

BIOFORTIFIED FODDER

- AN ENVIRONMENTALLY SUSTAINABLE MECHANISM TO SUPPLEMENT LIVESTOCK WITH TRACE ELEMENTS?

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Abstract

New Zealand agriculture utilises trace element supplements to protect livestock from fungal infection. For example Zinc (as zinc oxide) administered as an oral drench or intraruminal bolus, is used extensively to protect sheep and cattle from facial eczema. A large percentage of administered Zn is however excreted in faeces and there is published evidence to show that Zn levels in pastoral soils are increasing with time. The long-term environmental affect of this ongoing Zn input to soil is unknown.

In this paper we describe research into the efficacy of fodder with an elevated Zn concentration as a potential prophylaxis against facial eczema in sheep relative to a conventional drench. Our hypothesis is that Zn protection afforded by biofortified fodder may be realised at a relatively lower dose, thus limiting transfer of Zn into the pastoral environment. This may represent a more environmental sustainable mechanism to supplement livestock with trace elements than conventional options. Our mechanism of Zn administration can be described as the biofortification of food with essential trace elements.

During a controlled feeding trial, 20 sheep were administered one of four Zn treatments over seven days (conventional drench or biofortified / non-biofortified fodder). Blood and faecal samples were taken regularly and analysed for the constituent Zn concentration. Prescribed threshold levels for Zn in blood serum and faeces were used to gauge the likely effect of treatments in protecting against facial eczema. Both conventional ZnO drench and biofortified fodder (willow) increased the Zn concentration in blood and faeces. The drench increased serum levels to above the threshold level for protection, however Zn was rapidly excreted, and after a very short time levels had dropped to below the prescribed concentration. In comparison, serum and faeces Zn levels in the animals fed biofortified willow increased throughout the feeding period. The total amount of Zn administered to animals via willow was significantly lower than that administered via drench, and consequently serum and faeces concentrations did not increase above the prescribed threshold level. However, our results do show that biofortification has potential as a mechanism to deliver trace elements to animals.

Introduction

Trace elements (TEs) are a crucial component of animal diets. Deficiencies in one or more trace elements will cause a well-described reduction in animal health. Soil deficiencies of Cu, Co and Se are recognised in many parts of New Zealand, and fertilisation strategies have been adopted to fortify both soil and plants to overcome the resulting animal health issues. A trace element involved in a range of physiological processes is Zn. While Zn deficiency is not generally recognised in the New Zealand pastoral environment, high levels are used to protect against several production limiting animal-health conditions, in particular the condition known as facial eczema.

Facial eczema (FE) is a secondary response (photosensitivity) which occurs due to liver damage inflicted by the mycotoxin sporodesmin contained within spores of the saprophytic fungus *Pithomyces chartarum* (Bennison et al, 2010). Warm humid conditions at the end of summer promote the growth of the fungus which infect swards of grass. Spores are subsequently ingested, particularly by cattle and sheep. Acute exposure can lead to visible distress and death, but animals which have suffered chronic exposure, over a long time frame (often several seasons), show signs of significant damage to bile ducts and the liver (Smith and Towers, 2002). The liver enzyme gamma-glutamyl transpeptidase (GGT) is used as a biochemical indicator of facial eczema and is elevated in animals with either chronic or acute exposure to the disease.

There is no treatment for FE, but protection can be afforded through the oral administration of high levels of Zn. The traditional application is a drench or intra-ruminal bolus (15-20 mg Zn/kg liveweight per day), at a rate that is well above physiological requirement and that can be regarded as sub-toxic (Grace et al., 2010). The majority of Zn is excreted and the result of many years of FE prophylaxis is an increasing background concentration of Zn in NZ pastoral soils (Kim et al., 2008). The sustainability of current practice is poorly understood.

An alternative to the administration of inorganic forms of trace element is biofortification, a group of technologies which seek to increase the concentration of Zn and other trace element micronutrients in food or fodder pre-harvest using natural means (White and Broadley, 2005). Biofortification is today widely described as a technique to mitigate human disease and health conditions that are potentially caused by a dietary lack of essential trace elements (TEs), but the technology can potentially also play a role in animal nutrition.

The purpose of the described research was to assess the relative efficacy of biofortified fodder (willow and grass) and conventional drench, to raise blood and faecal Zn concentrations to a level where protection may be afforded against FE.

Methods and Materials

Twenty four female lambs, with weights ranging from 32-37 kg (average weight 34.5 kg), were randomly selected from a Massey University production farm on the 19 April 2010, and transferred into the animal production unit at the Palmerston North Campus of Massey University. The animals were randomly placed in individual pens, each with their own water and feed containers.

Control hay was fed to all animals for the first three days of the trial. At the end of the three day acclimation period, the 20 animals, in blocks of five, that were best responding to the trial conditions were assigned to one of four treatments (drench, willow, biofortified grass, control hay), and a controlled feeding regime was started. The first day of treatment was

defined as Day 0, and continued for seven days. From Day 8 to Day 10, all animals were placed back on the control hay diet, prior to release back onto the Massey University production farm. Feed and water were replaced daily.

