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Zinc-enriched and zinc-biofortified feed as a possible animal remedy in pastoral agriculture: Animal health and environmental benefits

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ABSTRACT

Zinc is used in New Zealand agriculture to protect livestock against fungal infection (facial eczema), a plementation can improve growth performance and feed efficiency. Zinc is commonly administere drench or bolus, but most is excreted in faeces. Mass balance calculations indicate that excreta is in the average Zn concentration in some NZ pastoral soils at a rate of 0.78 mg/kg/ha/yr. This repres unsustainable level of Zn increase that threatens environmental quality guidelines. To determine v current supplements could be replaced by naturally Zn-enriched or biofortified fodder, 20 sheep were istered one of four Zn treatments over seven days (drench, control hay, biofortified (Zn) hay and will a naturally elevated concentration of Zn; *Salix purpurea*, clone Pohangina). Blood and faeces were s regularly and analysed for Zn. Drench rapidly increased blood serum and whole blood Zn concentra above the established threshold for protection. The plant treatments also increased blood Zn relativ control, but there was insufficient Zn in the fodder to reach the threshold level. The concentration faeces was significantly increased by drench and willow. For animals fed willow, the faeces Zn conce remained elevated for longer than for those that were drenched. This was despite the animals on the treatment receiving only 11% of the Zn given to the drenched animals. We propose that willow, grow elevated in Zn to yield a higher concentration of Zn in fodder, may represent a biogeochemical syster supplementation where the trace element is cycled from soil, to plant, to animal, and back to soil. Suc tem could reduce the flux of trace elements entering the pastoral environment from animal remedi gate future environmental risk that may become apparent due to current farming practices, and pot lead to increased livestock production.

1. Introduction

Zinc (Zn) is an essential trace element for plants and all animals. Animal deficiencies are generally associated with Zn deficiencies in food/fodder (Branca and Ferrara, 2002; White and Broadley, 2005) and Zn supplementation can result in improved growth performance and feed efficiency (Herdt and Hoff, 2011). But high levels of Zn in soil can have the opposite effect. Zinc concentrations above a critical threshold level are toxic to plants and animals, and a build up of Zn in soil can have negative environmental and production implications. In New Zealand pastoral agriculture, Zn is utilised primarily as an animal remedy, particularly against facial eczema, a disease that affects ruminant animals and represents a significant production-limiting factor for New Zealand agriculture.

Facial eczema (FE) is a secondary response (a photosensitivi occurs due to liver damage inflicted by the mycotoxin sporidesm tained within spores of the saprolytic fungus *Pithomyces cha* (Gibson, 2008). Warm humid conditions at the end of summer p the growth of the fungus, which infects swards of grass with spo may be ingested by cattle and sheep. Acute exposure can lead to distress and death. Animals which have suffered chronic exposu several seasons, show signs of significant damage to bile ducts a liver (Smith and Towers, 2002). The liver enzyme gamma-gl transpeptidase (GGT) is used as a biochemical indicator of facial e and is elevated in animals with either chronic or acute exposure disease.

A preventative treatment against FE is the administration doses of Zn; 15–20 mg/kg LW/day (Grace et al., 2010), well abo tritional or physiological requirements. Administration must b to or during the exposure of animals to pasture with high spore counts; treatment is ineffective in curing animals tha clinical signs of photosensitivity (Towers, 1976). Zinc salt form of ZnO is more effective than other forms of Zn such

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metal when administered to animals via a drench or intraruminal bolus (Bennison et al., 2010). The effective protection afforded by these treatments can range from days/weeks (drench) to months (bolus). The healthy range of Zn in sheep blood serum is 9.2–18 μM (Grace et al., 2010), but levels between 20 and 30 μM are thought to provide protection against FE (Munday et al., 1997). The precise mode of action of Zn in preventing FE is unknown, and a direct association between the concentration of Zn in serum and prophlaxis has not been demonstrated. However, a general correlation between the serum Zn concentration and reduced GGT activity is reported in the literature. Recent data indicates that high Zn concentrations in the gastrointestinal tract may also protect against FE; this is potentially very important as most ingested Zn is excreted in faeces. Bennison et al. (2010) suggested that a threshold Zn concentration in faeces must be exceeded before protective effects against an increase in GGT levels are observed, and these authors suggested a concentration of 250 mg/kg fresh weight.

