

ETH Zürich

Department of Environmental Sciences

Report:

Compulsory Work experience in the Master of Environmental Science Programme

The Massey- Lincoln Biofortification Initiative: Sheep trial



supported by
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Personal Report

About New Zealand

New Zealand is located in the south west pacific comprising two main landmasses, the South- and the North Island and numerous smaller islands including Stewart Island. All these islands come to a landmass of 268680 square meters, which is comparable to the landmasses of the United Kingdom or the Philippines. The population of New Zealand is 4.3 million with 72% of the population living in the 16 main urban areas. About 15% of the population are Maori, the New Zealand indigenous people. The rest of the population are mostly European (68%) and Asian (9%) (Statistics New Zealand, 2009).

New Zealand's climate has a relatively small seasonal variation. In the North Island the climate is subtropical and in the South Island temperate. The average maximum temperature ranges from 20°C to 30°C in summer and from 10°C to 15°C in winter (Statistics New Zealand, 2009).

The geography of New Zealand varies a lot between the two islands. The South Island is divided through its length by the Southern Alps. The Southern Alps include 18 peaks over 3000 meters and numerous glaciers. The North Island is marked by volcanism with a volcanic plateau located in the centre of the island (Statistics New Zealand, 2009).

East Polynesians first arrived in New Zealand around 1300. They were the first settlers and are now known as the Maori. The first European to see New Zealand was Abel Tasman, a Dutch sailor, in 1642. He believed New Zealand to be part of Argentina. So he did not claim the discovery for the Netherlands. In 1769 James Cook arrived in New Zealand and claimed New Zealand for Britain. In 1840 the Treaty of Waitangi was signed. The Treaty established a British governor and recognized Maori ownership of their lands. Now New Zealand is an independent nation and part of the Commonwealth of Nations (Statistics New Zealand, 2009).

New Zealand has a developed economy with an estimated GDP of 115.624 NZD billion. The standard of living is comparable to Southern Europe with a GDP per capita of 27017 NZD in 2008. The service sector is the largest sector in the economy (68.8% of GDP), followed by manufacturing and construction (26.9% of GDP) and agriculture (4.3% of GDP) (Statistics New Zealand, 2009).

Agriculture is the main export industry together with Horticulture, Fishing and Forestry. Exports account to 24% of New Zealand's economic output thus are very important for the country. The main agricultural export commodities are milk powder, butter and cheese and meat and edible offal (Statistics New Zealand, 2009).

The main land use in New Zealand agriculture is pastoral farming with an increase in the land use for horticultural use. In Northland the major form of pastoral farming is beef cattle. In the Waikato and Taranaki regions dairy cattle make up the biggest part in pastoral farming and for the rest of

New Zealand sheep farming is the major pastoral land use. (Ministry of Agriculture and Forestry, 2003)

About Lincoln University

Lincoln University is located on the South Island of New Zealand, about 22 km south of the city of Christchurch. There are about 4500 students enrolled at Lincoln University with students from 60 different countries (Web 1). The study areas, which Lincoln University have to offer include Agriculture, Commerce, Computing, Environmental Science, Forestry, Horticulture, Hospitality, Tourism and Recreation, Sciences, Maori Studies, Landscape Architecture and Winemaking (Web 2).

Lincoln University was first founded in 1878 as a School of Agriculture. The organisation of the school was linked to Canterbury College, in Christchurch. Only in 1990 Lincoln University formally separated from the now called University of Canterbury and became a self-governing national university (Web 1).

Lincoln University is now governed by a council. The council is responsible for the strategic direction of the university and its performance. It also appoints the Vice- Chancellor which is responsible for the Management of the University. The Vice- Chancellor manages the key activities of the university through delegations to senior managers. The Faculties develop and give courses to students and perform research (Web 3). There are three faculties and the Foundation Studies and English Language Centre. The Foundation Studies and English Language centre gives students who don't have a tertiary qualification the opportunity to study at Lincoln University. The centre also offers courses in English for academic purposes. The three faculties include the faculty of Agriculture and Life Sciences, the Faculty of Commerce and the Faculty of Environment, Society and Design (Web 4).

The Faculty of Agriculture and Life Sciences represents a land- based philosophy including research in the areas of nutrition and health, soil and physical sciences, applied mathematics, statistics and computational modelling, biochemistry and cell biology, microbiology, toxicology, agronomy and plant science, food science, horticulture, viticulture and oenology, farm management, ecology and conservation and plant pathology. The faculty operates three Research Farms, a vineyard, a horticultural research area, a winery and a nursery. The faculty consists of four departments (Agricultural Sciences, Ecology, Wine, Food and Molecular Biosciences and Soil and Physical Sciences) (Web 5).