A jugular blood sample (5 mL) was collected from each of the 20 experimental sheep on Days 0, 2, 4, 7 and 10 using a vacupac container. The blood samples were centrifuged at 3000 RPM for 10 mins at 4°C. Serum was then separated from red blood cells, and both components frozen in preparation for analysis. The weight of the whole blood, serum and red blood cells was recorded.

A sample of faeces from each animal was also collected on Days 0, 2, 4, 7 and 10, then dried at 70°C until a constant weight was obtained.

Feed treatments

Biofortified hay was harvested from research plots at Lincoln University in Canterbury, where soil was amended with biosolids containing Zn up to a concentration of 250 mg/kg. Pasture was cut in March and April 2010 in preparation for the sheep trial. The grass was then dried, well mixed and packed for transport to Massey University. Willow (*Salix purpurea*, clone Pohangina) was harvested each day by cutting stems of one-year old trees growing at the RST Environmental Solutions Ltd. tree nursery in Aokautere near Palmerston North. The willow stems were coarsely chopped, then fed to the relevant sheep. Control hay was purchased from the animal production unit. For the zinc oxide drench, commercial ZnO (500 g) was mixed with water (500 mL) and 100 mL of Nutrimole suspending agent. Twelve mL (15 g) of this suspension was administered orally to each of the five treated sheep using a plastic syringe. A single dose of drench was given to the treated sheep on Day 0 of the feeding trial. The drenched animals were subsequently fed control hay for the duration of the trial.

A weighed amount of feed was presented to each animal at the start of controlled feeding on Day 0. The amount remaining was then recorded either daily, or twice daily, and feed topped up to a known weight. The cumulative amount of feed ingested over the seven day feeding trial could therefore be accurately calculated.

Analysis

The Zn concentration in all samples was determined using ICP-OES (Varian), at Lincoln University. Blood serum was analysed directly after 1:4 dilution of the samples. A known volume of red blood cells was first digested in nitric acid for 2 hours using a digestion block, then diluted to 25 mL with de-ionised water. Based on the weights of the plasma and red blood cells recorded, it was possible to express the Zn concentration as a function of serum, red-blood cell, and whole blood mass and volume.

The dry faeces samples were ground using a mortar and pestle. A subsample (0.5 g) was then 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 minutes 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 90% of the certified value.

Results

The concentration of Zn in each of the treatments, the amount of treatment administered over the seven days of controlled feeding, and the calculated total amount of Zn administered to the animals, is presented in Table 1. The Zn concentration in the serum and whole blood of the animals administered drench and willow for each sampling time is presented in Table 2. The Zn concentration in faeces is shown in Table 3.

Table 1. Description of the Zn treatments used

Treatment	[Zn] mg/kg ¹	Total treatment	Mass of Zn
Drench	365 g/kg	15 g on Day 0	5454 mg
Control hay	33.2	6387g	211 mg
Biofort. hay	64.0	4682 g	300 mg
Willow	258	2419 g	624 mg

Units are mg/kg unless stated otherwise

Discussion

Fodder vs drench for Zn delivery

Drench rapidly increased the Zn concentration in the blood of the treated sheep. The maximum serum Zn concentration was reached on Day 2 (30.7 μM). The guideline threshold for protection against FE is a serum Zn concentration of 20-30 μM (Munday et al., 1997), therefore animals in this trial were likely protected against FE from treatment to Day

Willow caused a significant increase in serum and whole blood Zn concentrations, but to an insufficient level to afford protection against FE (maximum value 14.37 μM on D2). There were no significant increases in serum or whole blood Zn concentration for the control animals and those fed biofortified hay.

Table 2. Whole blood and serum Zn concentration (drench and willow treatment)

Day	Whole blood [Zn] μM		Serum [Zn] μM	
	Drench	Willow	Drench	Willow
0	52.58 (2.27)	48.55 (1.47)	12.67 (0.78)	12.40 (0.64)
2	67.00 (3.23)	47.35 (2.34)	30.67 (3.37)	14.37 (0.80)
4	64.37 (3.61)	51.26 (1.59)	25.68 (2.91)	13.93 (0.88)
7	56.36 (4.10)	54.18 (1.89)	16.16 (2.06)	14.05 (0.96)
10	50.96 (2.05)	49.62 (7.28)	11.69 (1.20)	14.26 (1.12)

Values are mean and standard error (n=5)

Table 3. Faecal Zn concentration (drench and willow treatment)

Day	Faeces [Zn] mg/kg	
	Drench	Willow
0	223 (22.1)	224 (90.6)
2	3983 (448)	275 (49.9)
4	889 (214)	506 (85.8)
7	178 (39.2)	548 (27.7)
10	107 (8.33)	141 (4.88)

Values are mean and standard error (n=5)

Protection against FE may also be afforded by a high Zn concentration in the gastrointestinal tract. Bennison et al. (2010) suggested that a Zn concentration of 250 mg/kg fresh weight in faeces could afford prophylaxis. Drench resulted in an immediate increase in the faeces Zn concentration (3,983 mg/kg DW Day 2). This is equivalent to 1,328 mg/kg FW, well in excess of the threshold value. By Day 7, the faeces Zn concentration of the drenched animals had dropped to 59 mg/kg (FW), whereas the faeces Zn concentration of the sheep fed willow was still relatively high (183 mg/kg FW). While this is below the reported threshold it is not dissimilar. These animals potentially gained some protection against FE.

