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Abstract – Zinc oxide (ZnO) at pharmacological dosage can be supplemented in piglet diets in order to improve growth performance through adjusting gut health. In addition, chlortetracycline (CTC) is a common antibiotic in pig farms. However, there is a risk of bacterial resistance development. In this trial, CTC and ZnO were used to evaluate their effects on antibiotic resistance genes.

The experiment was performed with 4 diets: 2400 ppm of Zn from standard ZnO vs. 110 ppm of Zn from a potentiated ZnO source (HiZox[®]), with or without 300 ppm of CTC. Each treatment consisted of 10 piglets weaned at 25 days. DNA was extracted from feces (0, 2, 4, 7, 14 d), in order to quantify by qPCR the *Escherichia* group, some antibiotic resistance genes and related genes, like the *Escherichia coli* zinc transporter *zinT*.

As expected, both trials groups with high concentrations of ZnO reduced the *Escherichia* group, however low concentrations of HiZox and CTC showed the same trend. CTC increased numerically the development of various genes (*tetA*, *bacA*) after 4 days; without CTC, there were numerical differences between the group fed 2400 ppm of Zn from standard ZnO and the group fed 110 ppm of Zn from potentiated ZnO. Independent of CTC supplementation, pharmacological dosage of ZnO increased significantly the ratio of *zinT* copies to *Escherichia coli*.

Both ZnO at high level and CTC increase the development of antibiotic resistance genes, and may have an additive effect on these genes.

Objectives of the study

Chlortetracycline (CTC) and zinc oxide (ZnO) at pharmacological dosage may be added in piglets diets in order to prevent diarrhea and to improve growth performance during the post-weaning period. However, there is a risk of bacterial resistance development.

In a previous study (Vahjen 2012), the potentiated ZnO source HiZox[®] exhibited higher growth depressing effects on bacteria than standard ZnO. The objective of this study was to compare this new ZnO source and the regular ZnO at high dosage, with or without CTC, in order to evaluate their effects on antibiotic resistance genes.

Material and methods

Animals

40 piglets (Large White x Landrace) weaned at 25 days, with 2 piglets per pen, divided in 2 stables (with vs. without antibiotic)

Experimental diets

Wheat, barley, SBM, maize-based diets, during 14 days

[HZ] Basal diet + 110 mg/kg Zn from HiZox[®]

[HZ-CTC] [HZ] + 300 mg/kg of CTC

[ZnO] Basal diet + 2400 mg/kg Zn from standard ZnO

[ZnO-CTC] [ZnO] + 300 mg/kg of CTC

Measurements

Extraction of DNA from feces (0, 2, 4, 7, 14d)

Quantification of some genes by qPCR: *tetA* (tetracycline resistance), *bacA* (bacitracin resistance), *zinT* (*E. coli* zinc efflux protein)