The Department of Soil and Physical Sciences comprises 19 academic staff, research officers and postdoctoral fellows and again about the same number of technical and support staff. The range of research performed in the department is extensive but has increasingly focused on environmental issues in recent years. A number of current research projects involve the measurement and modelling of solute and microbial transport through soil. Other projects involve the measurement and modelling of gases generated within or absorbed by soils. Further examples of current projects

include investigations into soil- plant rhizosphere relationships, heavy-metal contamination of soil, soil remediation and quaternary geology and paleoclimate research (Web 6).

Personal Experiences

In autumn last year I contacted Rainer Schulin and asked him if he would have any recommendations for my internship abroad. He then told me about Brett Robinson and Lincoln University. He also told me that Brett happens to be in Switzerland for a short time then. I then contacted Brett and we met up one afternoon. The meeting was only very short. Brett introduced me to possible projects I could be working on in New Zealand. A few weeks later I decided to take on one of these projects in New Zealand, a biofortification project involving sheep. During my internship I was mainly responsible for this research project (see professional report). The project started in April. So before it started I already worked at Lincoln University for 1 and a half months. During this time I helped out with other research projects. I mainly helped with a survey of New Zealand soils. During these first few weeks I was slowly introduced to the methods used in their labs so when my biofortification project started I could work very independently.

I was very happy to be part of a research project from start to finish. During lab courses at ETH we would mostly only see one part of the research, either field work, lab work or the final analysis. So it was sometimes hard for me to always see the bigger picture in a project or to understand why a certain task had to be done. With my project at Lincoln University this was never an issue and I thoroughly enjoyed it.

I worked in a very good team. One could always ask questions and if something wasn't clear we discussed it together. Everyone was accepted and I always felt like my suggestions were just as valuable as those of others with more experience in the field. I especially enjoyed that there was no polite form of address. Everyone was called by their first name. This made it much easier for me to approach people and I also think that it leads to a more relaxed workplace. We had a morning tea break during which most people of the department came together. This way I got to know people on a more personal level.

Before my internship started there were a few communication problems. I only met Brett this one time very briefly in Switzerland before my internship started. Shortly after I decided to go New Zealand for my internship I left Switzerland to travel in Australia for 3 months. This trip was planned well before I met Brett. Because I was travelling and did not have access to internet continuously it made it very difficult for us to discuss the work contract and visa details. There were a few difficulties concerning my salary. It was said at first that the university could only pay my living arrangements on campus and not an actual salary as such because of tax reasons. I on the other hand did not want to live on campus. Brett kept asking for other possibilities in the department and worked out a way around this eventually. It was also very difficult for me to organise a visa for New Zealand while I was in Australia. I sent off my application in Australia and did not receive a visa for a

very long time. I and Brett both kept contacting the embassy but nothing happened. Two weeks before entering New Zealand I rang them again and would not hang up until they would give me my visa the next day. They finally realised that there was nothing wrong with my application and I could pick up my passport with a work visa. So previous to my arrival in New Zealand the communication was difficult but after that there were no more issues.

In my research project I could work very independently. It felt great to be able to do that. I also knew that if there was a problem I would always get help of someone. There was routine in the lab but mostly my work was very varied.

Financial boundaries were met with creativity. The soil science team built their own sieves to filter soils for example or used an old beer bottle to break up big soil pieces. We also used an old barbecue tong to collect the faeces samples when working with the sheep. I didn't really feel any pressure with time because my project was well planned and it was always discussed with Brett how much time I needed for my next job etc.

During the experiment with the sheep in Palmerston North we worked 7 days a week for two weeks because we had to feed the sheep every day. But other than that my working hours were really relaxed. As long as my work was done I was quite free. I could take an afternoon off if the weather was nice or if I wanted to discover more of New Zealand. I especially always felt supported with my travel plans around New Zealand and tips of where to go were always shared.

During my time at Lincoln University I definitely learnt a lot. The most important part was all the new experiences I made working in the lab. I also feel much more competent of working in a lab now. Furthermore I have less trouble approaching people who have more work experience than me because everyone was very helpful and relaxed in our department.

I had a great time in New Zealand and would definitely recommend the Soil Science Division for an internship to my friends.

Therefore I would like to thank Brett and Chris for their great support during my time in NZ. It was great working with and for you. The time we spent feeding sheep and cleaning up after them was always a great laugh and so were the hours together after work. Lynne was also a great support and I don't know what I would have done without you as you were always the one to answer my silly questions. Thank you Lynne!

