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Comparative *in vitro* study on survival of *Salmonella* Derby and *Streptococcus suis* serotypes 2 and 9 in compound feed and stomach contents of pigs

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Introduction

For some *Salmonella* serotypes – using oral pathways for infection – a protective influence of a coarse feed structure has been described[1,2]. Resulting low pH-values in stomach content as well as reduced invasion ability of *Salmonella* in the hind gut are possible explanations for these findings[2,3].
Although *Sc. suis* is thought to mainly use the upper respiratory tract as port of entry, one recent study[4] provides some evidence for an intestinal translocation. Therefore, the aim of this study was to investigate the influence of feed structure on the stomach barrier and whether *Sc. suis* has a comparable ability like *S.* Derby to pass it.

Material and methods

Survival in feed

Feed: conventional dry starter diet, based on wheat, barley, soybean meal, no antimicrobial additives

Experimental design:

Inoculation of 10 g feed with 1 ml PBS-bacteria suspension
(*S.* Derby: 1.88*10^8; *Sc. suis* serotype (s.t.) 2: 7.53*10^9; *Sc. suis* st. 9: 6.75*10^9 CFU/ml)
Incubation at room temperature, sampling at 3, 30, 60, 120 and 240 min post inoculation (p. i.) (see microbiological methods)

Survival in stomach content

Animals: weaned male piglets (n=5 per feed), BW at dissection 33,3±6,0 kg
Feed: conventional dry starter diet, fine pellet, coarse meal (FP vs. CM; see Tab.1)
Specimen: stomach content of the gastric fundus region, taken 6 h postprandial

Experimental design:

Inoculation of 10 g stomach content with 1 ml PBS-bacteria suspension
(*S.* Derby: 2.50*10^8, *Sc. suis* serotype 2: 1.82*10^8; *Sc.suis* Serotype 9: 1,70*10^8 KBE/ml)
Incubation at 37 °C in an shaking water bath, subsamples for each sampling in plastic bags sealed airtight, Sampling at 3, 60, 120 and 240 min p. i.

Table 1: Particle size distribution of compound feed for weaned piglets

diet	FP fine, pelleted	CM coarse, meal
> 1 mm (%)	5.01	52.71
< 0.2 mm (%)	54.18	25.83
GMD (µm)[5]	217	671

Physicochemical parameters:

pH in the stomach content of the fundus region was measured at every sampling

Microbiological methods:

Homogenization of samples, plating of 10-fold dilution series in PBS on specific culture media (*S.*Derby: Brilliance^{TM}Salmonella, BPLS; *Sc. suis*: StaphStrep Selective Supplement, Columbia Blood Agar with 6 % sheep blood, confirmation of *Sc. suis* identity by MP-PCR[6]

Calculation of Survival Rate (SR) = $\frac{\text{specific bacterial content [CFU/g] at timepoint}}{\text{dose of inoculation [CFU]}}$

Statistics:

Statistical analysis was performed by SAS 9.3 for Windows using PROC MEANS for differences between timepoints and student's T-Test/PROC GLM for those between groups or bacteria. Values are expressed as mean ± standard deviation. Differences were stated significant when p < 0,05.

Results and discussion

Regarding the survival in feed, a reduction of all bacteria occurred after 240 min, which was significant for both *Sc. suis* strains but not for *S.* Derby, which even showed growth at 30, 60 and 120 min p. i.. Counts of bacteria did not differ 3 min p. i.. Afterwards, *S.* Derby was detected in significantly higher numbers than *Sc. suis* at all points of time (see table 2).

Table 2: Survival rate of *S.* Derby, *Sc. suis* st. 2 and 9 in compound feed over 240 min

Time p. i.	*S.* Derby	*Sc. suis* st. 2	*Sc. suis* st. 9
3 min	0.875^{Aa} ± 0.166	0.797^{Aa} ± 0.176	0.978^{Aa} ± 0.035
30 min	1.08^{Aa} ± 0.278	0.593^{Bb} ± 0.217	0.482^{Bb} ± 0.102
60 min	1.24^{Aa} ± 0.295	0.263^{Cb} ± 0.020	0.213^{Cb} ± 0.079
120 min	2.03^{Aa} ± 1.81	0.257^{BCb} ± 0.058	0.125^{CDc} ± 0.053
240 min	0.889^{Aa} ± 0.187	0.133^{BCb} ± 0.095	0.079^{Db} ± 0.072

^{a,b} within a row, means without a common superscript differ ($P < 0.05$)
^{A,B} within a column, means without a common superscript differ ($P < 0.05$)

Values of pH in the stomach content differed significantly between the two feeding groups (FP 5,09 ± 0,464 vs. CM 2,47 ± 0,499; p<0,05). Values within one group were consistent over 240 min (see figure 1).

