Changing the presentation of pigs feed: a cost effective solution to reduce *Salmonella* spp. excretion

Philippe LEBEL[1], Philippe FRAVALO[1], Jessie LONGPRE[1], Benoît LAPLANTE[2], Daniel MASSE[3] and Ann LETELLIER[1]

[1]Chaire de Recherche en Salubrité des Viandes, Faculté de Médecine Vétérinaire, Université de Montréal, ST-HYACINTHE, Qc, Canada;

[2]F. Ménard, L'ANGE-GARDIEN, Qc, Canada;

[3]Agriculture Canada, SHERBROOKE, Qc, Canada

ABSTRACT

If some studies have attempted to mitigate the *Salmonella* spp. excretion in pigs by feed related interventions, none clearly demonstrated the impact of the presentation (mash or pellet and particular size). Thus this study aimed to determine if the modification of the pigs feed presentation alone can lower the *Salmonella* spp. excretion. To do so, 144 eight week aged piglets, previously confirmed as homogeneously in contact with *Salmonella*, were given diets that varied only by the particle size (500, 750 or 1250µm) and the texture (mash or pellet). They were individually sampled on day 0, 21 and 88 after feed transition for weight, blood and feces collection. After only 21 days, significantly less pigs excreted *Salmonella* spp. in the 500µm mash (1/24), the 1250µm pelleted (5/24), and 1250µm mash (5/24) ($P<0.05$) than in the 500µm pelleted (15/24) reference group. These differences should be consider in the perspective of a greater production ($P<0.05$) of butyric and propionic acids measured in colon contents from the 1250µm mash group (respectively 2179 and 3050mg/L) than in the 500µm pelleted group (respectively 1260 and 2493mg/L) while other short-chain fatty-acids concentration (acetic acid) didn't vary. Further analyses, qPCR tests, will allow determining any changes in the intestinal *Lactobacilli*, *E. coli* and *Bifidobacterium* populations. Finally, it should be noticed that for the two groups with significantly lower *Salmonella* spp. excretion (500µm mash and 1250µm pelleted) the feed conversion was equivalent to the 500µm pelleted group, most commonly used in the industry. In conclusion, this study is the first one to demonstrate and suggest how to mitigate *Salmonella* spp. excretion by only modifying the texture or the feed particle size without raising the production cost in swine.

INTRODUCTION

Modifying the pigs feed to mitigate the presence of *Salmonella* spp. in herds at the farm level while reducing the use of antibiotics is gaining in popularity over the years. Many solutions have been explored but none resulted in the elimination of the pathogen. These strategies are associated with beneficial modifications of the gut microbiota and its production of antibacterial element such has volatile fatty acids (Mountzouris, 2006). One promising strategy is the use of mash feed instead of the common pelleted feed (Lo Fo Wong, 2004). In her review comparing feed management practices and feed characteristics associated with *Salmonella* prevalence, O'connor *et al*. (2008) came to similar conclusions based on serological data. Unfortunately, none demonstrated a *Salmonella* spp. excretion reduction and many variables were present between the compared diets (composition, particle size, texture, heating, etc.). It is also important to notice that while promising on the *Salmonella* basis the use of mash feed often comes with a raise in the production cost. The goal of this study was to isolate the different factors possibly involved in a reduction of *Salmonella spp.* excretion on the farm when only the feed presentation is modified (mash/pellet/particle size).

MATERIAL AND METHODS

On farm

Nine hundred 5 weeks old piglets known to have been in contact with *Salmonella* spp. at the nursery were split (10 per pen) into 6 groups. Each group received a different diets varying only by their particle size (500, 750 or 1250 µm) or texture (mash or pellet). Pellet 500 being the reference group from the industry. Out of these, 144 pigs (24 from each diet, 2 per pen) were weighted on days: 0, 21, 46, 86. Individual blood and feces sample were taken as well as gut content at

slaughter. An aliquot of the feces was put into liquid nitrogen and later kept at -80° C for molecular biology analysis.

Blood analysis

Salmonella spp. seroconversion was followed by a commercial ELISA kit (Maxivet, St-Hyacinthe, Quebec, Canada) developed to detect serological response to more than 95% of *Salmonella* serotypes commonly found in Canada (Letellier, A, 2009). Seras were analysed by the diagnostic service of the Faculté de Médecine Vétérinaire of the Montreal University.

Salmonella spp. detection

We used a modified version of the ISO 6579 annexe D for the detection of *Salmonella* spp. in feces and environment (BPW 18-24h, MRSV 48h, isolation on BGS and XLD followed by biochemical and sero-agglutination confirmation).

Real-time PCR

DNA extraction (mechanical lysis followed by a phenol-chloroform extraction) was performed on all samples. Real-time PCRs, using the parameters shown in table 1, were performed on the samples of interest using an Eco® Illumina® real-time PCR with EvaGreen® qPCR mix as recommended by the manufacturer. For lactobacilli and enterobacteria, 15 ng of DNA and a final primer concentration 1 µM was used. For the *Bifidobacterium* genus, the amplifications reactions consisted of 10 ng of DNA with a final primer concentration of 0.25 µM. The standard curves for each reaction were obtained by diluting the precipitate of PCR product realized in the same conditions with the following reference strains *L. acidophilus* ATCC314, *E. coli* 25922 and *B. Longum* ATCC 15707. Results were expressed as log of copies/10 ng of DNA used.