Professional Report

Introduction

Trace elements (TEs) occur at small concentrations ($<1000 \text{ mg kg}^{-1}$) in organisms, yet they have a large biological effect as essential micronutrients. Some 35% of children and 20% of adults suffer from Zn deficiency, and 260 million suffer from iodine (I) or selenium (Se) deficiencies. Cobalt, selenium and zinc deficiencies are widespread in New Zealand soils. This leads to poor animal health and decreased productivity e.g. Facial Eczema in cattle. Most micronutrients that stock or humans ingest come from plants. Therefore, improving the plant uptake of micronutrients in deficient areas could benefit animal productivity and human health. Plant uptake depends on then total concentration of the micronutrient in the soil, the micronutrient's solubility and speciation, the pasture's species composition and other plant component that affect trace element uptake by the animal's gut. Biofortification aims to improve micronutrient fluxes between soil and plant as well as between plant and animals.

The Massey- Lincoln Biofortification Initiative investigates the use of biofortification to improve New Zealand's agriculture. Experiments involve the manipulation of the soil-plant system to increase uptake of specific trace elements, with the primary focus on Zn and Cu. Furthermore the biofortified biomass is fed to sheep at Massey University and trace elements in the cattle's blood are analyzed to track the micronutrient fluxes between plant and animal.

Material and Methods

More than 5 years ago two 10x5 m plots of pasture at Lincoln University were fertilized with biosolids. Biosolids are high in zinc and other trace elements. One of the plots was fertilised with biosolids containing 150mg Zn/kg and the other plot with biosolids containing 200 mg Zn/kg. The pasture was cut in March and April 2010 in preparation for the sheep trial. The grass was then dried, well mixed and packed up for the transport to Massey University on the North Island. Before the trial started the zinc levels in the biofortified hay were tested. The zinc concentration in the hay was 64.04 mg/kg, which equals to about twice as much zinc as in untreated hay (Diagram 1).

The trial was set up to have two treatments, a control and a positive control, with 5 replicas each. One of the treatments was the biofortified hay and the other treatment was willow. Willow leaves and stems are naturally high in Zinc. The positive control was a drench for the sheep and untreated hay and the control sheep had no treatment at all and were fed untreated hay. The willow leaves used in the trial, had an average zinc concentration of 258.13 mg/kg and the untreated hay had a concentration of 33.21 mg/kg. The willow was freshly cut every day of the trial and the untreated hay was provided by Massey University.

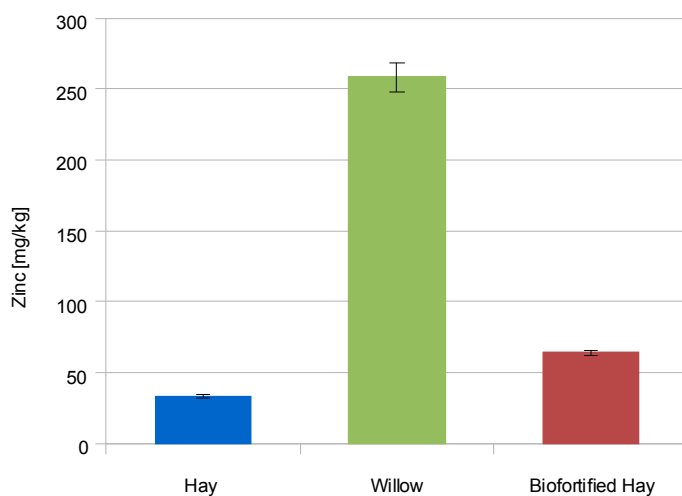


Diagram 1: Average amount of zinc in the treatments used in the trial

There were four treatments with 5 replicas each so there were 20 sheep used in the trial. The sheep were kept in individual boxes in a barn at Massey University for 14 days. The trial run for 10 days and 4 days before the trial started the sheep were put in their boxes and fed untreated hay. There was 24 sheep in case some would not get used to the new environment and not eat. Fortunately this did not happen so four sheep were kept in the barn but not used for the trial.

On day 0 the first blood samples and faeces samples were collected and right after the food was changed to willow leaves and biofortified hay and the drench was given to 5 sheep. The drench was

a mixture of Zinc Oxide and Nutrimol. Each sheep was given 11 ml of drench which contained a dose of 3g of zinc.