There is environmental concern associated with the high level of Zn that is added to the pastoral environment through animal excreta (Zn flux). The use of Zn as an animal remedy has increased the level of Zn in soil by approximately 100% relative to background levels for pastoral soils in some regions of New Zealand (Kim et al., 2008). Elevated concentrations of soluble Zn, beyond the sufficient range for organisms, will impair soil functioning through toxicity to plants and soil biota. A New Zealand environmental issue of emerging concern is the potential for future changes in the health of the pastoral ecosystem due to human-induced increases in regional soil Zn concentrations.

An alternative Zn remedy may be to produce stock fodder with an elevated Zn concentration. This concept is described by a group of technologies known as biofortification, which seeks to increase the concentration of Zn and other trace element micronutrients in food or fodder pre-harvest using natural means (White and Broadley, 2005). Robinson et al. (2005) reported that some varieties of willow (*Salix* spp.) accumulate inordinate concentrations of Zn relative to pasture growing in the same soil. Poplars and willows are used extensively in New Zealand for soil conservation and supplementary stock fodder during times of drought (Wilkinson et al., 1999). Both foliage and small twigs can be browsed by sheep and cattle (Douglas et al., 1996; Hathaway, 1986). In addition to providing an emergency food source, the use of poplars and willows as stock feed has proven health benefits including an improvement in fecundity (Barry and Kemp, 1991). These benefits may be derived from high protein, tannin or nutrient concentrations. The use of willow with a naturally elevated concentration of trace element as described here does not directly constitute biofortification. However, the action of increasing an already high trace element concentration through breeding or agronomic practices would meet the definition of biofortification.

A potential advantage of biofortification over conventional facial eczema prophylaxis may be realised in environmental management. Elevated soil Zn concentrations resulting from conventional drenching practices should increase Zn uptake in pasture and fodder crops. It is possible that such fortified fodder (naturally or through adoption of biofortification practice) could afford some protection against FE and thus reduce the need for further conventional drench- or bolus-based treatment. Were fortified fodders found to be more efficient in supplementing Zn, the total Zn burden on the pasture soil could potentially be reduced. We therefore hypothesise that continuous feeding with fortified fodder may elevate blood serum and/or faeces Zn levels to levels that afford protection against FE and improve general animal health, and result in a more efficient and environmentally sustainable Zn transfer to the animal compared to a conventional drench. To provisionally test this hypothesis, and to assess the likely mass-balance of Zn in the pastoral environment, we sought to compare blood and faeces Zn concentrations in sheep fed fortified fodder to animals administered a negative (hay) and positive (drench) control.

2. Materials and methods

2.1. Sheep

Twenty four female lambs, with weights ranging from 3
(average weight 34.5 kg), were randomly selected from
University production farm on 19 April 2010, and transfe
the Animal Production Unit at the Palmerston North Campu
sey University. The animals were randomly placed in indivic
each with their own water and feed containers.

Control hay (predominantly *Lolium perenne*) was fed to a
for the first three days of the trial. At the end of the three day
tion period, the 20 animals, in blocks of five, that were best
ing to the trial conditions were assigned to one of four tr
(drench, willow, biofortified grass, control hay), and a c
feeding regime was started. The first day of treatment wa
as Day 0 and treatment continued for seven days (Day 1 is
defined as 24 h after the initiation of treatment). From Day
10, all animals were placed back on the control hay diet, pr
lease back onto the Massey University production farm.
water were replaced daily.

A jugular blood sample (5 mL) was collected from each
experimental sheep on Days 0, 2, 4, 7 and 10 using a vacupac
er. The blood samples were centrifuged at 3000 RPM for
4 °C (Heraeus Megafuge). Blood serum was then separated
red blood cells and both components frozen in preparation f
sis. The weights of the whole blood, serum and red blood c
recorded. A sample of faeces from each animal was also col
Days 0, 2, 4, 7 and 10, then dried at 70 °C until a constar
was obtained.

