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Sex effects on porcine microbiota impact on alternative treatments for post weaning diarrhoea

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This pilot study investigated the role of ear biting behaviour performed by piglets in the week prior to weaning and other early life characteristics in the development of ear necrosis (EN) post-weaning.

Materials and methods

A total of 138 piglets born to 11 sows were housed in conventional farrowing crates with plastic slatted floors, one solid plastic heating mat, and one plastic toy attached to the wall. At weaning (28 d of age), they were mixed into 13 same sex pens of 9–10 pigs with single space feeders, plastic slatted floors, and two rubber floor toys. At three weeks of age, pigs were individually marked on their backs with hair dye to facilitate individual behaviour observations. All occurrences of ear biting behaviour were recorded by direct observation with continuous sampling (3-minute sessions; n = 24 minutes total/litter) over three days in the week prior to weaning. The identity of the bitten pig was recorded, and the average number of bites per observation session was calculated for each pig. Pigs were individually inspected for EN weekly from weeks 2 to 6 post-weaning (until transfer to finisher accommodation), and scored 0 to 5 according to severity. Scores were collapsed into 1] presence or absence of EN; 2] the maximum score the pig achieved during this time (MaxScore). Data were analysed using SASv9.4 (PROC Glimmix and Spearman correlations) to investigate associations between pre-weaning ear biting behaviour, birthweight, and litter characteristics (total piglets born, litter size at d1, mortality rate from d1 to day of behaviour observation) and EN.

Results

While 58.7% of pigs developed EN post-weaning, the majority received mild scores (Table 1) and no pigs received a score of 5. The average number of ear bites per 3 min observation session was 0.36 bites/litter. This equates to an average of 1.5 ear bites per minute occurring in a single litter when piglets are active in the week prior to weaning. There was no apparent association between ear biting and EN ($P > 0.05$). While total born ($P > 0.05$) and litter size at d1 ($P > 0.05$) were not associated with EN (data not shown), mortality rate had a moderate positive association with both EN of any severity ($r_{(136)} = 0.21$, $P = 0.01$), and EN MaxScore ($r_{(136)} = 0.21$, $P = 0.02$). Additionally, pigs that developed EN of any severity had higher birthweights than those that did not develop EN (1.57 ± 0.312 vs. 1.44 ± 0.293 kg, $P = 0.03$).

Table 1
Descriptive data (mean ± SE) of ear biting behaviour pre-weaning and early life characteristics and ear necrosis (EN) development post weaning.

	Maximum EN score				
Variable	0	1	2	3	4
Pigs (no., %)	57 (41.30)	43 (31.16)	15 (10.87)	18 (13.04)	5 (3.62)
Ear bites/3min obs (no.)	0.39 ± 0.39	0.32 ± 0.42	0.37 ± 0.30	0.34 ± 0.29	0.50 ± 0.16
Birthweight, kg	1.44 ± 0.29	1.54 ± 0.29	1.58 ± 0.35	1.62 ± 0.29	1.54 ± 0.39
Litter mortality, %	11.50 ± 10.11	15.66 ± 10.04	15.97 ± 10.10	16.31 ± 9.77	18.39 ± 8.46

Conclusion

The lack of an association between ear biting behaviour and EN could relate to the small number of pigs in the study and to the limited number that developed severe EN post-weaning. It may also be that the numerous stressors imposed on the pig at weaning outweighed any effect of behaviour recorded pre-weaning. Five pigs with an EN score of 4 received on average the numerical highest number of ear bites pre-weaning. This suggests the need for further research with more animals before rejecting the hypothesised link between ear biting behaviour and EN. The link between EN and higher birthweights supports on-farm findings where pigs with higher weaning weights had an increased risk of EN (Busch et al., 2008). Likewise, Diana et al. (2019) showed that the fastest growing pigs were more likely to develop EN. This suggests the possibility of an imbalance in the biological requirements of fast growing animals (e.g. nutritional, space etc.). Our preliminary findings indicate that further research on early life characteristics would help us better understand the role of internal, systemic factors in EN development.

