (22)	3.4 (1)	20.7 (6)						
		Feces, sample 2	50 (7)	7.1 (1)	14.2 (2)			7.1 (1)			21.4 (3)
		Cecal contents	35.7 (5)		14.3 (2)	35.7 (5)	14.3 (2)				
		Ileocecal lymph nodes				33.3 (1)	33.3 (1)	33.3 (1)			
	2	Feces, sample 1	100.0 (24)								
		Feces, sample 2	80.0 (4)		20.0 (1)						
		Cecal contents	68.4 (13)								31.6 (6)
		Ileocecal lymph nodes	100.0 (3)								
Total no. of isolates			312	138	120	60	29	23	1	1	23

[a] Product A1, butyric acid (active ingredient at 1.3 kg/ton of feed); product A4, a combination of short-chain organic acids (mixture of free acids and salts) and natural extracts (2.92 kg/ton); product A5+A6, a 1:1 blend of two commercial products consisting of medium-chain fatty acids, lactic acid, and oregano oil (3.71 kg/ton).

samples were collected from each of the 25 pens in each of the four compartments. Pen was not considered the experimental unit. This approach is justified by the fact that piglets from the nursery unit were randomly allocated to the pens in each compartment. Before transfer to the fattening unit, *Salmonella* colonization was confirmed in the nursery unit. The same *Salmonella* serotypes and pulsotypes were found in all finishing units, which indicates common sources already in the farrowing and nursery units. Thus, piglets were considered the main source of *Salmonella* contamination in the fattening unit, and the odds of having a *Salmonella* carrier in each pen within each compartment were considered equal. Therefore, piglet can be considered the experimental unit, although differences in transmission of *Salmonella* within a pen cannot be ruled out.

Cecal and lymph nodes were sampled after slaughter. Several researchers have reported that contamination with *Salmonella* serotypes from the lairage area can be found in the cecum and lymph nodes after only a couple of hours of exposure *(15, 16)*. Pigs in our study may have acquired *Salmonella* during transport and in the lairage. However, in the slaughterhouse care was taken that animals from the experiment were slaughtered immediately upon arrival, limiting the time between loading at the farm and actual slaughter. This protocol resulted in only a small number of *Salmonella* pulsotypes from cecal and lymph node samples that were not also found on the farm. In general, a few dominant pulsotypes were found both on the farm and at slaughter.

The feed additive with butyric acid as the active compound (at 1.30 kg/ton) was chosen for field testing based on the observed *Salmonella* reduction in the ileocecal lymph nodes in the challenge study *(22)*. In the present study, this product had no effect on *Salmonella* contamination of the ileocecal lymph nodes nor on *Salmonella* excretion. In the transmission study of De Ridder et al. *(9)*, feed supplement-

TABLE 3. *Distribution of the pulsotypes per feed treatment, fattening round, sample type, and serotype*[a]