Statistical analysis

5 experimental units per treatment

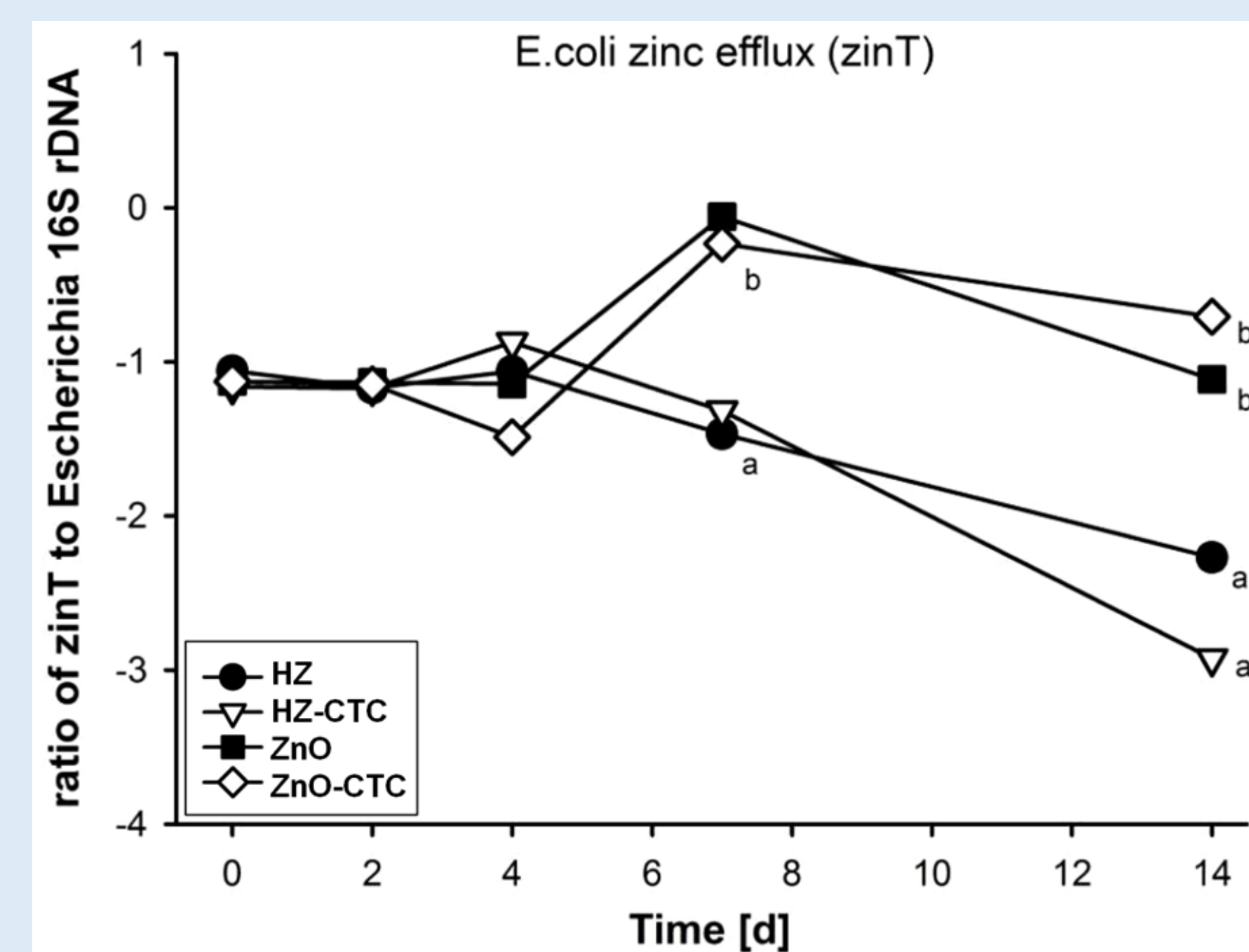
Software package SPSS

Non-parametric tests (Kruskal-Wallis, Mann-Witney-U)