Volatile fatty-acids (VFA)

At slaughter, ileal, caecal and colon contents from 165 pigs were sampled and stored at -20ºC for analysis of VFA. Samples were diluted with distilled water and centrifuge at 18000 rpm for 30 minutes. Supernatants were retained and Sulfuric acid (0.5M) was added. Samples were centrifuged at 13000 rpm for 15 minutes and the supernatants were used for further analysis. Internal standard (2-ethylbutyric) and resin were added. Samples were filtered and the vials were kept at 4ºC until they were analysed with the chromatograph.

Statistical analysis

All non-parametric data were tested with a logistic regression and parametric data by Student's T test. Statistical analyses were made with SAS 9.2.

RESULTS

Salmonella spp. excretion

As shown in tables 2 and 3, at day 0 there was no difference of excretion in between the groups. After only 21 days of specific diets, three groups (pellet 1250 µm, mash 500 µm and mash 1250 µm) showed significantly less *Salmonella* spp. excretion ($P<0.05$) than the reference group (pellet 500 µm) (Table 2).

Table 1. Real-time PCR parameters.

Group	Primers	Lenght	Cycles
Lactobacilli (Castillo, 2006)	LB-F : GCAGCAGTAGGGAATCTTCCA (Tm=57 °C) LB-R : GCATTYCACCGCTACACATG (Tm=57 °C)	≅200 pb	Init. Den : 10m@95°C 40 cycles: 15s@95° C 1m@60° C
Enterobacteria (Castillo, 2006)	Ent-F : ATGGCTGTCGTCAGCTCGT (Tm=60 °C) Ent-R : CCTACTTCTTTTGCAACCCACTC (Tm=60 °C)	364 pb	Init. Den. : 10m@95°C 40 cycles : 15s@95° C 1m@60° C
Bifidobacterium spp. (Matsuki, 2004)	Bif-F : CTCCTGGAAACGGGTGG (Tm=53 °C) Bif-R : GGTGTTCTTCCCGATATCTACA (Tm=53 °C)	550 pb	Init.: 5m@94°C 40 cycles : 20s@94°C 20s@55°C 50s @72°C

When comparing all the mash feed groups to the pellet feed groups (regardless of their particle size), the mash feed groups had significantly less *Salmonella* spp. excretion on three different dates: day 21 (P = 0.012), 88 (P = 0.002) and in the colon content at slaughter (P = 0.026) (Table 3).

Table 2. Number of pigs per group excreting Salmonella spp. on days 0 and 21. Legend: Data with (a) notice meaning: significantly different (P < 0.05) than data with (b) notice.

Diets	Day 0 (n = 24)	Day 21 (n = 24)
500 pellet	13	15 [(a)]
750 pellet	11	10
1250 pellet	12	5 [(b)]
500 mash	10	1 [(b)]
750 mash	10	10
1250 mash	11	5 [(b)]

Table 3. Total of pigs in the mash or pellet groups excreting Salmonella spp. on days 0, 21, 46, 88 and positive colon content at slaughter. Legend: Data with (a), (b) or (c) notice are different from each other.

Day	Mash (n = 72)	Pellet (n = 72)
0	31	36
21	16[(a)]	30[(a)]
46	9	14
88	5[(b)]	19[(b)]
Colon content	10[(c)]	21[(c)]

Seroconversion

Salmonella spp. seropositive pigs were only detected after 88 days of specific diet. No statistical difference was observed between the groups (total average of 25.7 % positive).

Table 4. Feed conversion at the end of the fattening period. Legend: Ratio of kg feed/kg weight gain. Ratio for pellet 500 µm (a) (reference group) is only different from the mash 1250 µm (b) ratio.

	Pellet	Mash
500	3.04 [(a)]	3.16
750	2.95	3.4
1250	2.99	3.45 [(b)]

Feed conversion

Feed conversion was not statistically different between pellet 500 (reference commercial feed) and mash 500. Only mash 1250 was significantly higher in comparison to reference group (Table 4).

Volatile fatty acids

Propionic acids concentration was significantly higher (P = 0.0012) in the colon content of pigs from the mash feed group (2750.62) in comparison to the pellet group (2389.91). The same observation were made for butyric acid (1823.38 vs. 1537.47, P = 0.0008). Moreover, butyric acid was present in significantly higher concentration in the mash 1250 (2179.07) than in any other group (P < 0.05).

Real-time PCR

The lactobacilli and enterobacteria groups and their ratio (lactobacilli/enterobacteria) were similar from group to group. On the other side, more *Bifidobacterium* spp. were detected in the mash feed groups compared to the pellet feed groups (all particle size together) and higher values in the mash 1250 µm compared to the 3 other groups tested on day 21 (P<0.05) were noted.

Table 5. Log of copies of Bifidobacterium spp. 16S RNA gene/10 ng of DNA from pig feces on day 0 and 21. Legend: Data with (a) or (c) noticed are significantly higher than data with respectively (b) or (d) notice.