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 biofortified fodder. In the current trial we did not expose animals to sporesdesmin, 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 biofortified treatments. However, our trial has recorded a significant and important observation, that serum Zn concentrations in ruminant animals (sheep) can be rapidly increased through biofortification of the animal's fodder.

Concerns over environmental compliance

The drenched animals were administered 5.45 g of Zn (Table 1). Assuming 95% is excreted, 5.18 g is added to soil each week by each animal. The FE period in New Zealand can last for up to 10 weeks. Therefore, at a stocking rate of 15 lambs per ha, the total input of Zn to soil is approximately 550 g per ha per FE season. Assuming that this Zn is retained in the top 10 cm of soil through adsorption to organic matter, the average increase in the zinc concentration of the surface soil is 0.78 mg/kg per year.

Kim et al. (2008) estimated that the Zn loading to pastoral soils of the Waikato region through animal remedies was 5-7 kg/ha/yr, at an average annual concentration increase of 0.7 mg/kg. The background concentration of Zn in Waikato soils is reported as 35 mg/kg, while the concentration for pastoral soils is 59.4 mg/kg. Jeyakumar et al. (2010) indicated that a safe guideline value (maximum concentration) for Zn in NZ pastoral soils could be as low as

100 mg/kg. Approximately 10% of Waikato pastoral soils exceeded this value. Therefore Zn loading to soil through the use of animal remedies may be an issue of possible future concern.

Practical application of biofortification for facial eczema prophylaxis: Animal remedy and environmental management

The willows used for this feeding trial were grown on a soil containing just 36 mg/kg Zn, yet there was a significant concentration effect of zinc in the willow biomass. If willows were to be grown on areas of known, higher Zn concentration in soil, then the concentration of Zn in the plant may be manifold higher. The willow species *Salix viminalis* (Gigantia) commonly used in New Zealand as a fodder tree species, has been reported to accumulate the Zn to a concentration in excess of 1,000 mg/kg growing on soil with 97 mg/kg Zn (Hermle et al., 2007).

A land management strategy can be envisaged where soils high in Zn are planted exclusively in willow. During the spring and summer months as these plants are growing, Zn will be accumulated into the above-ground biomass. During periods of high facial eczema risk (late summer, early autumn) stock could be allowed to graze the willow, either directly from the tree, or by way of a cut and carry system. Generally, in New Zealand, times of high facial eczema risk coincide with periods of low grass production at the beginning of a period of autumn growth. The willow would therefore represent a valuable source of fodder to sustain live weight gains. 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 (Hathaway 1986; Douglas et al. 1996). 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 2001). The distribution of excrement concentrated in Zn as a result of willow digestion could be limited through holding animals in the willow 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.

In this scenario we propose that pasture is not suitable for use as biofortified fodder because of the low bioaccumulation factor of Zn for pasture recorded in our trial. Biofortified pasture in this experiment had no clear, significant effect on blood or faeces chemistry. However, the relatively high bioaccumulation factor of Zn for willow leads us to believe that use of biofortified willow as fodder has potential as an animal remedy. The total amount of Zn ingested by the animals fed willow was approximately 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 was the same (serum) or higher 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 the Zn in the fodder is being slowly released from the stomach. Tannins in willow biomass may be the cause of this slow release, and represent a further beneficial effect in the use of biofortified willow as an animal remedy.

Drenching is expensive and time consuming, requiring that the sheep be mustered and manually administered the drench. In contrast, supplying biofortified fodder could be less expensive and more efficient. A key unknown that is pertinent to this strategy is the extent to which Zn in biofortified fodder provides protection against sporidesmin, and the length of time after ingestion of biofortified fodder this effect is apparent for. These questions are being addressed in ongoing research.

Conclusions

Biofortified fodder (willow) can raise the blood and faecal Zn concentration of sheep. But at the rate administered in this research, levels to protect against FE were not reached. Growth of willow on a soil with a higher concentration of Zn may lead to a biofortified fodder than can act as an animal remedy.

Consideration of the amount of Zn excreted by animals during conventional drench treatment shows that current agricultural practices may not be environmentally sustainable. The conclusion of our work is that biofortification for prophylaxis could represent a more sustainable solution than current practice, where a closed loop of Zn transfer from soil to plant to animal is achieved. Ongoing research is seeking to further elucidate this hypothesis.

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