2.2. Feed treatments

Biofortified hay was harvested from research plots at Lin
versity in Canterbury, New Zealand where soil was amen
biosolids to contain Zn up to a concentration of 250 mg/kg
(predominantly *L. perenne*) was cut in March and April 201
aration for the sheep trial. The grass was then dried, well m
packed for transport to Massey University. Willow (*S.*
clone Pohangina) was harvested each day by cutting stem
year old trees growing at the RST Environmental Solutions
nursery in Aokautere near Palmerston North. The willo
were coarsely chopped, then fed to the relevant sheep. Co
was purchased from the animal production unit. For the z
drench, commercial ZnO (500 g) was mixed with water
and 100 mL of Nutrimole suspending agent. Twelve mL
this suspension was administered orally to each of the fiv
sheep using a plastic syringe. A single dose of drench was
the treated sheep on Day 0. The drenched animals were subs
fed control hay for the duration of the trial.

A weighed amount of feed was presented to each anim
start of controlled feeding on Day 0. The amount remai
then recorded either daily, or twice daily, and feed toppe
known weight. The cumulative amount of feed ingested
seven day feeding trial could therefore be accurately calcula

Hay was fed to animals as dry weight feed. Willow was f
imals on the basis of fresh weight feed. A bulk sample of wi
dried at 70 °C until constant weight, and the dry weight cc
factor calculated.

2.3. Analysis

The Zn concentration in all samples was determined using
(Varian), at Lincoln University. Blood serum was analysed
after 1:4 dilution of the samples. A known volume of red b
was digested in nitric acid for 2 h using a digestion block, the

to 25 mL with de-ionised water. Based on the weights of the serum and red blood cells recorded, it was possible to express the Zn concentration in the serum, the red blood cells, and by calculation, in the whole blood mass and volume. The dry faeces samples were ground using a mortar and pestle, and dry herbage samples using a Cyclotec sample mill. Subsamples (0.5 g) were weighed into a microwave digestion container and pre-digested in concentrated nitric acid (5 mL) overnight. The following day the samples were microwave digested (700 W) at 75 °C for 15 min then diluted to 25 mL with deionised water.

For quality assurance, a reference material (Wageningen 100 GR94) was analysed alongside the experimental samples. The analytical result differed by less than 10% from the certified value.

The Zn bioaccumulation factor for the biofortified fodder is calculated in this work as the concentration of Zn in the fodder divided by the concentration of Zn in the soil in which this fodder was grown.

2.4. Statistical treatment of data

Analysis of Variance (ANOVA) was performed using the SAS 9.1.2 statistical software package to determine significant differences between Zn concentrations as a function of both treatment, and days after treatment.

3. Results and discussion

3.1. Zinc ingestion

Table 1 shows the Zn concentration of each treatment, and the amount consumed over the seven days of controlled feeding. The total amount of Zn administered through drenching was an order of magnitude greater than for the control or plant treatments. There were variations in the amount of feed consumed by animals on each treatment. The fresh weight of willow consumed was considerably lower than the dry weight of hay consumed by animals on the three hay treatments (control, drench and hay). However, despite the low feed intake, there was no apparent loss of animal condition during the trial. The Zn intake of animals on the willow treatment was higher than that on the control or hay treatment, and was 11% of that administered to the drenched animals. The Zn intake of animals fed willow was at least twice that of the animals fed hay (control or biofortified).

3.2. Zinc concentration in sheep blood

Animal bodies contain no significant mobilisable stores of zinc and therefore a continuous dietary supply is necessary to meet the requirements of nutrition (Grace et al., 2010). Blood is therefore a key indicator of the Zn status of animal physiology and concentration will change as a function of the amount of Zn in an external supply (fodder or drench). In the context of mineral nutrition, blood is comprised of two fractions, blood serum (or plasma) and red-blood cells (the erythrocyte fraction), and Zn is unevenly partitioned between these two fractions. 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). In the current study Z centrations are reported for both the serum fraction and the blood fraction. The results can in this way be directly comp published guideline values for serum Zn, but can also be con in the context of whole blood chemistry. For some trace ele for example selenium, the mineral concentration of the red cell fraction is important in making sound conclusions on mine trition (Herdt and Hoff, 2011).