Feed treatment	Round	Sample type	Typhimurium	Mbandaka	Derby	Livingstone	Agona	Anatum
Control	1	Feces, sample 1	6.5, **6.8**, 6.13	**5.1**	**3.2**			
		Feces, sample 2	**6.8**	**5.1**	**3.2**, 3.3	**4.1**		
		Cecal contents	6.2, **6.8**, 6.11, 6.13	**5.1**	**3.2**, 3.4	**4.1**		
		Lymph nodes	6.2, 6.13			**4.1**		
	2	Feces, sample 1	**6.8**, **6.9**, 6.21					
		Feces, sample 2	**6.8**, **6.9**	**5.1**, 5.4	**3.2**	**4.1**, 4.3	**1.1**	
		Cecal contents	**6.8**	**5.1**		**4.1**, 4.2	**1.1**	
		Lymph nodes	6.12, 6.16			**4.1**, 4.2	**1.1**	**2.1**
A1	1	Feces, sample 1	6.1, **6.9**, 6.11, 6.16	5.3	**3.2**			**2.1**
		Feces, sample 2	**6.9**, 6.15	**5.1**		**4.1**	1.2	**2.1**
		Cecal contents	**6.9**		3.1	**4.1**		**2.1**, 2.2
		Lymph nodes	**6.8**, **6.9**		3.3	**4.1**		2.1
	2	Feces, sample 1	**6.8**, **6.9**	**5.1**	**3.2**			
		Feces, sample 2	**6.8**, **6.9**	**5.1**	**3.2**	**4.1**		
		Cecal contents	**6.9**, 6.19		**3.2**, 3.3	**4.1**	**1.1**	
		Lymph nodes	**6.9**, 6.17	**5.1**	**3.2**	**4.1**	**1.1**	
A4	1	Feces, sample 1	**6.8**, **6.9**			**4.1**		
		Feces, sample 2	**6.8**, **6.9**, 6.16		**3.2**			
		Cecal contents	**6.8, 6.9**	**5.1**	**3.2**	**4.1**, 4.2		
		Lymph nodes	**6.8**, **6.9**					
	2	Feces, sample 1	**6.9**	**5.1**, 5.4	3.1	4.2		**2.1**
		Feces, sample 2	**6.9**	**5.1**	3.1	4.2	**1.1**	
		Cecal contents	**6.9**, 6.18	**5.1**	**3.2**	**4.1**		
		Lymph nodes		**5.1**, 5.5	**3.2**	**4.1**		
A5+A6	1	Feces, sample 1	**6.8**, **6.9**, 6.11, 6.16	**5.1**, 5.4	**3.2**	**4.1**		
		Feces, sample 2	6.14, 6.22	**5.1**	**3.2**			**2.2**
		Cecal contents	**6.8**, 6.16	**5.1**, 5.2		**4.1**, 4.3	**1.1**	
		Lymph nodes				**4.1**	**1.1**	**2.1**
	2	Feces, sample 1	**6.9**					
		Feces, sample 2	6.6	**5.1**				
		Cecal contents	6.3, 6.4, 6.6					
		Lymph nodes	6.2, 6.7					
Total no. of pulsotypes			20	5	4	3	2	2

[a] Predominant pulsotypes are indicated in bold.

ed with coated calcium butyrate (ca. 2 kg/ton of feed) significantly reduced the number of pigs excreting *Salmonella* and the number of *Salmonella*-contaminated postmortem samples compared with the control group. The transmission of *Salmonella* between pigs also was lower in the treatment group than in the control group. In the present study, the commercial calcium butyrate product was not coated. Boyen et al. *(3)* found that coated butyric acid decreased the levels of fecal shedding and intestinal colonization in *Salmonella*-infected piglets, whereas uncoated butyric acid had no effect. As recommended previously *(3),* supplemented fatty acids should be coated to be able to reach the lower gastrointestinal tract instead of being metabolized by the microbiota in upper gastrointestinal tract and absorbed by epithelial cells.

Product A4 contained formic acid, acetic acid, propionic acid, and sorbic acid and natural extracts as active compounds, with a total organic acid concentration of 2.92 kg/ton (as measured by Michiels et al. *(22)*). This additive performed the best in the seeder model; it significantly reduced the amount and duration of *Salmonella* shedding after challenge. However, in the present study this product had no impact on *Salmonella* shedding. This result could be strain dependent; in the seeder model study the animals were challenged with only one well-defined *Salmonella* Typhimurium strain (MB2486), whereas in the present field trial each sampling occasion revealed that the pigs were naturally colonized with several serotypes and pulsotypes. Different serotypes and/or strains may differ in their susceptibility to the effects of organic acids. Strains also can become resistant to organic acids over time. For example, on a poultry farm a persistent *Salmonella* Paratyphi B var. Java strain became more resistant to SCFAs during long-term treatment of the drinking water *(26)*. The farm in the present study has a known *Salmonella* history, and certain strains may have been circulating on this farm for a long time. De Busser et al. *(7)* found that acidification of the drinking water with a commercial blend containing formic acid, propionic acid, acetic acid, and sorbic acid at 0.25 to 0.4% on four farms with naturally infected animals had no significant effect on the shedding and prevalence of *Salmonella* in the pigs at slaughter. However, these animals were given this drinking water additive only during the last 2 weeks of the fattening period, which could explain the limited effect. Another explanation for the limited effect could be the low concentration of active ingredients. Creus et al. *(5)* found

that feed containing relatively high concentrations of organic acids (0.8 to 1.2% lactic and formic acid) successfully reduced *Salmonella* prevalence in fattening pigs.