Throughout the trial it was monitored how much each sheep ate. And their feed was normally filled up twice a day. The blood samples were taken by vet Dr. Dave West of Massey University. The blood and the faeces samples were taken on Day 0, Day 2, Day 4, Day 7 and Day 10 of the trial. On day 7 the treatments were stopped and all sheep were fed untreated hay again until the trial was finished on day 10.

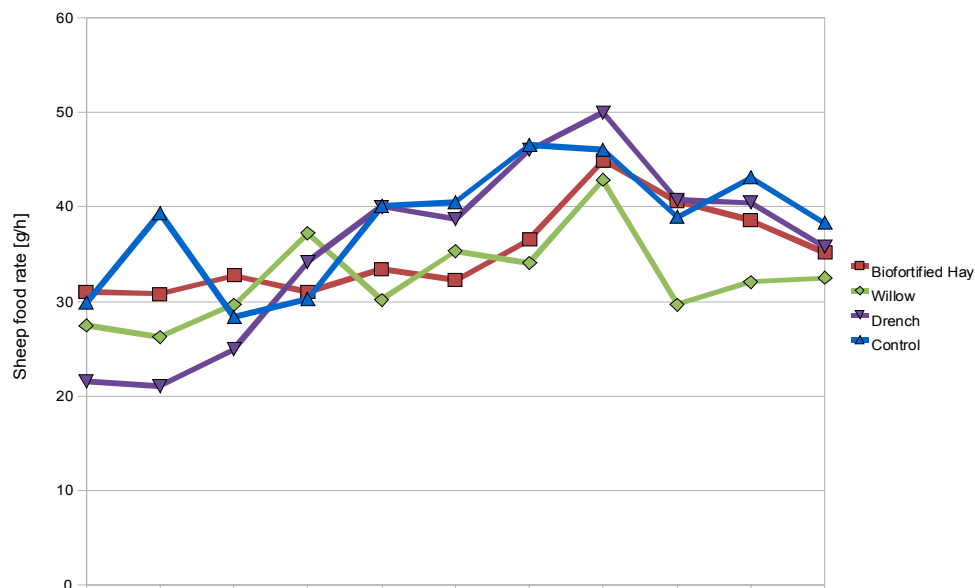


Diagram 2: Amount of food that was eaten by the sheep, grouped by treatment, in grams per hour over the course of the trial.

After each day of sampling the blood samples were kept in a fridge for 24 hours. After the 24 hours the bloods were centrifuged for 10 minutes with 3000 Rpm and the serum was separated from the whole blood. The whole blood and the serum were then kept in the freezer. The faeces samples were put in an oven on 105°C for drying.

After the trial was over the faeces and blood samples were taken to Lincoln University for the lab analysis.

All samples had to be prepared for the final analysis on the ICP. The blood serum was diluted 1:4 and could be analyzed straight away after that. The whole blood had to be digested on a digest block. All of the samples were put in glass containers and 10ml of nitric acid were added to each sample. The glass containers were then put on a digest block at 100°C for at least 2 hours. Then the samples were diluted to 25ml with deionized water.

The dry faeces samples were ground with a mortar and pestle. 0.5 g of the faeces powder was weighed in a microwave container. 5ml of nitric acid were added and the mixture was left over

night. The next morning the solution was put in the microwave. The microwave heated up to 75°C and 700 Watts during 15 minutes and then stayed at that level for 10 minutes. After the digest the samples were diluted to 25ml with deionized water.

All measurements were carried out on an ICP- mass- spectrometer (Varian) combined with a nebulizer (Varian).