Table 1
Detailed description of the Zn treatments used during the controlled feeding trial.

Feed treatment	Zn concentration	Total treatment administered[a]	Zn
Drench	365 g/kg (drench)	15 g (12 mL) on Day 0	5454 mg
	33.2 mg/kg DM (diet)	5713 g (±460 g)	190 mg
Control hay	33.2 mg/kg DM	6387 g (±463 g)	211 mg
Biofortified hay	64.0 mg/kg DM	4682 g (±519 g)	300 mg
Willow	258 mg/kg DM	2419 g (±1037 g)	624 mg

[a] The total treatment administered for each feed is cumulative dry matter ingested presented as an average for the five animals and standard error. All fodder concentrations and quantities are dry matter (DM).

3.2.1. Serum zinc concentration

The blood serum Zn concentration of the control anim creased from Day 0 to Day 10 ($P<0.01$) (Table 2). This is att to a lower concentration of Zn in the control hay relative to concentration in the grass the animals were grazing in th This established a dropping baseline Zn concentration for the treatment that must be considered in the presentation of our To delineate the effects of the various treatments against this v base line, the results in Table 1 are also presented in terms of p age change for each treatment relative to the concentration o treated animals on Day 0 (Fig. 1).

Administration of the ZnO drench resulted in an immedi crease in the serum Zn concentration of the treated sheep r to the serum Zn concentration of those animals on Day 0 (T Fig. 1). However, the serum Zn concentration began to rapi crease during the days after administration (maximum 30.7 μM on Day 2). The serum Zn concentration of the drench mals was significantly higher than that for the other treatm Day 2 and Day 4 ($P<0.01$). By Day 7 the serum Zn concentrat the drenched animals was the same as that for the sheep fed By Day 10 the serum Zn concentration of the drenched anim decreased relative to the concentration at Day 0 (Fig. 1) a the same as that recorded in the serum of the animals that re the control treatment.

The significant decrease in the serum Zn concentration of th trol animals was not observed for the animals fed willow (Ta The animals fed willow therefore recorded a relative incr blood serum Zn concentration for all days when compared serum Zn concentration for those animals on Day 0 and to the animals, although the magnitude of this increase was not as g that for drench (Fig. 1).

The serum Zn concentration of the animals fed biofortifi was lower at all sampling times relative to the initial concen on Day 0 (Fig. 1). However, when compared to the blood seru centration of the control animals on Day 10, the Zn concentrati significantly higher, and the same as the serum concentration sheep fed willow (Table 2).

Table 2
Serum and whole blood Zn concentration as a function of time for each tr Values are mean and standard error ($n=5$). Significance testing (Duncan's Range Test) is for concentration as a function of day for each treatment. Me the same letter are not significantly different ($P<0.05$).

Day	Drench	Willow	Biofortified hay	Control
Serum Zn concentration μmol/L				
0	12.66 (0.78) C	12.40 (0.64) A	15.64 (1.48) A	15.53 (0.4
2	30.67 (3.37) A	14.37 (0.80) A	13.64 (1.11) AB	13.58 (0.7
4	25.68 (2.91) B	13.93 (0.88) A	13.58 (1.55) AB	12.23 (0.3
7	16.16 (2.06) C	14.05 (0.97) A	13.03 (0.71) B	13.73 (0.2
10	11.70 (1.20) C	14.27 (1.12) A	14.18 (0.82) AB	9.97 (0.2
Whole blood concentration μmol/L				
0	52.58 (2.27) BC	48.54 (1.47) A	47.12 (3.31) AB	48.19 (2.6
2	67.00 (3.23) A	47.35 (2.35) A	48.41 (5.25) A	46.12 (2.8
4	64.37 (3.61) A	51.26 (1.59) A	42.82 (2.04) B	44.56 (3.0
7	56.36 (4.10) B	54.18 (1.89) A	42.53 (3.03) B	45.45 (3.1
10	50.96 (2.05) C	49.62 (7.28) A	42.95 (3.51) B	43.96 (2.8