The anti-*Salmonella* activity of MCFAs has been reported in several in vitro trials *(3, 21, 29)*. However, in the challenge seeder model study *(22),* the feed treatments based on MCFAs did not reduce *Salmonella* shedding and colonization. Because other in vivo trials for the reduction of *Salmonella* in pigs are lacking, the MCFA blends were tested in the field but at a higher concentrations in the feed. The two MCFA feed products were mixed 1:1, resulting in a blend with an organic acid dosage of 3.71 kg/ton. This combined feed product significantly reduced *Salmonella* prevalence in finishing pigs; both the *Salmonella* excretion rate at slaughter age on the farm and the *Salmonella* contamination rate at the slaughterhouse were reduced. The higher concentration of active ingredients in this combination supplement compared with other commercial products may explain its effectiveness. During this treatment, *Salmonella* Derby was almost completely absent and *Salmonella* Typhimurium was the dominant serotype, especially during the round 2, and some *Salmonella* Typhimurium pulsotypes were observed only during this treatment. Thus, the *Salmonella* reduction may have been due to the decline in *Salmonella* Derby, but this hypothesis needs further investigation.

Regardless of the effect of the feed treatments, this particular farm had a very high prevalence of *Salmonella* shedding in their pigs. On average, 27% of the control pigs were excreting *Salmonella* at the end of the fattening period. However, this percentage is probably an underestimation of the actual percentage of *Salmonella*-colonized pigs because the majority of pigs shed *Salmonella* intermittently. In a longitudinal study, Pires et al. *(25)* found that nearly half of the examined *Salmonella*-positive pigs were culture positive on only one of eight sampling occasions, and a shedding duration of 2 to 4 weeks has been reported *(18, 25)*. Few other studies have included within-herd prevalence of *Salmonella*-excreting pigs at slaughter age; most often an overall *Salmonella* prevalence among a large number of pigs across several farms is reported. In another Belgian study, within-herd prevalence of *Salmonella* shedding on closed farms was 0% to 6.8% among slaughter age pigs *(28)*. In U.S. studies, within-herd prevalence on *Salmonella*-positive pig farms has been reported as 2 to 84% *(6, 12, 14)*. In most studies, the prevalence of *Salmonella*-excreting pigs decreased toward slaughter age on farrow-to-finish farms *(23, 25, 28)*. As explained by Nollet et al. *(23)* and Pires et al. *(25),* pigs may become infected with *Salmonella* in the farrowing unit and may start shedding in the farrowing unit or after transport to other units. This shedding scenario may also have occurred on the farm in the present study as suggested by the fact that the same serotypes and pulsotypes were found in all finishing units, which indicates common sources in the farrowing and nursery unit or the outside environment rather than in all finishing units separately.

In conclusion, only one feed product with an elevated concentration of MCFAs (3.71 kg/ton) had a significant influence on *Salmonella* shedding in slaughter age pigs on the farm and on the *Salmonella* prevalence in ceca and lymph nodes in pigs at slaughter. Although this finding is promising, further investigation is needed, such as the cost-efficiency of this combined feed product and the effect on the animals (e.g., feed conversion). The studied farm had a very high *Salmonella* prevalence and a high number of serotypes and pulsotypes. This type of study should be repeated on other farms with lower *Salmonella* prevalence to determine whether the other feed treatments could affect the *Salmonella* prevalence under these conditions.

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