Results

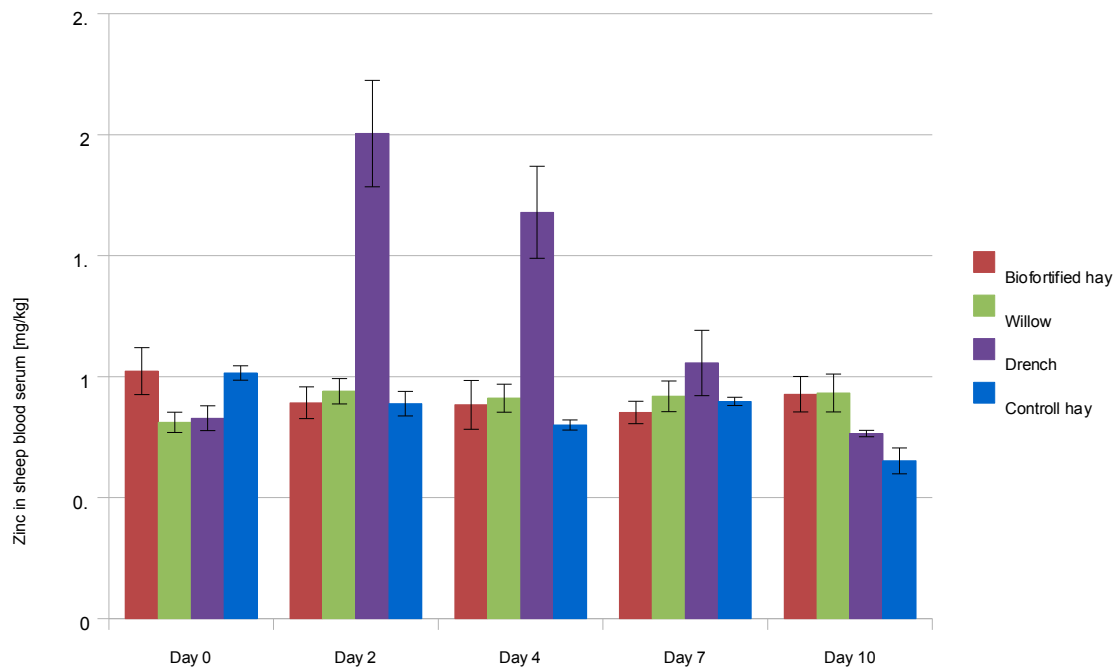
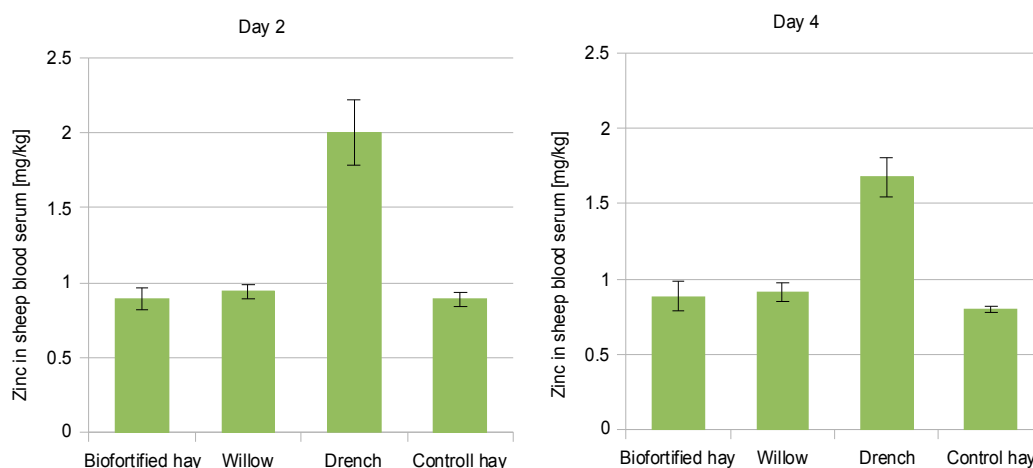


Diagram 3: Amount of zinc in sheep blood serum over the ten days of the trial.

The zinc in the sheep blood serum increases a lot after the drench was given to the sheep on day 0. This increase is clearly visible in the blood serum taken on day 2 (Diagram 3). The control sheep have 2.00mg/kg zinc in their blood serum on day 2 and 1.68 mg/kg on day 4. In the blood serum taken on day 7 the zinc levels have reduced a lot and on day 10 the effect of the drench is no longer visible. The biofortified hay treatment shows a decrease in the zinc measured in the blood serum from day 0 to day 7 from 1.02 mg/kg to 0.85mg/kg. Between day 7 and day 10 there is an increase to 0.93mg/kg. The willow treatment shows a steady increase of the zinc levels in the sheep blood serum between day 0 (0.81mg/kg) and day 10 (0.93mg/kg). These results are shown in more detail in diagrams on the following page.