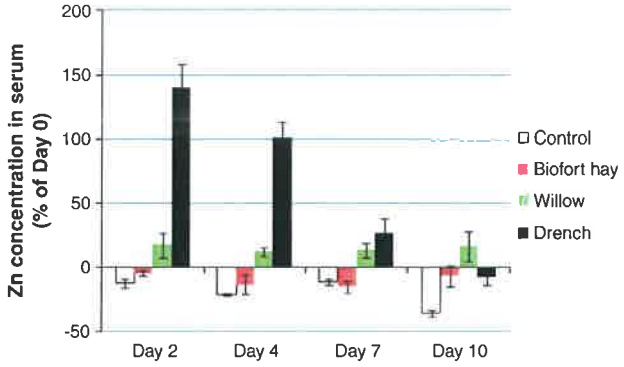

Fig. 1. The relative concentration of Zn in the blood serum of sheep for each experimental treatment as a percentage of the Zn concentration in the same animal on Day 0 (mean and standard error).

3.2.2. Whole blood zinc concentration

The whole blood Zn concentration of the control animals again decreased through the 10 day feeding trial ($P<0.01$) (Table 2). Therefore, the Zn concentration in the whole blood is again presented in terms of percentage change for treatments relative to the concentration on Day 0 (Fig. 2).

Animals administered the ZnO drench showed a rapid increase in whole blood Zn (Fig. 2), but the concentration decreased after Day 2 (maximum value 67.0 μM on Day 2) (Table 2). By Day 10 the blood Zn concentration of the drenched animals was the same as the concentration of these animals on Day 0 ($P<0.01$).

The animals fed willow recorded a nominal increase in the whole blood Zn concentration relative to Day 0 for all days after Day 2 (Fig. 2). This is in contrast to a significant reduction in the concentration of Zn in the whole blood of the control animals. The concentration of whole blood Zn increased over the 7 days of willow feeding, reaching a maximum concentration of 54.2 μM on Day 7. By Day 7 the whole blood concentration of the willow-fed animals was the same as that of the animals that were drenched.

The concentration of Zn in the whole blood of the animals fed biofortified hay did not show a clear trend. There was a slight increase in the Zn concentration for these animals on Day 2 relative to Day 0 (Fig. 2), however for all other sampling times there was a relative decrease (significant at $P<0.06$). The whole blood Zn concentration of animals fed biofortified hay was the same as that for the control animals throughout the trial.

Our results show that drenching with ZnO immediately increased the blood serum and whole blood Zn concentration of the treated animals to levels prescribed as necessary to protect against FE. The maximum serum Zn concentration was reached on Day 2 (This declined on Day 4 (25.7 μM), and had decreased by n on Day 7 (16.2 μM). Protection against FE is afforded by a concentration of 20–30 μM (Munday et al., 1997). Therefor in this trial had diminished protection from sometime betv 4 and 7. This observation supports the recommended dre animals once a week with the drench dose used in this wo

The Zn concentration in the serum and whole blood of th fed the control diet significantly decreased during the feeding trial. This is attributed to a reduction in the Zn conc of the hay these animals were given, relative to their diet ir However, the Zn concentration in the serum and blood of th fed willow did not change. Zinc in the willow offset the dec served for the control animals. We can therefore state that t treatment caused an increase in serum and whole blood Z trations relative to the control animals at each sampling to the willow animals on Day 0. However, this Zn supplen was insufficient to afford protection against FE based on lite ures (maximum value for serum 14.4 μM on Day 2). There v crease in the serum Zn concentration of the animals fed b hay relative to the control animals by Day 10 of the trial. this effect was less pronounced than that for willow, and ag ficient to reach the reported threshold value for protection a The data indicate that the serum fraction is more respons supply than the whole blood fraction.

