

# SERUM AND FAECAL ZINC CONCENTRATIONS IN SHEEP FOLLOWING LOW DOSE ORAL ZnO TREATMENT

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## Introduction

Facial Eczema (FE) (Pithomycototoxicosis) is a disease in farm animals caused by the fungus *Pithomyces chartarum* which, under favourable conditions, can rapidly spread through pastures (Di, 2009; Smith and Towers, 2002). In New Zealand, high dosages of Zn oxides and Zn sulphates are used as a prophylaxis against FE. Zinc is commonly administered via a drench or bolus, but most is excreted via faeces. High doses of Zn may cause negative effects on animal health and the accumulation of Zn excreted by animals in soil is threatening to environmental quality guidelines in some regions (Anderson et al. 2012). Ongoing research at Massey is investigating if Zn-enriched fodder can protect animals against FE (biofortification) with a lower total Zn intake. This would have benefits for both animals and soil health.

A serum Zn concentration which protects against FE is defined (20 µmol Zn/L serum), however there is also some evidence that zinc in faeces (250 mgZn/kg FW) will also afford prophylaxis (Bennison et al. 2010; Munday et al. 1997). However it is not clear which mode of physiological zinc (i.e. blood or gut) least to most effective protection against FE (Morries et al. 2004). Biofortification research to date suggests that it may not be possible to achieve this serum concentration with fodder, but the target faeces concentration is realistic (Anderson et al. 2012). As part of ongoing work to investigate the potential relevance of biofortified fodder to FE protection, a study was conducted to investigate if faecal and serum Zn concentration can be separately manipulated through oral Zn supplementation. This is an important first step towards a challenge trial where animals are infected with FE; a trial which would qualify both the mode of protection against FE and the efficacy of biofortification as an alternative prophylaxis.

## Materials and methods

Nine rams, with weights ranging from 30.0 kg to 40.7 kg (average weight 35.1), were randomly selected from a Massey University production farm and transferred in to the Large Animals Teaching Unit (LATU), Massey University. They were randomly organised into three groups of three and each group was orally treated with 3 different treatments of ZnO. The treatments were, 5 mg Zn/kg/day for three days (3 doses), 5 mg Zn/kg /12 hours for three days (6 doses) and 10 mg/kg Zn, at time zero as a single dose. These doses are all significantly lower than those administered to animals in the field. Blood and faeces were sampled at four-hour intervals for three days. A subsample of the faeces was dried at 65 °C, ground using mortar and pestle, and stored after sieving through a 1-mm sieve. The rest of the fresh faecal samples were stored at 4 °C. The blood samples collected in a heparin tubes were centrifuged at 3000 rpm for 10 minutes at 4 °C. Serum was then separated from the plasma, and the whole blood, serum and plasma were frozen in preparation for analysis.

## Analysis

The Zn concentrations in all samples were determined using flame atomic absorption spectrometry (FAAS: GBC AvantaΣ). Blood serum was analysed directly after a 1:4 dilution of the samples. A known volume of plasma was first digested in 65% nitric acid for 2 hours using a digestion block, and then diluted to 25 mL with de-ionised water. A subsample (0.5 g) of dried faeces was weighed and pre-digested in concentrated nitric acid (5 mL) overnight. The following day the samples were digested using a digestion block at 120 °C for 2 hours, then diluted to 25ml with deionized water. For quality assurance, a reference material (Wageningen 100 GR94) was analysed alongside the experimental samples. The analytical result was within 92% of the certified value. The recorded Zn concentration was compared to guideline serum and faeces Zn concentration levels that are thought to signal protection against FE.

## Results and discussion

The Zn concentration in the dry faeces of the animals at each treatment and sampling time is presented in Figure 1. High Zn concentration in the gastrointestinal tract of the animal has been suggested to protect against FE. Bennison et al. (2010) stated that a Zn concentration of 250 mg/kg fresh weight in faeces could afford prophylaxis. Single dose treatment (10 mg/kg) rapidly increased the Zn concentration in faeces to a maximum value of 1174 mg/kg DW, which was calculated as 308 mg/kg FW. However, this reduced below the threshold for protection after 24 hours. The Zn concentration in faeces gradually increased up to 1670 mg/kg in animals treated twice a day and remained significantly elevated for longer than those treated once a day and above the threshold for protection. This indicates that the regular administration of low-level zinc is likely to elevate Zn above the target concentration relative to a single and higher- level Zn administration.