Diet	Day 0	Day 21
Pellet 500	3.52	$3.25^{(b)}$
Pellet 1250	3.62	$3.32^{(b)}$
Mash 500	3.40	$3.39^{(b)}$
Mash 1250	3.52	$4.23^{(a)}$
Total pellet	3.57	$3.29^{(d)}$
Total mash	3.46	$3.81^{(c)}$

DISCUSSION

Salmonella positive pigs

Many published article related that the use of mash feed instead of pellet feed reduces the number of seropositive pigs in a herd (Lo Fo Wong, 2004; Farzan, 2009). In contrast, no difference in seroconversion was observed between the groups in our study. On the other hand it is to our knowledge the first study to demonstrate the reduction of the number of pigs excreting *Salmonella* spp. by only modifying the feed presentation. Our results indicate for the first time that a simple modification of the particle size or the texture can reduce pigs *Salmonella* spp. excretion. In addition, since the only diet to have a significantly higher conversion ratio than the reference group (pellet 500 µm) is the mash 1250 group, it would be possible to only vary one parameter such as particle size or texture and have a *Salmonella* spp. benefit while being costly effective.

Volatile fatty acids

At slaughter, the reduction of the presence of *Salmonella* spp. was observed where an increase of propionic and butyric acids concentration in the colon was also seen. These Short Chain Fatty Acids (SCFA) are known to down-regulate the expression of SPI1 a pathogenicity island necessary to cell invasion (Van Immerseel, 2006). Similar observation and hypotheses have been made with bigger particle size feed and coliform count in other studies (Mikkelsen, 2004).

Microbiota

Bifidobacterium spp. being producers of butyric acid, a link can be made between its increases in the same groups where higher butyric and propionic concentrations were found (Mountzouris, 2006).

Conclusion

To this date our data have provided us with a cost effective solution (mash 500 µm) to cure *Salmonella* spp. from the farm level of the pig production. Moreover, the data obtained from the mash 1250 µm and the mash vs. pellet regardless of the particle size have given us good clues on the mechanism by which a simple presentation change in the pigs feed can reduce *Salmonella* spp. excretion in the herd. Further analysis of the microbiota at different time and with different targets bacteria will give us an extended look to the real impact of the feed presentation on the pigs gut health and its beneficial impact on the reduction of *Salmonella* spp.

ACKNOWLEDGEMENT

Thanks to F. Ménard for the experimental design and help on the farm (special thank to Guy Maynard and Martine Messier for their help). Thanks to all the students on the CRSV team for the help on the farm and in the laboratories.

REFERENCES

1. Castillo, M. 2006. *Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR.* Veterinary Microbiology. Vol. 114. 165-170.

2. Farzan, A., Friendship, R. M., Dewey, C. E., Warriner, K., Poppe, C., Funk, J. 2009. *Evaluation of the Risk Factors for Shedding Salmonella with or without Antimicrobial Resistance in Swine Using Multinomial Regression Method*. Zoonose and Public Health. Vol. 57. 85-93.

3. Letellier, A., Beauchamp, G., Guévremont, E. D'Allaire, S. Quessy, S. *Risk Factors at Slaughter Associated with Presence of Salmonella on Hog Carcasses in Canada.* Journal of Food Protection. Vol. 72. No. 11. 2326-2331.

4. Lo Fo Wong, D. M. A., Dahl, J., Stege, H., van der Wolf, P. J., Leontides, L., von Altorock, A., Thorberg, B. M. 2004. *Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds*. Preventive Veterinary Medicine. Vol. 62. 253-266

5. Matsuki, T. Et al. 2004. *Quantitative PCR 16s rRNA-Gene-Targeted Species-Specific Primers for Analysis of Human Intestinal Bifidobacteria.* Applied and Environmental Microbiology. Vol. 70. No. 1. 167-173

6. Mikkelsen, L. L., Naughton, P. J., Hedemann, M. S., Jensen, B. B. 2004. *Effects of Physical Properties of Feed on Microbial Ecology, and Survival of Salmonella enterica Serovar Typhimurium in Pig* Gastrointestinal Tract. Applied and Environmental Microbiology. Vol. 70. No. 6. 3485-3492.

7. Mountzouris, K. C., Balaskas, C. Fava, F., Tuohy, K. M., Gibson, G. R., Fegeros, K. 2006. *Profiling of composition and metabolic activities of the colonic microflora of growing pigs fed diets supplemented with prebiotic oligosaccharides*. Anaerobe. Vol. 12. 178-185.

8. O'Connor, A.M. et al. 2008. *Feeding management practices and feed characteristics associated with Salmonella prevalence in live and slaughtered market-weight finisher swine: A systematic review and summation of evidence from 1950 to 2005*. Preventive Veterinary Medicine. Vol. 87. 213-228.

9. Van Immerseel, F., Russel, J. B., Flythe, M. D., Gantois, I., Timbermont, L., Pasmans, F., Haesebrouck, F., Ducatelle, R. 2006. *The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy*. Avian Pathology. Vol. 35. No. 3. 182-188.