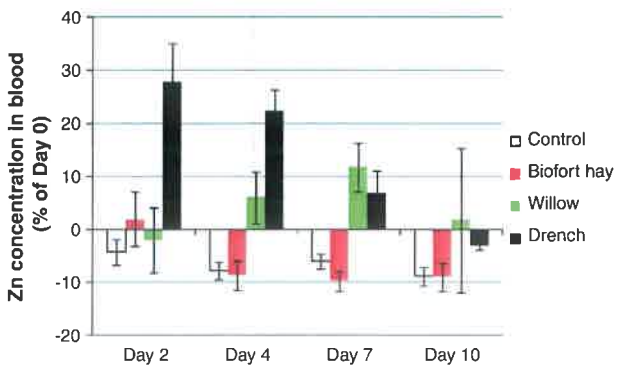

Fig. 2. The relative concentration of Zn in the whole blood of sheep for each experimental treatment as a percentage of the Zn concentration in the same animal on Day 0 (mean and standard error).

3.3. Zinc concentration in sheep faeces

The absolute concentration of Zn in the faeces of the a each treatment, for each sampling time, is shown in Fig. 3. there was a significant increase in the concentration of Zn i ces of the drenched animals to a maximum value of 3983 m There was a significant increase in the faeces Zn concentrat animals fed willow on Days 4 and 7 relative to these anima 0 ($P<0.01$) (Fig. 3). While the Zn concentration in the fae drenched animals decreased after Day 2, faecal Zn in th fed willow increased to a maximum value of 548 mg/kg Day 7. On Day 4, the Zn concentration in the faeces for

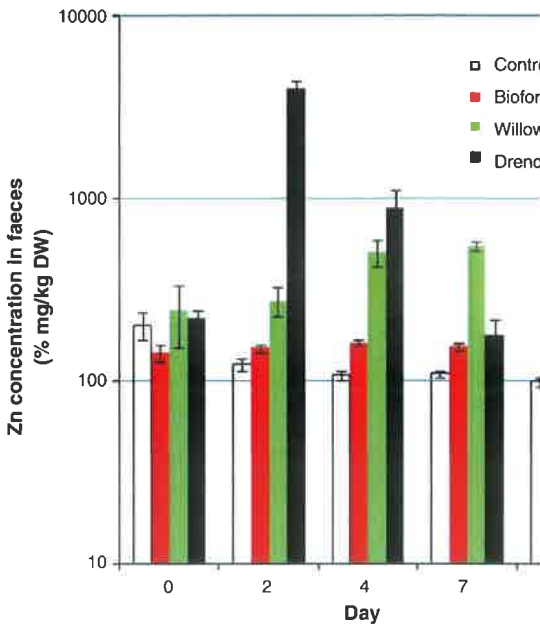

Fig. 3. The absolute concentration of Zn (mg/kg dry weight) in the faeces of ministered the four treatments for each day of sampling (mean and stand

treatments was not significantly different ($P<0.01$) (willow 506 mg/kg; drench 889 mg/kg). On Day 7 the Zn concentration in the faeces of the sheep fed willow was higher than that in the sheep administered drench. By Day 10 the faeces Zn concentration for the drench- and willow-treated animals was the same as the concentration for that animal on Day 0.

The faeces Zn concentration of the animals fed biofortified hay did not change from Days 0 to 7, but was significantly lower on Day 10. The faeces Zn concentration of the animals fed the control diet decreased on Day 2, but did not change thereafter.

3.4. Efficacy of treatments to protect against facial eczema

Serum Zn concentration is used as an indicator of FE protection due to the reported negative correlation between the serum Zn concentration and serum GGT activity. The threshold values to afford protection against FE have been generated through clinical trials where the relationship between serum Zn concentration, GGT activity and clinical affects have been investigated. These clinical trials have used inorganic forms of Zn as an FE prophylaxis (ZnO and Zn metal), not organic forms of Zn. There is insufficient data to suggest that the same threshold values can be used in the consideration of the level of protection that might be afforded by fortified fodder. In the current trial we did not expose animals to sporidesmin, and therefore, as there was no reason to expect an increase in the activity of liver enzymes (specifically GGT), we did not measure the GGT activity in the serum of the animals. We therefore have insufficient data to state whether any protection has been afforded against FE using the elevated Zn fodder treatments. However, our trial has recorded that the serum and whole blood Zn concentration of ruminant animals (sheep) can be manipulated through amendment of an animal's diet to include biomass with an elevated concentration of Zn.