Acknowledgements

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References

Busch, M.E., Dedeurwaerdere, A., and Wachmann, H. 2008. The development and the consequences of ear necrosis in one herd. 20th International Pig Veterinary Society Congress, 278.

Diana, A., Boyle, L.A., Manzanilla, E.G., Leonard, F.C., Calderon Diaz, J.A., 2019. Ear, tail and skin lesions vary according to different production flows in a farrow-to-finish pig farm. Porcine Health Management 5 (19).

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99. Sex effects on porcine microbiota impact on alternative treatments for post weaning diarrhoea

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Application

Differential microbiota composition between males and females may indicate that sex-specific therapeutical intervention approaches could improve their efficacy.

Introduction

We previously described that the broad-spectrum antimicrobial-alternative peracetic acid (PAA) derived by precursor hydrolysis was able to decrease diarrhoetic symptoms in piglets (Galgano et al., 2023), in similar fashion as zinc oxide (ZnO). Here, we further explored possible interactions between these interventions and piglet sex.

Materials and methods

Four treatments (control, 3100 ppm in-feed ZnO, 50 ppm and 150 ppm of in-water PAA), were administered to 6 pens/treatment (14-day trial) with 2 pigs per pen, 6 rooms (4 pens/room) and balanced for sex. In-water PAA treatments were prepared daily from mixing different ratios of PAA precursors. We report here data on stomach and caecal microbial composition via 16S rRNA sequencing (515Fb-816Rb). Linear mixed model was carried out in R via using the package lme4 for performance and MaAsLin2 for taxonomical data, including fixed (treatment and sex) and random effects (rooms/pens/pigs).

Results

The taxonomical analysis of the composing genera through the gut locations analysed revealed both compositional differences between sexes and in response to treatments in males and females. In the stomach, the relative abundance of both *Fibrobacter* and *Sharpea* variated significantly when analysing the microbiota of males and females ($P < 0.05$, $Q < 0.05$). Moreover, 97 genera were significantly differentially abundant as a consequence of treatment administration in either males or females. Of these, *Actinobacillus* was found to be significantly less abundant ($P < 0.05$, $Q < 0.05$) in the males given 150 ppm of PAA (0.1% ± 0.1%) compared to all other treatment-sex combinations (0.8% ± 0.6%).
In the caecum, the relative abundance of 27 genera was significantly different when comparing the microbiota of males and females in general ($P < 0.05$, $Q < 0.05$), amongst which *Lactobacillus* was significantly enriched in females (23.57 % ± 8.53 %) compared to males (14.21 % ± 8.63 %). In parallel, 135 genera were significantly differentially abundant ($P < 0.05$, $Q < 0.05$) in the interaction of treatment and sex. Amongst these genera, *Ruminococcus* was less abundant in males given ZnO (0.09% ± 0.07%) compared to all other treatment-sex combinations, and in the caecal content of females administered ZnO (0.42% ± 0.42%) compared to control females (1.65% ± 1.04%) (Fig. 1).

Conclusions

Treatments targeting the modulation of gut microorganisms could achieve different effects in males and females, likely due to differences in microbiota composition between the two sexes. Amongst others, we found that 150 ppm of PAA resulted in a reduction of *Actinobacillus* in the