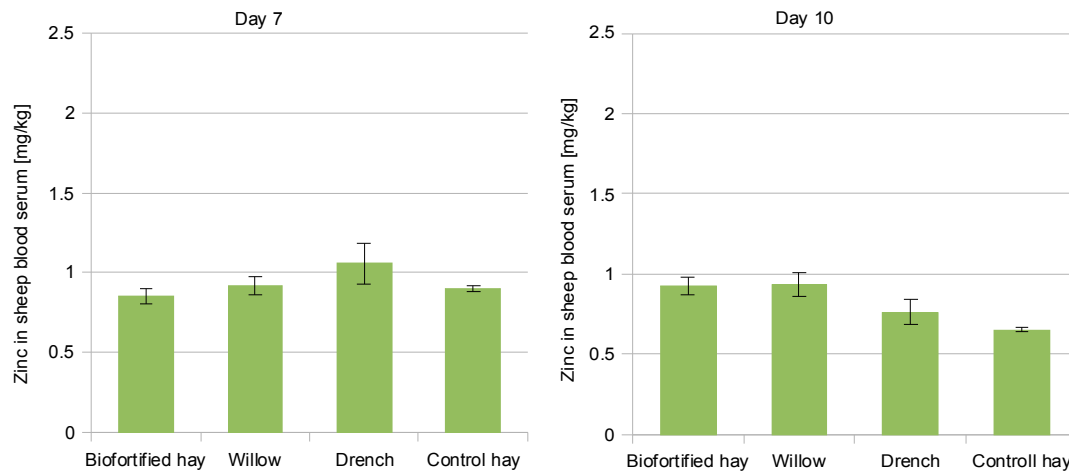


Diagram 4: Zinc in sheep blood serum in more detail. Each diagram stands for a day of the trial on which samples were taken. Day 0 is not shown in detail.

The results for the amount of zinc in the sheep faeces show a similar picture than the results for the sheep blood serum.

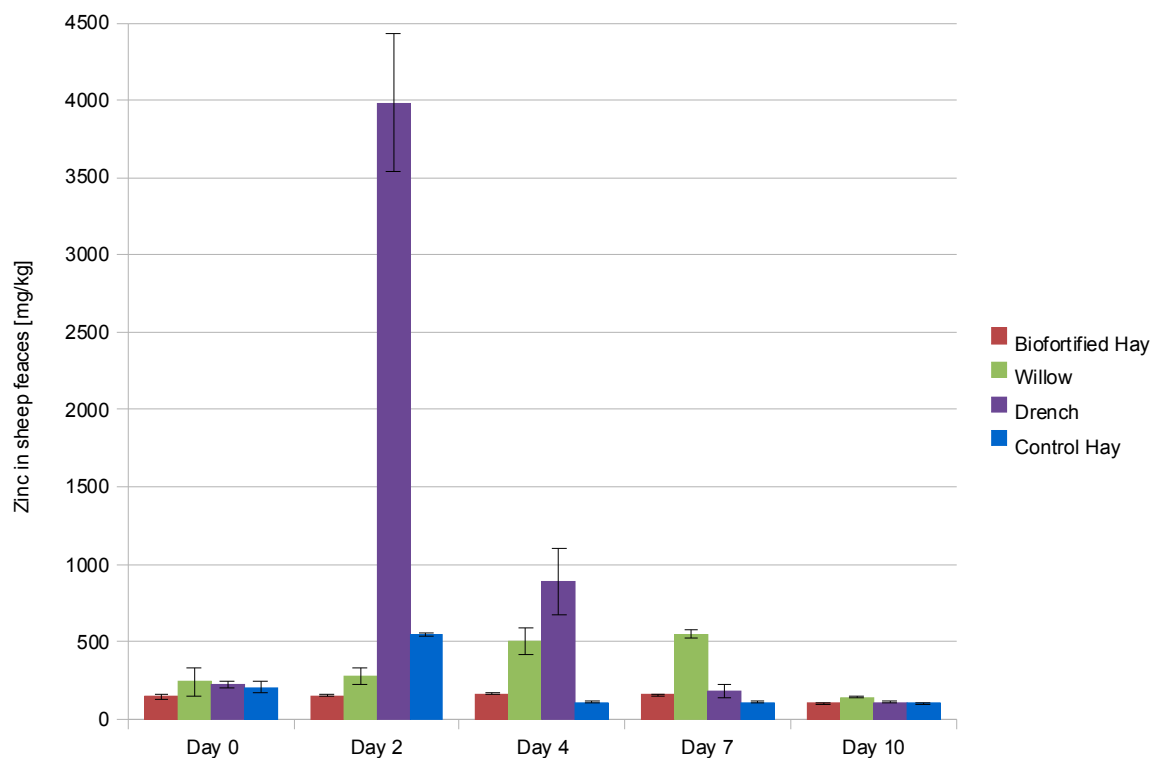


Diagram 5: Amount of zinc in sheep faeces over the 10 days of the trial.