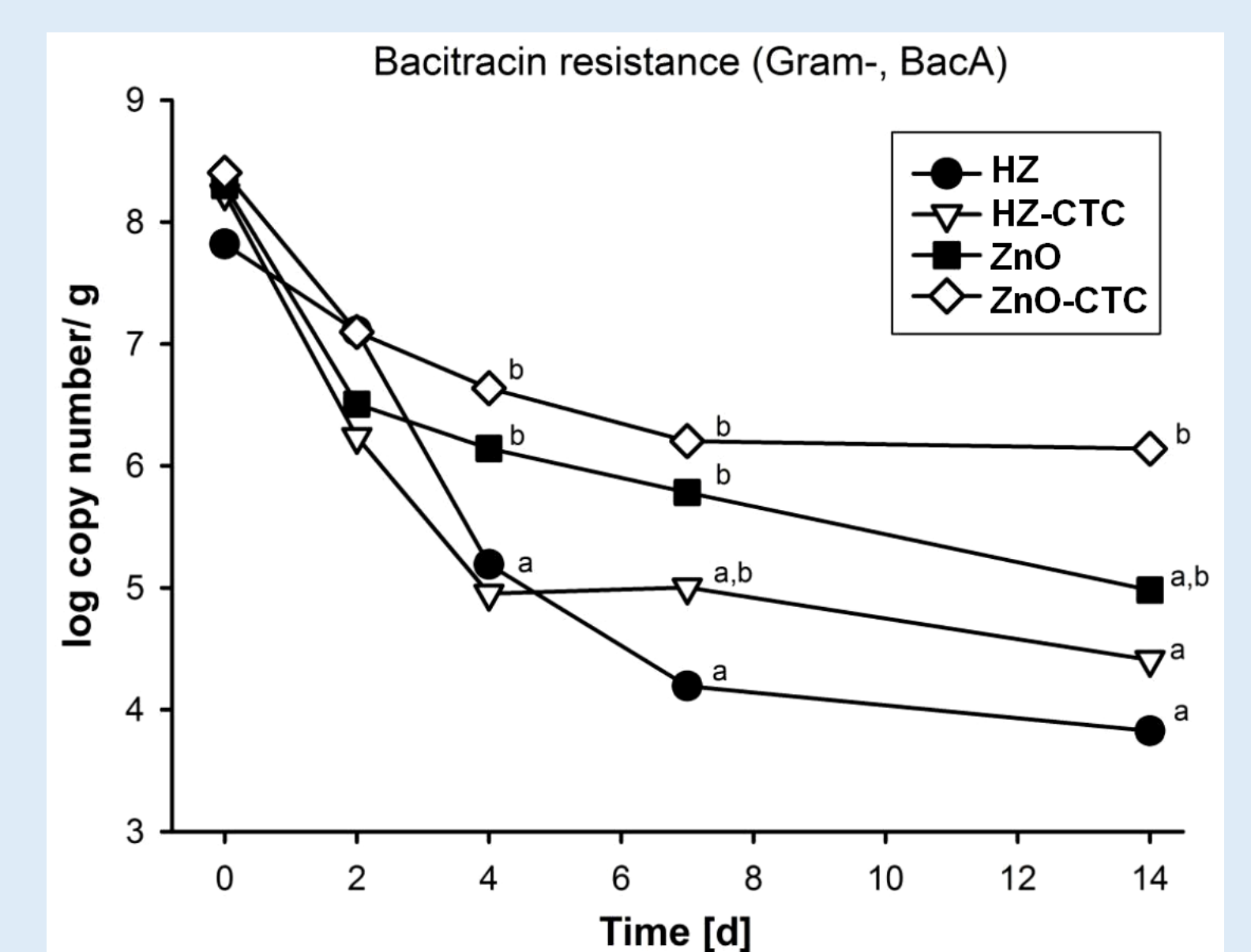
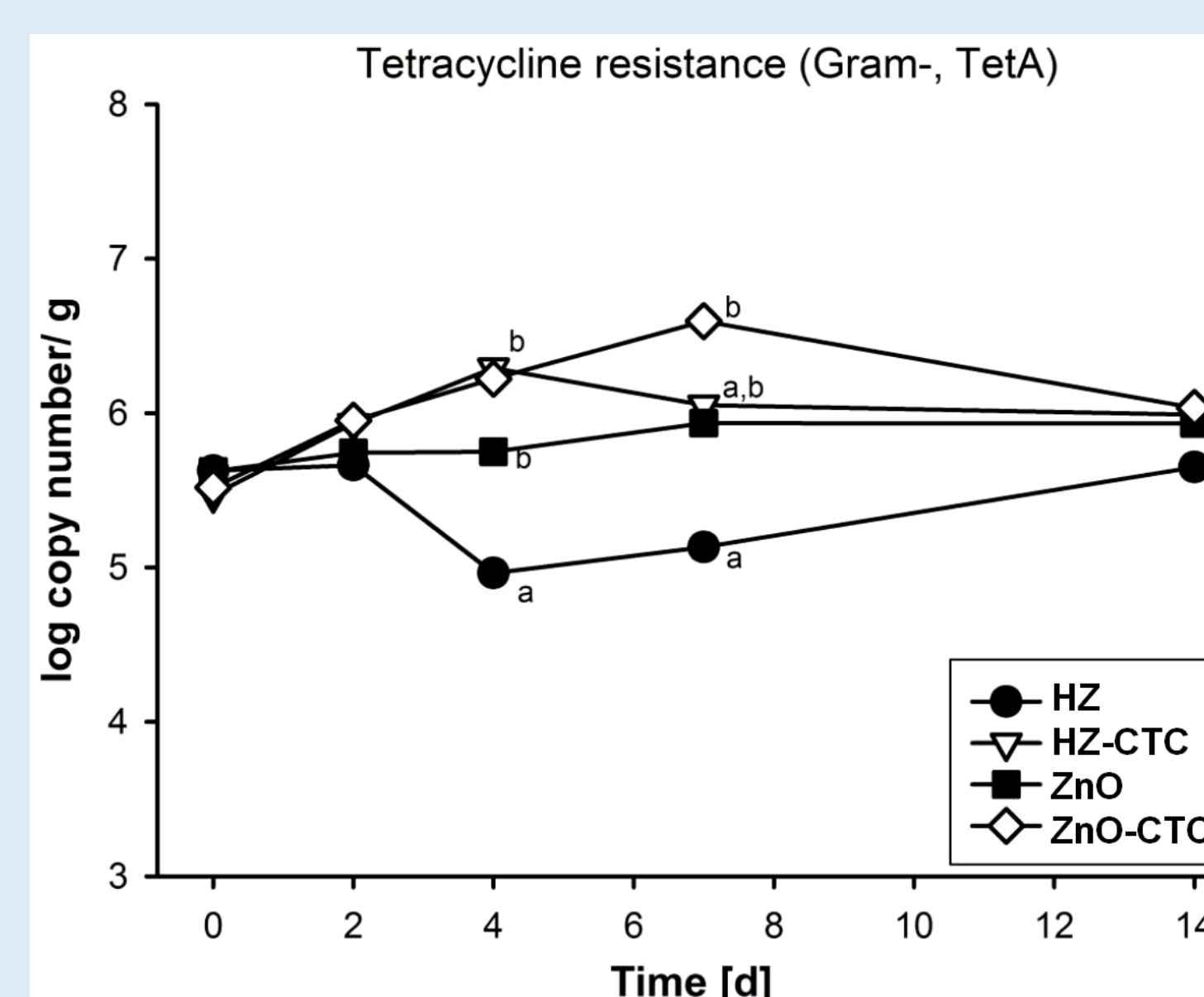
Level of $p < 0.05$ is considered significant

Results and discussion

A clear effect of high dosage of ZnO was found for the ability of *E. coli* cells to expel Zn ions, with the zinc transporter *zinT*:



In addition, CTC increased the development of various resistance genes, including *tetA* and *bacA*:



The impact of pharmacological dosage of ZnO was also visible in the higher copy numbers of antibiotic resistance genes, especially for *BacA*: after 6 days, the copy numbers were numerically (with CTC) or significantly (without CTC) higher with the groups fed high dosages of ZnO, compared to the groups fed HiZox[®].

A phenomenon of cross-resistance can be observed in this trial: ZnO and CTC can favor respectively *zinT* gene development and tetracycline resistance, but also resistance against other antibiotics like bacitracin. For the gene *BacA*, interestingly, the differences between the treatments remained significant at the end of the trial; however, after 14 days, the *Escherichia* group recovered and no difference were visible between the treatments groups, as noted in earlier studies (Vahjen 2016).

Conclusion

In conclusion, both ZnO at high level and CTC increase the development of antibiotic resistance genes, and may have an additive effect on these genes.

References

W. Vahjen, J. Zentek and S. Durosoy, JAS (90), 2012, 334-336
W. Vahjen, A. Roméo and J. Zentek, JAS (94), 2016, 359-363