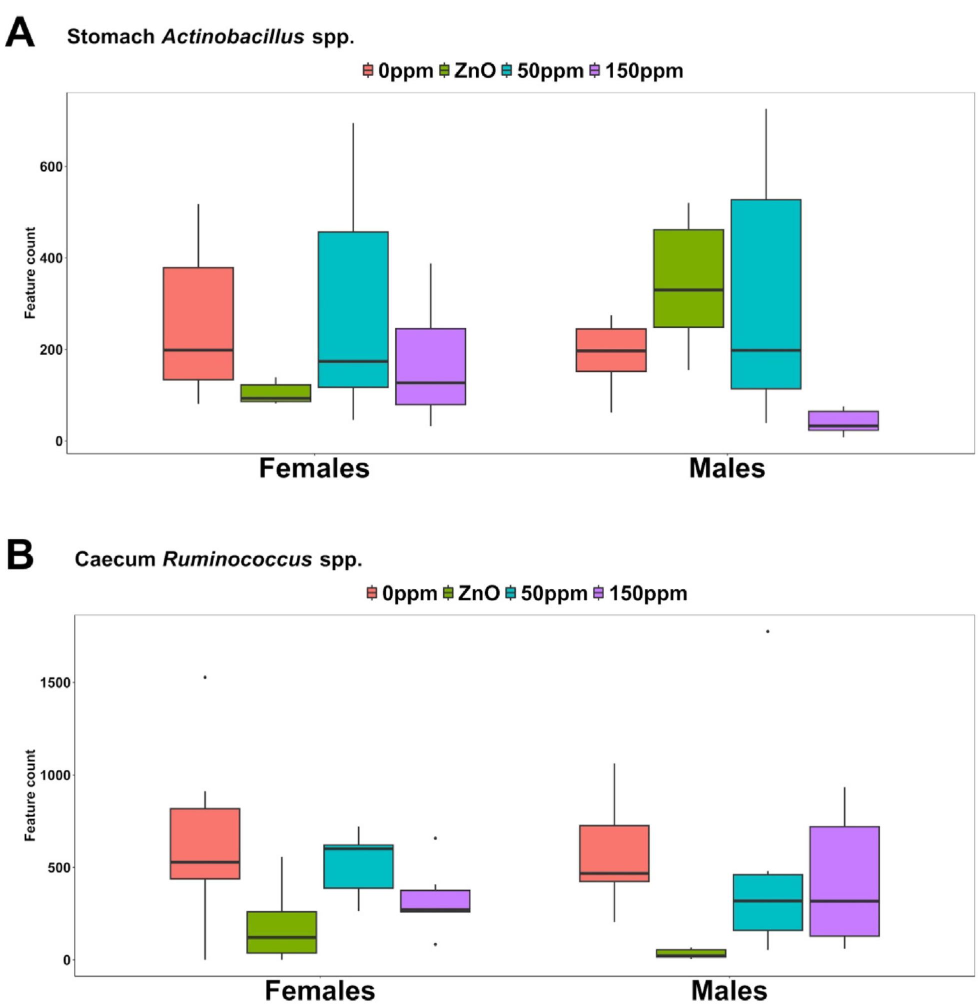

Fig. 1. *Feature count of Actinobacillus* spp. In the stomach (A) and *Ruminococcus* spp. In the caecum in males and females through the different treatments.

stomach of male pigs. Interestingly this genus had previously been found in association to infection in porcine epidemic diarrhoea (Tan et al., 2020), likely pointing towards a beneficial effect of this PAA level of inclusion in males. In parallel, we found that *Lactobacillus*, whose enrichment in the lower gut is usually associated with healthier pigs was more abundant in females than males in general, whilst probiotic genera, such *Ruminococcus* (Sun et al., 2019) were reduced in both females and males given ZnO. Our findings could thus indicate that interventions based on the modulation of the gut microbiota may benefit from being sex-tailored in order to enhance their therapeutic effect.

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References
Galgano, C.L., Fellows, A., Houdijk, J.G.M., 2023. Animal – Sci. Proc. 14, 2, 361–362.
Sun, J., Du, L., Li, X., Zhong, H., Ding, Y., Liu, Z., Ge, L., 2019. Sci. Rep. 9, 18675.
Tan, Z., Dong, W., Ding, Y., Ding, X., Zhang, Q., Jiang, L., 2020. Genes (Basel) 11 (1), 44.