Administration of the ZnO drench resulted in an immediate increase in the Zn concentration of the faeces of the treated animals (3983 mg/kg DW). At a dry weight content of sheep faeces of 30%, this is equivalent to a fresh weight concentration of 1196 mg/kg, well in excess of the reported threshold value for protection (250 mg/kg FW; Bennison et al., 2010). The willow diet resulted in a significant increase in the Zn concentration in the faeces. By Day 7, the Zn concentration in the faeces of the drenched animals had dropped to 54 mg/kg (FW), whereas the Zn concentration in the faeces of the sheep fed willow was still relatively high; 165 mg/kg (FW). While this is below the reported threshold, it is not dissimilar. There is the potential that these animals did gain some protection against sporidesmin and therefore against FE. No elevation of faeces Zn to a level that could likely begin to afford protection against FE was recorded for the control animals or those fed biofortified hay.

3.5. Environmental consideration of facial eczema prophylaxis

The data generated during the feeding trial allows for general mass balance calculations to explore the environmental fate of Zn administered to animals for FE prophylaxis. The drenched animals in this trial were administered a dose containing 5.45 g of Zn (Table 1). This is a minimum amount of Zn that can be expected to be administered to sheep each week during the facial eczema period in a pastoral environment. Assuming 95% of this Zn is excreted (substantiated by the faeces Zn concentration of the drenched animals), 5.18 g of Zn per week is added to soil by each treated animal. The facial eczema challenge period in New Zealand can last for up to 10 weeks (on average). Therefore, assuming a stocking rate of 15 lambs per hectare, the total input of Zn to soil in the form of excreta is approximately 550 g per ha per eczema season. Further assuming that this Zn is retained in the top 10 cm of soil through adsorption to organic matter, this would result in an average increase in the Zn concentration of the surface soil by 0.78 mg/kg per year (bulk 1.0 t/m^3).

Our average annual soil-Zn increase concentration agre that proposed by Kim et al. (2008). These authors condu study investigating the flux of Zn in pastoral soils of the Wai gion and estimated that the Zn loading to soil through the us imal remedies was 5–7 kg/ha/yr, at an average annual concer increase of 0.7 mg/kg. The average background concentratio in Waikato soils is reported as 35 mg/kg, while the average tration for pastoral soils is 59.4 mg/kg. In a recent review of t cal concentration of Zn in the soil, Jeyakumar et al. (2010) ca the concentration of total soil Zn that promoted a 10% yield re in poplar and pine trees to be 120 and 103 mg/kg respectiv though the critical concentration was higher for soil bacteria a corrhiza. This was for a pastoral soil amended with biosolids t been contaminated with Zn. Consideration of these values s revision downwards of the intervention concentration of tot soil from the current value of 300 mg/kg (NZWWA, 20 100 mg/kg. Approximately 10% of pastoral soils sampled by al. (2008) had a soil Zn concentration above the interventi of 100 mg/kg. Assuming the annual concentration increases re here, the majority of soils sampled may exceed this investigati within 40 years. This is clearly an issue of potential concern; concentration in New Zealand pastoral soils is steadily increa

These calculations assume that the animals evenly distribu ces across 1 ha, which is not a realistic assumption. Excreme be applied to concentrated areas, leading to localised hot spot plied soil Zn. Shorter or longer periods of FE risk will result i variation of the amounts of input both up and down. This Zn sents an anthropogenic input that is poorly contained and has tial to readily interact with the productive environment. The e future environmental risk that may be incurred through this p is unknown.

3.6. Practical application of biofortification for facial eczema prop animal remedy and environmental management

The willows used for this feeding trial were grown on a s taining just 36 mg/kg Zn. The biofortified hay was prepared vesting grass from soil amended with biosolids that had a t concentration of 250 mg/kg. The increased bioaccumulation for willow (7.17) relative to the biofortified hay (0.26) hig the ability of willow to accumulate Zn. If willows were to be on areas of known, higher-concentration (and thus environ risk) soil, then the concentration of Zn in the plant may be m higher. The willow species *Salix viminalis* (Gigantia) common in New Zealand as a fodder tree species, has been reported t mulate Zn to a concentration in excess of 1000 mg/kg grow soil with 97 mg/kg Zn (Hermle et al., 2007).