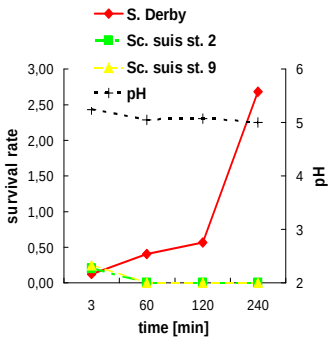

Figure 1: Survival rate of *S.* Derby, *Sc. suis* st. 2 and 9 in stomach content, (diet FP), over 240 min; pH of digesta

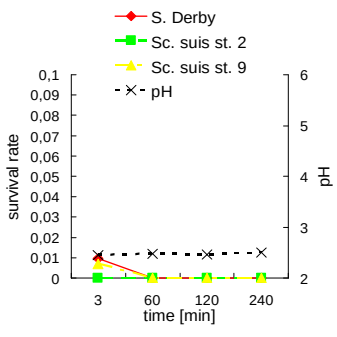

Figure 2: Survival rate of *S.* Derby, *Sc. suis* st. 2 and 9 in stomach content, (diet CM), over 240 min; pH of digesta

Effects of different diets on survival of microbiota were obvious for all strains used (see fig. 2 and tab. 3).
None of the *Sc. suis* strains could be detected for longer than 3 min in stomach content CM. Reduction of these strains was significantly less after 3 min in stomach content FP, but after 60 min significant decrease of both strains occured also under these conditions. None of the *Sc. suis* strains was detectable for longer than 60 min.

Table 3: Survival rate of *S.* Derby, *Sc. suis* st. 2 and 9 in stomach content (diet: fine pellet, FP vs. coarse meal , CM) over 240 min

Feed	Bacteria	3 min p. i.	60 min p. i.	120 min p. i.	240 min p. i.
FP	*S.* Derby	0.128^{Aa} ± 0.049	0.403^{Ba} ± 0.135	0.569^{Ca} ± 0.191	2.68^{Da} ± 1.32
	Sc. suis st. 2	0.199^{Aa} ± 0.192	0.000^{Bb} ± 0.000	0.000^{Bb} ± 0.000	0.000^{Bb} ± 0.000
	Sc. suis st. 9	0.252^{Aa} ± 0.267	0.005^{Bb} ± 0.007	0.000^{Cb} ± 0.000	0.000^{Cb} ± 0.000
CM	*S.* Derby	0.010^{Aa} ± 0.016	0.000^{Ba} ± 0.000	0.000^{Ba} ± 0.000	0.000^{Ba} ± 0.000
	Sc. suis st. 2	0.000^{Ab} ± 0.000	0.000^{Ba} ± 0.000	0.000^{Ba} ± 0.000	0.000^{Ba} ± 0.000
	Sc. suis st. 9	0.007^{Aab} ± 0.015	0.000^{Ba} ± 0.000	0.000^{Ba} ± 0.000	0.000^{Ba} ± 0.000

^{A,B} within a row, means without a common superscript differ within one feed ($P < 0.05$)
^{A,b} within a column, means without a common superscript differ within one feed ($P < 0.05$)

In contrast to this, initially reduced counts of *S.* Derby were followed by a significant increase in stomach content FP over 240 min. *Salmonella*-counts in this environment were always significantly higher than those of the *Sc. suis* strains used.
Importantly, regarding stomach content CM, counts of *S.* Derby were significantly lower than in the other feeding group. Detection of bacteria was possible for no longer than 3 min.
While *Sc. suis* could not be detected for longer than 60 min, regardless of the feed structure, *S.* Derby was only eliminated in stomach content of the feeding group CM, but not in FP. Regarding these results, a stomach passage of *Sc. suis in vivo* seems at least questionable, although the mechanisms underlying the bacterial reduction remain unclear and a difference between detectable and real bacterial count ("viable but not culturable") cannot be excluded. The acidic conditions in digesta of the gastric fundus region following consumption of a coarse meal are probably due to the higher DM-content and a resulting pH-gradient[7]. A protective influence of a improved stomach barrier (characterized by a low pH) could be assumed also for other bacteria using an oral route of invasion.

Conclusion

In conclusion, *Sc. suis* st. 2 and 9 were reduced faster than *S.* Derby in compound feed.
For the first time an efficient killing of *Sc. suis* serotypes 2 and 9 in different stomach contents was shown *ex vivo*. In contrast, numbers of *S.* Derby decreased substantially only at the quite low pH in the stomach content after feeding a coarse meal diet. Feeding a coarse meal resulted in lower pH-values of the gastric fundus region than feeding of a fine pellet, which could be taken as a sign of improved gastric barrier function.

Literature: [1]VISSCHER, C.F. et al. (2009): J. Anim. Physiol. Anim. Nutr. 93, 423-430; [2]MIKKELSEN, L.L. et al. (2004): Appl. Environ. Microbiol., 70, 3485-3492; [3]GANTOIS, I. et al. (2006): Appl. Environm. Microbiol. 72, 946-949; [4]SWILDENS, B. (2004): Vet. Microbiol. 103, 29-33; [5]WOLF, P. et al. (2012):Übers. Tierernähr. 40, 21-64; [6]SILVA, L.M.G. et al. (2006) Vet. Microbiol., 115, 117-127; [7]KÖTTENDORF et al. (2008): Proc. Soc. Nutr. Physiol. 17, 110

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