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100. Does removing Zinc Oxide affect post-weaning blood chemistry?

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Application
This study shows that removing Zinc Oxide (ZnO) from post-weaning diets does not negatively affect C-reactive protein (CRP), Haptoglobin (Hp), Tacrolimus (TAC) or Cortisol levels in the blood.

Introduction
There is an urgent need to seek alternative strategies to cope with the absence of ZnO in piglet diets. The European swine industry has been banned from using ZnO due to its effect on the environment. Modifications of Crude Protein (CP) and Crude Fibre (CF) have been suggested as nutritional measures to reduce the risk of post-weaning diarrhoea (PWD) in the absence of ZnO. This work sought to evaluate the impact of different post-weaning diets varying in levels of CP, CF and the addition of dietary supplements on piglet post-weaning performance.

Materials and methods
720 pigs, housed in mixed-sex pens (10 pigs/pen, 12 pens/treatment) and weaned at 28 days of age, were offered one of six treatments (T) constructed of a Starter 1 diet (16.25 MJ/kg DE, 1.65 % Lys) for 13 days. Treatments were: **T1**, conventional diets (20% CP, 2.11% CF) with ZnO (2500 mg/kg); **T2**, conventional diets, no additional ZnO; **T3**, conventional diets with antibiotic (Apramycin,100 mg/kg), no additional ZnO; **T4**, 18% CP diets (3.5% CF), no additional ZnO; **T5**, 16% CP diets (3.5% CF), no additional ZnO; **T6**, 18% CP diets (2.49% CF) and supplemental amino acids (commercial product), no additional ZnO. All treatments were formulated at the same Energy/Lysine ratio. On day 0 and 7 blood samples were taken from two average piglets per pen (one male and one female). Samples were collected in 2 ml lithium heparin tubes, centrifuged at 7000 rpm for 15 min, and frozen at – 22 °C pending analysis. Data were analysed in R studio using the nlme package to perform GLM models. Body weight was included as a covariate and where appropriate, batch was included as a random factor in the models. The alpha level of significance was set at $P < 0.05$.

Results
Cortisol concentrations were significantly lower in the T1 treatment compared to T3 at day 7 ($P < 0.05$). Tendencies were observed on C-reactive protein (CRP; $P = 0.057$) and Haptoglobin (Hp; $P = 0.067$) on day 0 and 7, respectively. Both of these were a result of low concentrations of CRP and Hp in the T4 treatment compared to T2 (CRP), and T4 to T6 (Hp) (Table 1).

Table 1
The effect of dietary treatment on plasma C-reactive protein (CRP), Haptoglobin (Hp), Tacrolimus (TAC) and cortisol concentration in the blood of piglets at Day 0 or 7 of age (mean ± SEM).

	Day 0				Day 7			
	CRP (mg/L)	Hp (g/L)	TAC (mmol/L)	Cortisol (µg/dL)	CRP (mg/L)	Hp (g/L)	TAC (mmol/L)	Cortisol (µg/dL)
T1	8.17	0.261	0.216	5.16	7.54	1.08	0.170	1.37[a]
T2	13.7	0.294	0.239	4.54	8.36	0.721	0.186	1.58[ab]
T3	12.7	0.375	0.228	3.72	16.1	1.01	0.178	2.13[b]
T4	6.52	0.110	0.210	3.51	9.87	0.540	0.177	1.54[ab]
T5	11.6	0.357	0.214	3.52	14.0	0.843	0.185	2.09[ab]
T6	10.1	0.220	0.250	4.52	15.5	1.24	0.171	1.97[ab]
SEM	1.78	0.1040	0.0161	0.540	4.950	0.2130	0.0110	0.206
	Probabilities							
Treatment	0.057	0.521	0.380	0.188	0.626	0.067	0.862	**0.029**

[a,b] Means within in a column with different superscripts differ significantly (P<0.05).