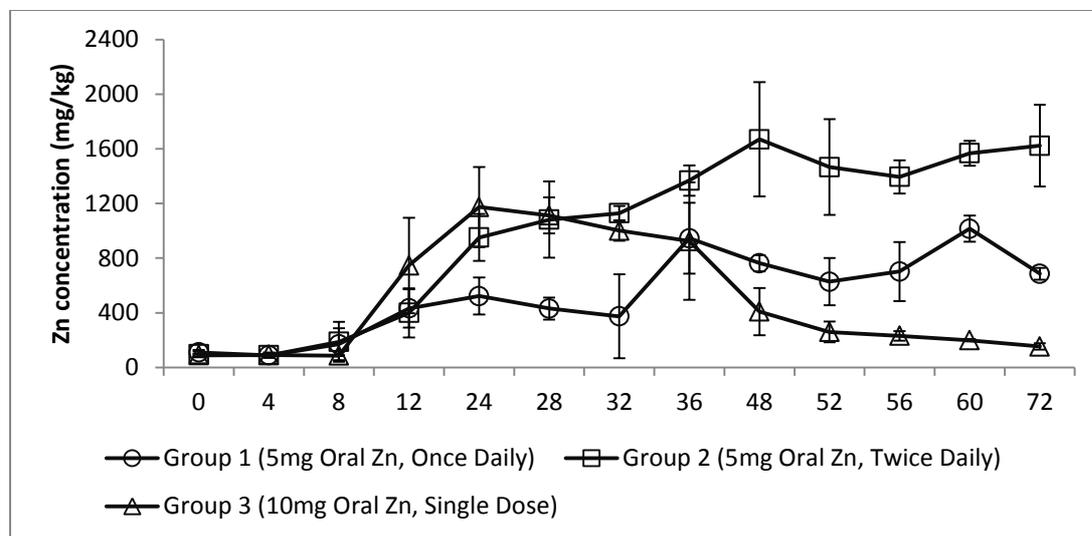


Figure 1. Zn concentration in dry faeces after oral application of Zn at 3 different rates and time combinations

Animals lack in the ability to store zinc in their bodies for later usage and hence they require a continuous supply, to ensure that they meet the required amounts of Zn (Grace et al., 2010). Therefore, blood plays an important role in indicating the concentration of Zn available in the animals, as it changes with the amount of Zn in the external supply. Deficiency levels or prophylaxis targets for Zn are generally made for the serum fraction, as serum results are

generally considered to reflect the mineral status of the transport pool of trace elements (Herdt and Hoff, 2011). All treatments increased the blood serum Zn concentration to the established threshold (20  $\mu\text{mol Zn/L}$ ), 8 hours after commencing the experiment. However, the serum Zn concentration thereafter reduced below the threshold level prescribed as necessary to protect against FE. There were no significant differences observed in the blood serum Zn concentration among the treatments. This indicates that the regular administration of Zn at either high or low levels of concentration may influence the Zn concentration in the blood serum within a short period in protecting against FE.

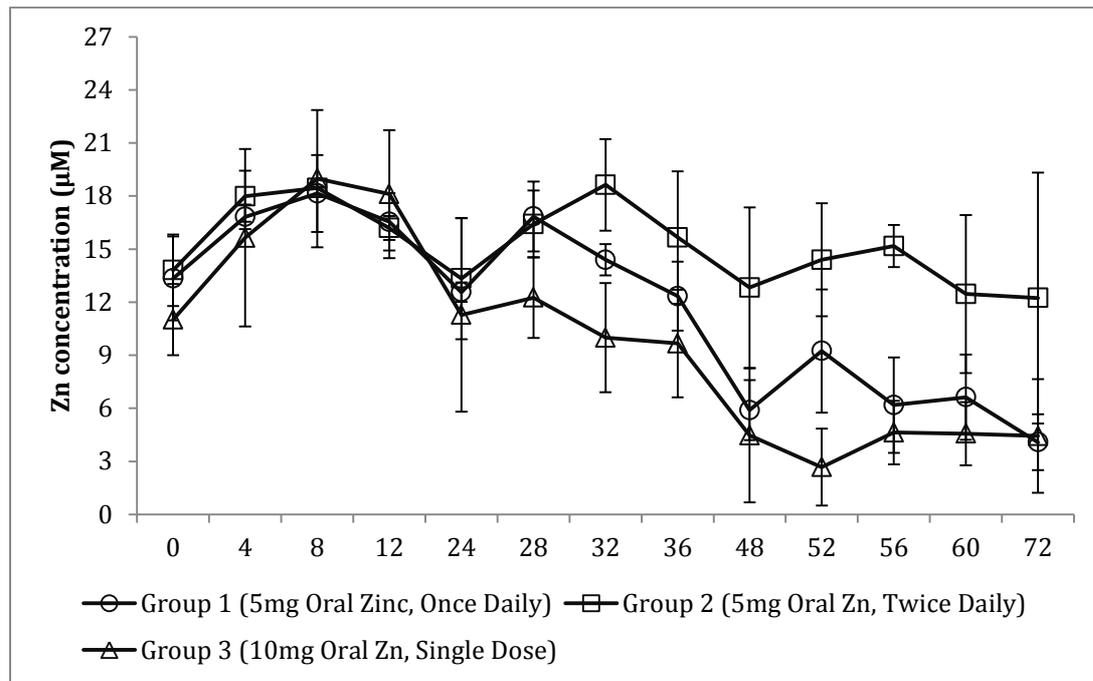


Figure 2. Zn concentration in blood serum after oral application of Zn at 3 different rates and time combinations

The concentration of Zn in the blood plasma of the animals did not show a clear trend among the treatments (Data not presented here). The Zn concentration in the whole blood increased from 32  $\mu\text{mol Zn/L}$  to a maximum of 60  $\mu\text{mol Zn/L}$ , for the animals treated with Zn twice a day. However, there were no significant differences among the treatment means observed in Zn concentrations for whole blood or blood plasma.

### Conclusion

The regular administration of low-level zinc had limited effect on the serum Zn concentration of the sheep, but had a major effect on their faeces Zn concentration. This experiment therefore proved that serum and faeces Zn concentration can be separately manipulated. This finding is an important step towards feeding trials and a FE challenge to quantify the value of biofortified fodder as a treatment option to protect against this important production-limiting disease.

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