The drenched sheep show a huge increase in the zinc levels in their faeces on day 2 after they were drenched on day 0. The zinc reaches 3983.07mg/kg on day 2 and 888.93mg/kg on day 4. On day 7 and day 10 the levels are under the level of day 0 (223.33mg/kg) with 178.31 mg/kg and 107.40 mg/kg. The treatment with biofortified hay shows an increase in the zinc between day 0 and day 4 from

142.46mg/kg to 162.58 mg/kg. Between day 7 and day 10 this amount is reduced back to 102.70 mg/kg. The willow treatment shows an increase in the zinc levels in the sheep faeces right through to day 7. The levels are 242.17mg/kg on day 0 and 547.86 mg/kg on day 7. On day 10 the zinc levels are back to 140.98 mg/kg. These results are also shown in more detail in diagrams on the following page.

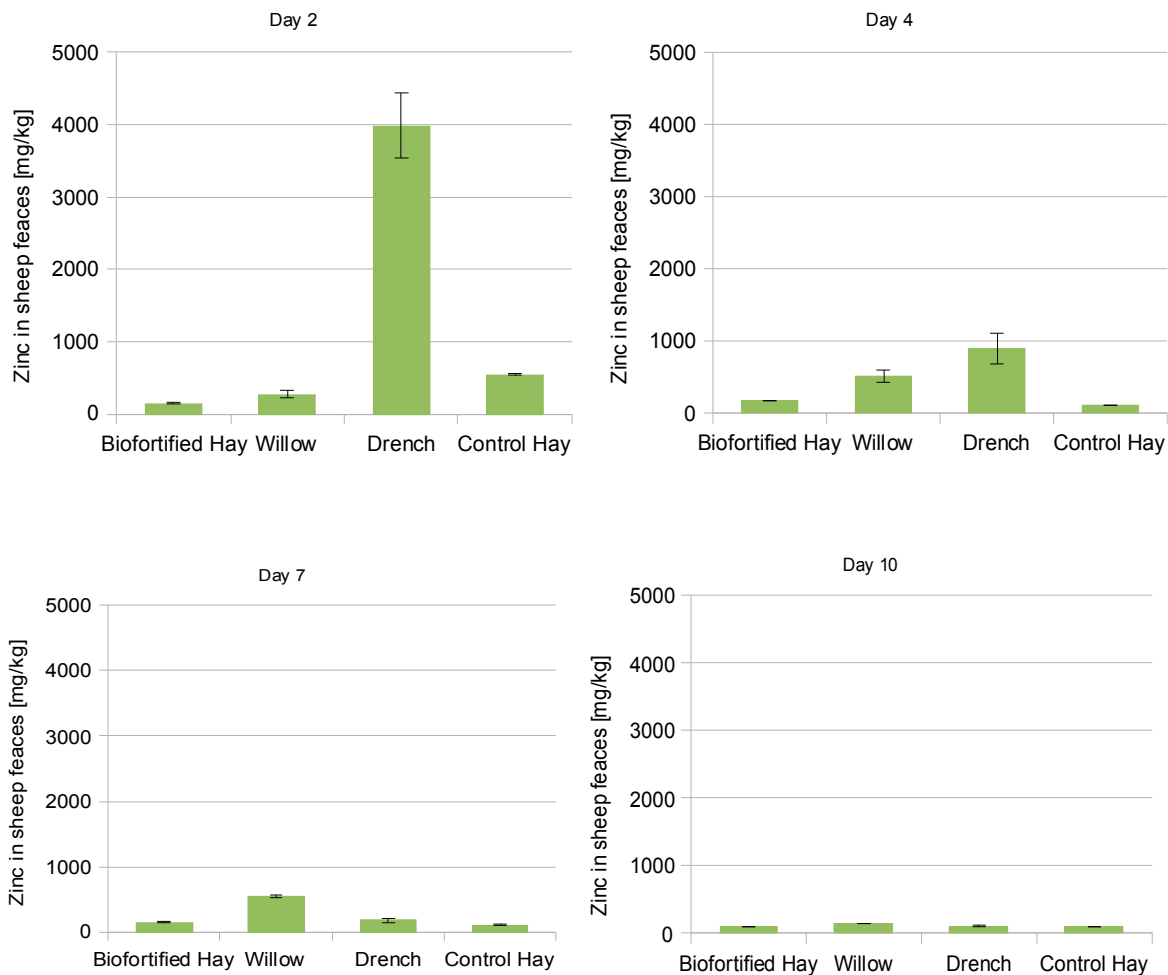


Diagram 6: Zinc in sheep faeces in more detail. Each diagram stands for a day of the trial on which data was collected. Day 0 is not shown in detail.