Drenching is expensive and time consuming, requiring t sheep be mustered and manually administered the drench. trast, supplying fortified fodder could be less expensive and m ficient. A land management strategy can be envisaged whe critically high in Zn are planted exclusively in willow. This wou resent an active attempt to increase the Zn content of willow t agronomic practice (an example of biofortification). During the and summer months as these plants are growing, Zn will be a lated into the above-ground biomass. During periods of high fa zema risk (late summer, early autumn) stock could be allo graze the willow, either directly from the tree, or by way o and carry system. Generally, in New Zealand, times of high fa zema risk coincide with periods of low grass production at the ning of a period of autumn growth. The willow would th represent a valuable source of fodder to sustain live weigh The distribution of excrement concentrated in Zn as a result of digestion could be limited through holding animals in the

areas during the periods of high eczema risk. In subsequent seasons this Zn would be cycled from soil back into the trees, limiting the need for further Zn input into the agricultural system. This would not only represent a sustainable system for animal trace element nutrition, but a sustainable system for the management of potential trace element contaminants in soil.

In this scenario we propose that biofortified pasture is not suitable for use as fortified fodder because of the low bioaccumulation factor of Zn for grass (hay). Biofortified pasture in this experiment had no clear, significant effect on blood or faeces chemistry. But the relatively high Zn bioaccumulation factor for willow leads us to believe that use of willow as biofortified fodder has potential as an animal remedy. The total amount of Zn ingested by the animals fed willow was only 11% of that ingested by the animals administered drench. However, by Day 7 of the trial the Zn concentration in serum, whole blood and faeces were the same (serum and whole blood) or higher (faeces) for the sheep fed willow relative to the drenched animals. The concentration of Zn in blood serum, whole blood and faeces for the sheep fed willow increased throughout the period of controlled feeding. This indicates that Zn in the fodder is being slowly released from the stomach. A key factor in the dietary supplementation of animals is the bioavailability of the trace elements in the fodder. Compounds such as tannins and oxylates inhibit the uptake of trace elements, including Zn and Fe, while uptake is promoted by ascorbates and citrates (Fuxia et al., 2009). Under the conditions of the current trial, tannins in the willow biomass may have retarded the release of Zn into the blood stream. This could represent a further beneficial effect of biofortified willow as an animal remedy.

A key unknown that is pertinent to this strategy is the extent to which Zn in naturally fortified/biofortified fodder provides protection against sporidesmin, and the length of time after ingestion of the fodder this effect is apparent for. These questions are being addressed through ongoing research.

3.7. The general health context of the research results

Zinc deficiencies in livestock are less widely quantified than deficiencies in the human population, but will occur for the same reasons. Deficiency of all trace elements will lead to decreased production and economic return. A general theme of literature is that supplementation of Zn beyond normal physiological requirements will lead to increased livestock growth performance relative to control populations (Herdt and Hoff, 2011; Lee et al., 1999). Our data shows that Zn-fortified fodder (biofortified or naturally enriched) will readily increase the serum and whole blood Zn concentration of sheep. Therefore, the positive effects of Zn biofortification of animal fodder may extend beyond just prophylaxis for fungal infection. In this sense, biofortification for animal health could be considered a research direction of similar importance to that for human health.

4. Conclusion

The findings of this research support biofortification as a valid mechanism to increase the blood concentration of Zn in sheep. Willow, which has a bioaccumulation factor greater than that of grass (hay), is the most promising candidate as fortified fodder. Selective planting of willow on soil with an elevated concentration of zinc, or harvest of native willow with a higher concentration of zinc than that used in this study (258 mg/kg), could yield Zn-enriched fodder that can more readily increase blood or faeces concentration to above levels prescribed as necessary to protect against facial eczema. A planting strategy, where willow is grown on soil that is becoming gradually contaminated due to the amount of Zn entering the environment through the use of conventional animal remedies (agronomic biofortification), to the sustainable biogeochemical recycling of trace elem minimise the need for new inputs of Zn into the pastoral env An increased level of Zn in animal diets beyond normal physic nutritional requirement achieved through the use of biofor low fodder may promote the additional benefits of improve performance and feed efficiency.

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