Discussion

This project is a part of the Massey Lincoln Biofortification Initiative as described in the Introduction. The Initiative also supports other research projects involving biofortification with biosolids and biochar. Combined with these other research projects the flux of trace elements between soil, plants and animals can hopefully be shown. This is the first time in biofortification research that animals were involved.

The results of this particular project show that the treatment of sheep with zinc oxide through drenching as commonly practised on farms in New Zealand is not sustainable. With the drench the zinc levels in the sheep's blood rise to poisonous levels on the first day and after three days there are no more effects left from the drench. To keep the zinc levels up in the sheep blood serum sheep would need to be drenched once or twice a week. This is impossible to be practised on farms in New Zealand. Furthermore the amount of zinc in the sheep's faeces that is excreted onto the soil could possibly lead to toxic zinc levels in soils. Right after the sheep were drenched a decrease in food eaten by the sheep was also observed.

Neither the willow nor the biofortified hay treatment showed satisfactory results. The amount of zinc in the feed would need to be higher especially with the biofortified hay. But on day 10 the willow and the biofortified hay treatment show significantly higher levels of zinc in the sheep blood serum than the drench treatment. This leads to the conclusion that the willow treatment as well as the biofortified hay treatment could be more sustainable practice on New Zealand farms than the drench.

The result show that there is a lot of zinc found in the sheep faeces. This zinc is not absorbed by the sheep. The excretion of a lot of the zinc occurs with all the treatments. This amount is especially high if compared with the little amount that is absorbed by the sheep in the blood serum. There is no obvious explanation of why the sheep cannot absorb more zinc. It would be extremely helpful if the absorption of trace elements by the animals gut could be explained.

More research in this area is necessary to solve these questions. This was the first research project of its kind for the Massey- Lincoln Biofortification Initiative. Because of this there is still a lot of room for improvement. The sheep or cattle would have to spend a longer period in their individual boxes. Especially the period before the trial starts is essential. With this project the period before the trial was only four days. The hay that was fed to the animals as a control treatment was of less quality than the pasture. This can be seen in the results. Other than that it seems that it would be easier and more controllable if all animals were in separate metabolic boxes. This way urine samples could be collected as well. And it would not be possible for samples to be mixed up between animals. The trial itself could be longer and more data point could be attained. Furthermore the feed could be improved. The biofortified hay would have to contain a higher amount of zinc than in this

experiment. In this trial the biofortified hay and the control hay was grown on very different soils. If all treatments could be grown in similar conditions the experiment would be improved as well.

Brett has presented results of the biofortification Initiative including the sheep trial at the international conference on environmental pollution and clean bio/phytoremediation in Pisa in June this year. His presentation and the Abstract will be attached in the Appendix of this report.

References:

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Appendix:

Abstract Phytoremediation Conference, Pisa June 2010:

Biofortified fodder production during the phytoremediation of biosolid-amended land

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Countries with sewage treatment plants produce some 27kg of dry biosolids per person per year. Many biosolids are placed in landfills or burned because of concerns about pathogens and, in some cases, their high concentrations of heavy metal and organic contaminants. Yet biosolids represent a valuable source of soil nutrients, including phosphorus, of which global supplies are dwindling. Moreover, the heavy metal concentrations in biosolids are comparable to those found in some phosphate fertilisers. Some of the heavy metals in biosolids, such as Zn and Cu, are essential Trace Element Micronutrients (TEMs) for plants and animals. Biofortification is the augmentation of TEM concentrations, pre-harvest, in food and fodder crops.

We used fodder crops to reduce nitrate and heavy metal leaching as well as killing pathogens in biosolid amended soil. These are all objectives of conventional phytoremediation. We sought to reduce plant uptake of non-essential trace elements and other contaminants that may compromise fodder quality, while simultaneously augmenting the concentrations of TEMs. Further amendments, which include biochar, were used to manipulate the balance of essential trace elements and toxic heavy metals. The resultant fodder crop contained no more heavy metals than biomass from an unamended soil, while levels of some TEMs were increased by up to 300%. Animal trials were used to quantify the rate of transfer of TEMs from the biofortified fodder to blood serum.

Using phytoremediation to promote better human and animal health requires a whole systems approach. Understanding of the soil-plant system permits the recovery beneficial TEMs from waste material into plants while limiting plant uptake of toxic elements and compounds. There is a lacuna of information on the interactive effects of many trace elements. Here, we detail our understanding of the effect of phytoremediation in the context of producing of biofortified fodder crops.

Presentation Phytoremediation Conference, Pisa June 2010:

